

Cell wall modifications during fruit ripening: when a fruit is not the fruit

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Textural changes that lead to softening of fruits are accompanied by loss of neutral sugars, solubilisation and depolymerisation of the polysaccharides of the cell wall, and rearrangements of their associations, as the result of the combined action of several cell wall-modifying enzymes, acting in both pectic and hemicellulosic fractions. Recent studies on the structure of the plant cell wall have disclosed a large number and type of biochemical linkages between the components. Such linkages are potential targets for enzymatic action and draw attention to the putative involvement of several members of enzymes able to act and modify its structure in a developmental and coordinated way. Extensive work on fruit ripening has been done using tomato (*Solanum lycopersicum* [*Lycopersicon esculentum* Mill.]) as a plant model and the information concerning fruits other than model species is fragmented and incomplete. However, recent data from the literature had disclosed that differences exist between fruits, and even between cultivars of the same fruit species. These differences exist in the type and extent of the modification of the polysaccharides of the cell wall and in the expression and regulation of cell wall-modifying enzymes. In addition, genetic manipulation of cell wall-modifying genes re-opened the discussion about the real effect of these enzymes in the cell wall and their role in fruit softening. Moreover, the function of each enzyme has been proposed based on its homology with other

annotated sequences, but, in most cases, confirmation of activity *in planta* and substrate specificity remains to be investigated. This aspect and recognized limitations of the *in vitro* enzymatic activity assays also need to be considered when discussing their role. This paper provides a critical review on the current knowledge concerning these differences and emphasises the need of using other species and more accurate methodologies to investigate general mechanisms and fruit specificities of softening among different fleshy fruits.

Introduction: the significance of fruit ripening and associated textural modifications

Once regarded as a senescent phenomenon, fruit ripening is now considered as a well coordinated and genetically determined process of tissue differentiation. Events like pigment accumulation and volatile production are included among universal ripening changes but do not occur usually in senescence (Brady, 1987). Fruit ripening is a crucial physiological process for plants, since it represents the terminal stage of development in which the matured seeds are released. Therefore, it is the mechanism responsible for the reproduction of flowering plants. The modifications on the biochemistry, physiology and structure of the ripening organ that are developmentally altered to influence appearance, texture, flavour and aroma, have the evolutionary objective of render the fruit attractive and palatable, to attract a variety of seed-dispersing organisms. To meet this reproductive role, ripening fruits undergo many physiological and biochemical modifications that include conversion of starch to sugars, alterations in the pigment biosynthesis and accumulation, biosynthesis of flavour and aromatic volatiles, and changes in the cell wall ultra-structure and metabolism which are thought to result in loss of firmness of the pulp.

From the horticultural and commercial viewpoint, ripening confers both positive and negative attributes to the fruit. Ripening impacts various quality and nutritional characteristics, including fibre content and composition, lipid metabolism and level of several vitamins and antioxidants (Kalt, 2001). Ripening also imparts desirable flavour and colour, but the changes in fruit firmness increase its susceptibility to the attack from pathogens and, in the latter stages of ripening or after long storage periods, it confers undesirable texture to the consumer's perception. These aspects are the major contributors to fruit loss in the postharvest period

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and have a significant commercial and economical importance (Giovannoni, 2001). In fact, excessive softening of fruits is the main factor responsible for limitations of shelf-life, transportability and storage, for increased occurrence of physical damages during handling, and for higher susceptibility to pests and diseases. Fruit firmness and texture also affect the integrity of processed fruits. From the consumer's point of view, texture is the principal quality attribute for the acceptance in the market. In the majority of fleshy fruits, textural quality is generally more important than aromatic properties (Johnson & Ridout, 2000) and while consumers in different countries may have differing preference for the various quality attributes, there is a universal need to produce firm fruits, that are free from physiological disorders and pathogen decay. Many markets are increasingly using fruit firmness as a guide to ensure that fruits delivered to customers have the required textural characteristics year round (Johnston, Hewett, & Hertog, 2002).

The metabolic events responsible for the textural changes in fruits are believed to involve loss in turgor pressure, degradation and other physiological changes in the composition of membranes, modifications in the symplast/apoplast relations, degradation of starch, and modifications in the cell wall structure and dynamics. Although the relative contribution of each event in fruit ripening is not clear, and probably depends on the species, changes in cell wall composition, especially cell wall mechanical strength and cell-to-cell adhesion, have been considered to be the most important factors (Fischer & Bennett, 1991; Hadfield & Bennett, 1998).

In the most consensual model, the plant cell wall is composed by xyloglucan molecules in which short lengths are hydrogen bonded to restricted areas of cellulose, forming a tether that reinforces the primary cell wall. This xyloglucan-cellulose framework is embedded in an amorphous pectin matrix composed of polyuronides together with a domain of other less abundant components, including phenolic compounds, structural proteins, enzymes and receptor-interacting molecules (Bootten, Harris, Melton, & Newman, 2004; Cosgrove, 2001). The complexity in structure of these individual components of the cell wall and the different ways by which they are linked together have been extensively reviewed in the last years (Brummell, 2006; Carpita & Gibeaut, 1993; O'Neill, Ishii, Albersheim, & Darvill, 2004; Vicente, Greve, & Labavitch, 2006; Vincken *et al.*, 2003; Willats, McCartney, Mackie, & Knox, 2001; Zykwin-ska, Ralet, Garnier, & Thibault, 2005). This knowledge is fundamental to understand the significance of the enzyme-driven action in the polysaccharide backbones or side groups during fruit softening. Biochemical studies indicate that the structural changes and rearrangements of the cell wall structure during ripening occur mutually in pectins, hemicelluloses and cellulose (Huber, 1983; Seymour, Colquhoun, Dupont, Parsley, & Selvendran, 1990) as the result, at least in part, from the activity of members of cell

wall-modifying enzymes and proteins from the same families that promote tissue growth and extension (Fischer & Bennett, 1991). The disassembly of the cell wall structural network probably involves the concerted and synergistic action of several different enzymatic activities, where one family of cell wall-modifying enzymes may mediate the activity of another, resulting in ordered cell wall modifications (Rose & Bennett, 1999).

The developmental of rational and effective approaches to improve texture and shelf-life depends on understanding the biological basis of fruit ripening. Despite the role of fleshy fruits both as an unique aspect of plant development and an important portion of the human diet, the molecular basis of fruit ripening have been mostly studied using tomato (*S. lycopersicum* [*L. esculentum* Mill.]) as a plant model for climacteric fruits and strawberry (*Fragaria ananassa* Duch.) for non-climacteric fruits. Information about the ripening-related cell wall modifications of other species is fragmented and largely incomplete.

Botanical, compositional and developmental differences between fruits

Different fruits differ markedly in their botanical origin, polysaccharide and protein composition, cell wall structure, enzymatic metabolism, growing and ripening pattern, or softening behaviour. These differences that reflect pulp firmness, rate of softening and overall texture are now recognized not only between different species but also between different cultivars, varieties and selections from the same species.

Different fruits have distinct botanical origins. The botanical definition of fruit is a seed receptacle developed from an ovary. This definition encompasses a very wide range of fruit types like composite, simple or multiple fruits, dry fruits or fleshy fruits. Fleshy fruits can also be classified as dupe types, berry types, pome types. Even within the same fruit type important differences can be observed. For instances, apples (*Malus × domestica* Borkh.) contain about 25% in volume of air space between the cells, in contrast with 5% in European pears (*Pyrus communis* L.) (Knee, 1993) and both apples and pears are classified as pome fruits. Apple has a relatively high content of hemicellulose and cellulose (compared with berries and with cherry (*Prunus cerasus* L.) for hemicellulose) or papaya (*Carica papaya* L.) (for cellulose) and low content in lignin (compared with cherry or pear). On the other hand, apples display a very low content in proteins, in contrast with other fruits, such as berries, where the protein content is higher. Accordingly to their softening behaviour, fruits can be divided into two categories: those that soften greatly as they ripen (e.g. tomato, peach (*Prunus persica* L.), strawberry) and those that soften moderately as they ripen (like apple, nashi pear (*Pyrus pyrifolia* Nakai) or cranberry (*Vaccinium macrocarpon* Aiton.)) (Bourne, 1979). While fruits from the first category have a poor relationship between firmness at harvest and after storage, in fruits from the

second category, firmness after storage tends to be correlated with firmness at harvest.

Differences also occur concerning swelling of the cells. Swelling results from water penetrating into larger microfibrillar spaces created by the separation of cells in synchrony with pectic solubilisation, which occurs independently of depolymerisation (Redgwell *et al.*, 1997). Swelling only occurs in species that soften to a melting texture, like tomato (Crookes & Grierson, 1983), strawberry (Redgwell *et al.*, 1997), European pear (Ben-Arie, Kislev, & Frankel, 1979) or kiwifruit (*Actinidia deliciosa*) (Hallett, MacRae, & Wegrzyn, 1992), and does not occur in fruits like apple or nashi pears, that soften moderately and are characterised by a crisp fracturable texture.

In pome fruits, the phases of fruit growth and fruit softening are well separated. However, in fruits like avocado (*Persea americana* Mill.), cell division in the pericarp continues until shortly before ripening and in strawberry, softening starts before the fruits stop growing, so considerable overlapping of growth and ripening process occur.

Considering this wide range of fruit types, it is not surprising that they also differ in their respective metabolisms. These differences are expected to influence and to reflect distinct biochemical modifications of the cell wall and distinct basis of their regulation. Thus, ripening and softening of distinct fruits may proceed via different mechanisms so the question must be addressed for individual fruits and, in some cases, the results obtained from one cultivar may not be properly extended to all cultivars that belong to the same species.

Cell wall modifications in structure and composition during fruit ripening

Changes in the structure of the cell wall are associated with dissolution of the middle lamella and disruption of the primary cell wall (Crookes & Grierson, 1983). Structural changes in pectin, hemicellulose and cellulose together are assumed to be responsible for the alteration of cell wall structure during ripening-related loss of firmness (Huber, 1983; Seymour *et al.*, 1990). These changes include not only solubilisation and depolymerisation of the polysaccharides but also rearrangements of their associations (Redgwell, Fischer, Kendal, & MacRae, 1997; Rose, Hadfield, Labavitch, & Bennett, 1998).

The most remarkable aspect of the biochemical changes that occur in the cell wall components of fruits during ripening is that, depending on the fruit species, different modifications of the polysaccharides may occur and may occur at distinct extents. Modifications of the pectin polysaccharides may result from two processes: solubilisation and depolymerisation. Although solubilisation is considered to be an universal feature of pectin modifications, depolymerisation seems to occur additionally in some species. Similarly, some fruits soften without detectable depolymerisation of xyloglucan and other matrix glycans, while in others, ripening-related depolymerisation of these compounds appears

to occur. Table 1 summarizes the current knowledge on the major modifications in the cell wall polysaccharides during ripening, according to a given fleshy fruit species. Some fruits, like apples, seem to soften without extensive depolymerisation on the polysaccharides of the cell wall. This contrasts with the mechanism reported in the climacteric model plant, tomato, where most significant modifications of the cell wall properties were reported and include both depolymerisation and solubilisation of polyuronides (Brummell & Labavitch, 1997; Campbell, Huysamer, Stotz, Greve, & Labavitch, 1990; Huber & O'Donoghue, 1993), and depolymerisation of hemicelluloses, including xyloglucan (Brummell, Harpster, Civello, *et al.*, 1999; Harpster, Dawson, Nevins, Dunsmuir, & Brummell, 2002; Huber, 1983; Maclachlan & Brady, 1994; Sakurai & Nevins, 1993; Tong & Gross, 1988). In fact, although the amounts of cellulose and xyloglucan remain constant during tomato ripening (Maclachlan & Brady, 1994), a significant decrease in the relative molecular weight of the tightly bound xyloglucan fraction was noticed, coincident with the onset of softening (Maclachlan & Brady, 1994). According to the same authors, the molecular weight of cellulose does not decrease or decreases slightly.

Together with solubilisation of pectins, loss of neutral sugars from side-chains of pectins seems to be a common mechanism that accompanies ripening in all fruit species (Gross & Sams, 1984). Likewise, although loss of galactose has been considered as the most important event, according to the species, some neutral sugars are preferentially lost. Loss of galactose is very pronounced in species like tomato, peach, muskmelon (*Cucumis melo* L.) and apple, but does not seem to have the same importance in plums (*Prunus* spp.), apricots, European pears, blueberries (*Vaccinium angustifolium* Ait.) or raspberries (*Rubus idaeus* L.) (Brummell, 2006; Gross & Sams, 1984). For instances, arabinose and xylose are the main neutral sugar lost during ripening of European pears and apricots, respectively (Gross & Sams, 1984). It is notable that cell walls from berries, which are relatively soft fruits, have a low content of galactose (Gross & Sams, 1984).

Based on known events considered to be common to all fleshy species, structural universal changes associated with softening are likely to be those concerned with loosening of the cell walls and loss of cell cohesion, which can be or not accompanied by actual cell wall degradation. Pectin solubilisation may result from the loss of a cohesive pectin matrix, un-cross-linking of pectin molecules with each other, as the result of loss of galactosyl residues or other neutral sugars in the form of neutral galactose-rich side-chains of rhamnogalacturonans (Redgwell, Melton, & Brasch, 1992; Seymour *et al.*, 1990). These changes usually result on an apparent dissolution of the pectin-rich middle lamella region and, as ripening progresses, the cell wall becomes increasingly hydrated as the pectin-rich middle lamella is modified. The changes in cohesion of the pectin gel govern the ease with which cell can be separated from another,

Table 1. Summary of the fruit species which suffer (✓) and which do not suffer (X) significant polysaccharide depolymerisation during ripening

Fruit	Depolymerisation of		References	
	Pectin	Hemicellulose	Pectin	Hemicellulose
Apple	X	X	Yoshioka <i>et al.</i> (1992)	Percy <i>et al.</i> (1997); Siddiqui <i>et al.</i> (1996)
Avocado	✓ ^a	✓	Huber and O'Donoghue (1993); Sakurai and Nevins (1997); Wakabayashi, Chun, & Huber (2000)	O'Donoghue and Huber (1992); Sakurai and Nevins (1997)
Fig	✓	✓	Owino, Nakano, Kubo, and Inaba (2004)	Owino <i>et al.</i> (2004)
Grape	✓	✓ ^a	Yakushiji <i>et al.</i> (2001)	Yakushiji <i>et al.</i> (2001)
Kiwifruit	✓	✓	Redgwell <i>et al.</i> (1992)	MacRae and Redgwell (1992); Redgwell, Melton, and Brasch (1991)
Mango	✓		Muda, Seymour, Errington, and Tucker (1995); Prasanna <i>et al.</i> (2004)	
Melon	✓	✓	McCollum <i>et al.</i> (1989); Ranwala <i>et al.</i> (1992); Rose <i>et al.</i> (1998)	McCollum <i>et al.</i> (1989); Rose <i>et al.</i> (1998)
Papaya	✓	✓ ^c /X ^d	Ali <i>et al.</i> (1998); Lazan, Selamat, and Ali (1995); Manrique and Lajolo (2004); Paull <i>et al.</i> (1999)	Manrique and Lajolo (2004); Paull <i>et al.</i> (1999)
Peach	✓ ^b	✓	Brummell <i>et al.</i> (2004); Dawson <i>et al.</i> (1992); Hegde and Maness (1998); Muramatsu, Tanaka, Asakura, and Haji (2004)	Brummell <i>et al.</i> (2004); Hegde and Maness (1998)
Pear	✓	✓	Yoshioka <i>et al.</i> (1992)	Yoshioka (1993)
Persimmon		✓		Cutillas-Iturralde <i>et al.</i> (1994)
Strawberry	X	✓	Huber (1984)	Huber (1984)
Tomato	✓ ^a	✓	Brummell and Labavitch (1997); Huber and O'Donoghue (1993)	Brummell, Harpster, Civello, Dawson, <i>et al.</i> (2002); Huber (1983); MacLachlan and Brady (1994); Sakurai and Nevins (1993); Tong and Gross (1988)

^a Unripe to mid-ripe.
^b Mid-ripe to full ripe.
^c Paull *et al.* (1999).
^d Manrique and Lajolo (2004).

which in turn affects the final texture of the ripe fruit. In species in which depolymerisation of pectins and hemicelluloses occur, additional events are likely to be involved, so probably this mechanism presents differences and specificities, according to individual fruits.

Parallels and differences in the temporal pattern of ripening-related cell wall modifications in distinct fruits

Knowledge about the temporal pattern in which these modifications take place is important to the overall understanding of the whole process. Using “Charentais” melon (*C. melo* L.), a fruit which softens in a very short period of time, a sequential pattern of polysaccharide modifications was proposed for the first time (Rose *et al.*, 1998). The results obtained suggest that early events in melon fruit softening are associated with the regulated disassembly of a tightly bound fraction of xyloglucan and the later softening, which accompanies overripe deterioration, is associated with pectin disassembly (Rose *et al.*, 1998). However, this early loss of xylose-rich polymers was not observed in melting flesh peaches (Brummell, Dal Cin, Crisosto, & Labavitch, 2004). On the other hand, during pectin disassembly, loss of galactose, possibly from pectin-associated galactan side-chains, is proposed to contribute to early polygalacturonase (PG)-independent solubilisation of covalently linked pectins prior to or coincident with the onset

of significant fruit softening. Solubilised pectins are subsequently subject to depolymerisation in the later stages of ripening through the action of *endo*- and/or *exo*-acting polygalacturonases (Dawson, Melton, & Watkins, 1992). Studies in kiwifruit and peach confirm the assumption that pectin solubilisation occurs prior to depolymerisation (Brummell *et al.*, 2004; Redgwell *et al.*, 1992). Depolymerisation of polyuronides is initiated at mid-softening in fruits like avocado or tomato, or during late softening in other fruits, including melon and peach (reviewed in Brummell, 2006).

Since much of the wall-bound pectins are highly branched in unripe fruits, this would presumably limit attack by *endo*-PGs, unless the removal of side-chains makes the molecule more labile to the enzyme. Supporting this assumption, it is known that arabinan in pear protects pectins from PG degradation (Ahmed & Labavitch, 1980a). However, the hypothesis that loss of neutral sugars from branched areas of pectic polysaccharides is the first and main event responsible and determinant to fruit softening has been challenged. Based on observations from other species, a correlation between cell wall swelling and pectin solubilisation, but not with galactose loss, has been established in kiwifruit (Redgwell *et al.*, 1997; Redgwell & Percy, 1992), which argues with the assumption that removal of galactose is required for pectin solubilisation. Further evidences came from the observation that galactose

Table 2. Summary of experimental results cited in the literature, which correlate mRNA accumulation and enzymatic activity of members of cell wall-modifying enzymes with ripening in selected fruits

Fruit	mRNA accumulation	Enzymatic activity
Apple	<p><i>PG</i>: Atkinson <i>et al.</i> (1998), Wakasa <i>et al.</i> (2006)</p> <p><i>β-Gal</i>: Ross <i>et al.</i> (1994)</p>	<p><i>PG</i>: Abeles and Biles (1991), Bartley (1978), Knee (1982), Goulao <i>et al.</i> (2007), O'Beirne and Van-Buren (1983), Siddiqui <i>et al.</i> (1996), Yoshioka <i>et al.</i> (1992), Wu <i>et al.</i> (1993)</p> <p><i>PME</i>: Goulao <i>et al.</i> (2007), Klein, Hanzon, Irwin, Shalom, and Lurie (1995), Knee, Sargent, and Osborne (1977), Yoshioka <i>et al.</i> (1992)</p> <p><i>β-Gal</i>: Bartley (1974), Dick <i>et al.</i> (1990), Goulao <i>et al.</i> (2007), Knee (1973), Ross <i>et al.</i> (1994), Yoshioka <i>et al.</i> (1995)</p> <p><i>AFase</i>: Dick <i>et al.</i> (1990), Goulao <i>et al.</i> (2007), Yoshioka <i>et al.</i> (1995)</p> <p><i>EGase</i>: Abeles and Biles (1991), Goulao <i>et al.</i> (2007)</p> <p><i>XET</i>: Goulao <i>et al.</i> (2007), Percy <i>et al.</i> (1997), Vincken <i>et al.</i> (1998)</p>
Avocado	<p><i>Expa</i>: Wakasa <i>et al.</i> (2003)</p> <p><i>PG</i>: Kutsanai <i>et al.</i> (1993)</p> <p><i>β-Gal</i>: Tateishi, Inoue, and Yamaki (2002)</p> <p><i>EGase</i>: Cass <i>et al.</i> (1990), Christoffersen <i>et al.</i> (1984), Tonutti, Cass, and Christoffersen (1995), Tucker <i>et al.</i> (1987)</p>	<p><i>PME</i>: Awad and Young (1980)</p> <p><i>β-Gal</i>: De Veau <i>et al.</i> (1993), Tateishi <i>et al.</i> (2002)</p> <p><i>EGase</i>: Awad and Young (1979), Christoffersen <i>et al.</i> (1984), Kanellis and Kalaitzis (1992), O'Donoghue and Huber (1992), Pesis <i>et al.</i> (1978)</p> <p><i>β-Xyn</i>: Ronen <i>et al.</i> (1991)</p>
Banana	<p><i>PG</i>: Asif and Nath (2005)</p> <p><i>PL</i>: Dominguez-Puigjaner <i>et al.</i> (1997), Marín-Rodríguez, Orchard, and Seymour (2002), Medina-Suarez <i>et al.</i> (1997), Pua, Ong, Liu, and Liu (2001)</p> <p><i>EGase</i>: Medina-Suarez <i>et al.</i> (1997)</p>	<p><i>PG</i>: Ali <i>et al.</i> (2004), Pathak and Sanwal (1998)</p> <p><i>PME</i>: Ali <i>et al.</i> (2004), Brady (1976)</p> <p><i>PL</i>: Marín-Rodríguez <i>et al.</i> (2002), Payasi, Misra, and Sanwal (2004), Payasi and Sanwal (2003)</p>
Blackberry	<p><i>EGase</i>: Abeles and Takeda (1989)</p>	<p><i>PG</i>: Abeles and Takeda (1989)</p>
Grape	<p><i>PME</i>: Davies and Robinson (2000), Nunan <i>et al.</i> (2001)</p> <p><i>PL</i>: Nunan <i>et al.</i> (2001)</p> <p><i>β-Gal</i>: Barnavon <i>et al.</i> (2000), Nunan <i>et al.</i> (2001)</p> <p><i>EGase</i>: Nunan <i>et al.</i> (2001)</p> <p><i>XTH</i>: Nunan <i>et al.</i> (2001)</p>	<p><i>PG</i>: Nunan <i>et al.</i> (2001)</p> <p><i>PME</i>: Nunan <i>et al.</i> (2001)</p> <p><i>β-Gal</i>: Barnavon <i>et al.</i> (2000), Nunan <i>et al.</i> (2001)</p> <p><i>EGase</i>: Nunan <i>et al.</i> (2001)</p> <p><i>XET</i>: Nunan <i>et al.</i> (2001)</p>
Kiwifruit	<p><i>PG</i>: Wang <i>et al.</i> (2000)</p> <p><i>XTH</i>: Schroder, Atkinson, Langenkamper, and Redgwell (1998)</p>	<p><i>β-Gal</i>: Bonghi <i>et al.</i> (1996), Wegrzyn and MacRae (1992)</p> <p><i>XET</i>: Percy <i>et al.</i> (1996), Redgwell and Fry (1993), Redgwell <i>et al.</i> (1992), Vincken <i>et al.</i> (1998)</p>
Melon	<p><i>PG</i>: Hadfield <i>et al.</i> (1998), Rose <i>et al.</i> (1998)</p>	<p><i>β-Gal</i>: Fils-Lycaon and Buret (1991)</p>
Muskmelon	<p><i>EGase</i>: Lester and Dunlap (1985)</p>	<p><i>PG</i>: McCollum <i>et al.</i> (1989)</p> <p><i>β-Gal</i>: Ranwala, Suematsu, and Masuda (1992)</p>
Papaya	<p><i>EGase</i>: Paull and Chen (1983)</p> <p><i>PG</i>: Lester <i>et al.</i> (1994), Morgutti <i>et al.</i> (2006), Zhou <i>et al.</i> (2000)</p> <p><i>PME</i>: Zhou <i>et al.</i> (2000)</p> <p><i>EGase</i>: Bonghi <i>et al.</i> (1998), Trainotti, Pavanello, and Zanin (2006), Trainotti, Spolaore, Ferrarese, Casadoro (1997), Zhou <i>et al.</i> (2000)</p> <p><i>XTH</i>: Hayama, Shimada, Ito, Yoshioka, and Kashimura (2001)</p>	<p><i>PG</i>: Ali <i>et al.</i> (2004)</p> <p><i>PME</i>: Ali <i>et al.</i> (2004), Lazan <i>et al.</i> (1995), Paull <i>et al.</i> (1999)</p> <p><i>β-Gal</i>: Ali <i>et al.</i> (1998), Lazan and Ali (1993), Lazan, Ali, Liang, and Yee (1989), Lazan, Ng, Goh, and Ali (2004)</p> <p><i>PG</i>: Brummell <i>et al.</i> (2004), Jin, Kan, Wang, Lu, and Yu (2006), Manganaris <i>et al.</i> (2006), Morgutti <i>et al.</i> (2006), Pressey and Avants (1973, 1978)</p> <p><i>PME</i>: Brummell <i>et al.</i> (2004), Manganaris <i>et al.</i> (2006)</p> <p><i>β-Gal</i>: Brummell <i>et al.</i> (2004), Jin <i>et al.</i> (2006)</p> <p><i>AFase</i>: Brummell <i>et al.</i> (2004), Jin <i>et al.</i> (2006)</p> <p><i>EGase</i>: Bonghi <i>et al.</i> (1998), Brummell <i>et al.</i> (2004), Hinton and Pressey (1974), Trainotti <i>et al.</i> (2006)</p>

Table 2 (continued)		
Fruit	mRNA accumulation	Enzymatic activity
Pear	<p><i>Expa</i>: Hayama <i>et al.</i> (2000, 2001, 2003), Obenland <i>et al.</i> (2003)</p> <p><i>PG</i>: Fonseca <i>et al.</i> (2004), Yoshioka <i>et al.</i> (1992)</p> <p>β-Gal: Mwaniki <i>et al.</i> (2005), Tateishi <i>et al.</i> (2001, 2005b)^a</p> <p><i>AFase</i>: Tateishi <i>et al.</i> (2005a)^a</p> <p>β-Xyl: Itai <i>et al.</i> (1999)^a</p> <p><i>EGase</i>: Hiwasa, Kinugasa, <i>et al.</i> (2003)</p> <p><i>XTH</i>: Hiwasa, Kinugasa, <i>et al.</i> (2003), Hiwasa <i>et al.</i> (2003a)^a</p> <p><i>Expa</i>: Hiwasa, Rose, <i>et al.</i> (2003)</p>	<p><i>PG</i>: Ali <i>et al.</i> (2004), Pressey and Avants (1976)</p> <p><i>PME</i>: Ahmed and Labavitch (1980b)</p> <p>β-Gal: Ahmed and Labavitch (1980b); Fonseca <i>et al.</i> (2004), Kitagawa, Kanayama, and Yamaki (1995)^a; Mwaniki <i>et al.</i> (2005), Tateishi and Inoue (2000), Tateishi <i>et al.</i> (1996)^a</p> <p><i>AFase</i>: Ahmed and Labavitch (1980b); Tateishi and Inoue (2000)^a; Tateishi <i>et al.</i> (1996, 2005a)^a</p> <p>β-Xyl: Itai <i>et al.</i> (1999)^a</p> <p><i>EGase</i>: Ahmed and Labavitch (1980b); Yamaki and Matsuda (1977)^a</p>
Persimmon		<p><i>Expa</i>: Rose, Cosgrove, Albersheim, Darvill, and Bennett (2000)</p> <p><i>PG</i>: Cutillas-Iturralde <i>et al.</i> (1993), Matsui and Kitagawa (1989)</p> <p><i>XET</i>: Cutillas-Iturralde <i>et al.</i> (1994)</p>
Strawberry	<p><i>PG</i>: Redondo-Nevado <i>et al.</i> (2001), Trainotti, Spinello, Piovano, Spolaore, and Casadoro (2001)</p> <p><i>PME</i>: Castillejo, de la Fuente, Iannetta, Botella, and Valpuesta (2004)</p> <p><i>PL</i>: Benítez-Burraco <i>et al.</i> (2003), Medina-Escobar <i>et al.</i> (1997)</p> <p>β-Gal: Trainotti <i>et al.</i> (2001)</p> <p>β-Xyl: Bustamante <i>et al.</i> (2006), Martínez, Chávez, and Civello (2004)</p> <p><i>EGase</i>: Harpster <i>et al.</i> (1998), Llop-Tous <i>et al.</i> (1999), Manning (1998), Spolaore <i>et al.</i> (2003), Trainotti <i>et al.</i> (1999)</p> <p><i>Expa</i>: Civello, Powell, Sabehat, and Bennett (1999), Dotto <i>et al.</i> (2006), Harrison, McQueen-Mason, and Manning (2001)</p>	<p><i>PG</i>: Abeles and Takeda (1990), Huber (1984), Nogata, Ohta, and Voragen (1993)</p> <p><i>PME</i>: Abeles and Takeda (1990)</p> <p>β-Xyl: Bustamante <i>et al.</i> (2006), Martínez <i>et al.</i> (2004)</p> <p><i>EGase</i>: Abeles and Takeda (1990)</p>
Tomato	<p><i>PG</i>: Dellapenna, Watson, Liu, and Schuchman (1996)</p> <p><i>PME</i>: Eriksson <i>et al.</i> (2004), Frenkel, Peters, Tieman, Tiznado, and Handa (1998), Hall <i>et al.</i> (1994), Harriman <i>et al.</i> (1991), Ray, Knapp, Grierson, Bird, and Schuch (1988)</p> <p>β-Gal: Carey <i>et al.</i> (1995), Smith and Gross (2000), Smith <i>et al.</i> (1998, 2002)</p> <p><i>AFase</i>: Itai <i>et al.</i> (2003)</p> <p><i>EGase</i>: Brummell <i>et al.</i> (1997), del Campillo and Bennett (1996), Catalá, Rose, and Bennett (2000), Gonzalez-Bosch <i>et al.</i> (1996), Lashbrook <i>et al.</i> (1994), Milligan and Gasser (1995)</p> <p>β-Xyn: Itai <i>et al.</i> (2003)</p> <p><i>XTH</i>: Arrowsmith and de Silva (1995)</p> <p><i>Expa</i>: Brummell, Harpster, and Dunsmuir (1999), Catalá <i>et al.</i> (2000), Rose <i>et al.</i> (1997, 2000)</p>	<p><i>PG</i>: Eriksson <i>et al.</i> (2004), Pressey (1987), Tucker and Grierson (1982)</p> <p><i>PME</i>: Eriksson <i>et al.</i> (2004), Gaffe <i>et al.</i> (1994), Harriman <i>et al.</i> (1991), Koch and Nevins (1989), Pressey and Avants (1982), Tieman and Handa (1994), Tucker <i>et al.</i> (1982)</p> <p>β-Gal: Ali <i>et al.</i> (2004); Campbell <i>et al.</i> (1990), Carey <i>et al.</i> (1995), Carrington and Pressey (1996), Eriksson <i>et al.</i> (2004), Pressey (1983), Sozzi <i>et al.</i> (1998), Smith and Gross (2000), Watkins <i>et al.</i> (1988)</p> <p><i>AFase</i>: Campbell <i>et al.</i> (1990), Itai <i>et al.</i> (2003), Sozzi, Fraschina, <i>et al.</i> (2002)</p> <p><i>EGase</i>: Babbitt <i>et al.</i> (1973), Campbell <i>et al.</i> (1990), Hobson (1968), Huber (1985), Maclachlan and Brady (1994)</p> <p>β-Xyn: Itai <i>et al.</i> (2003)</p> <p><i>XET</i>: Eriksson <i>et al.</i> (2004), Maclachlan and Brady (1994)</p> <p><i>Expa</i>: Rose <i>et al.</i> (2000)</p>

^a Japanese pear.

loss is prevented by inhibiting the action of endogenous galactosidase, but this inhibition does not affect the rate of softening or the degree of pectin solubilisation (Redgwell & Harker, 1995). Moreover, some pectin solubilisation precedes galactan loss early in softening (Redgwell *et al.*, 1992) and neither depolymerisation nor removal of pectic

side-chains is necessary for the initial solubilisation of some pectic polymers in kiwifruit (Redgwell *et al.*, 1992). The authors suggest that degalactosylation can only occur after solubilisation, perhaps due to the fact that solubilisation renders the substrate accessible to β -galactosidases. In tomato, softening occurs before β -Gal activity or galactose

loss is detected (Carrington & Pressey, 1996). However, in avocado, a considerable net loss of galactose and arabinose occurs prior to significant softening (Cutillas-Iturralde, Zarra, & Lorences, 1993) and β -Gal was shown to significantly increase the solubility and to decrease the molecular size of pectins, through removal of galactose residues (De Veau, Gross, Huber, & Watada, 1993).

The expression and activity of cell wall-modifying enzymes

The plant cell wall contains many enzymes able to modify matrix polysaccharides, including several types of endoglycanases ordered to cleave the backbone of matrix hemicelluloses or pectins; glycosidases that may remove side-chains, thus allowing greater interactions between polysaccharide backbones; transglycosylases that may cut hemicelluloses and ligate them together, or esterases and acetylases that can remove methyl or acetyl groups from pectins and cleave ester linkages between polysaccharide chains. Such enzymatic changes reflect the degradation of a mixture of polysaccharides due to the coordinate action of multiple enzymes and alter the physical properties of the wall by changing the viscosity of the matrix, or by altering the pattern of ligation of the hemicelluloses to cellulose. The way these enzymes act to promote the coordinated disassembly of each particular cell wall component or network is the main question addressed.

For several decades, the research was focused only on the role of PG as the main responsible for ripening-associated pectin modifications, contributing to fruit softening. The role of PG had received massive attention in the past due to its significance in the climacteric model fruit. In fact, tomato fruit ripening and softening are accompanied by massive increases on PG mRNA abundance (ca. 2000-fold), immunological detectable protein and enzyme activity (Brady, MacAlpine, McGlasson, & Ueda, 1982; Campbell *et al.*, 1990; DellaPenna, Alexander, & Bennett, 1986; Grierson & Tucker, 1983). However, applications of antisense technologies in tomato fruits have questioned and deemphasise that hypothesis (see below). Cell wall-modifying enzymes are usually classified in pectolytic and non-pectolytic, according to the specific class of polysaccharides used as substrate. Among pectolytic enzymes are endo- and exo-polygalacturonases (endo- EC 3.2.1.15; exo- EC 3.2.1.67), pectate lyases (PL; EC 4.2.2.2), pectin methylesterases (PME; EC 3.1.1.11), pectin acetylsterases (PAE; EC 3.1.1.-), β -galactosidases (EC 3.2.1.23) or α -L-arabinofuranosidases (EC 3.2.1.55). These enzymes are able to cleave or modify the nature of the polysaccharide backbone or to remove neutral sugars from branched side-chains. Non-pectolytic enzymes are responsible for hemicellulose modifications and include endo-1,4- β -glucanases (EGase; EC 3.2.1.4), endo-1,4- β -xylanases (EC 3.2.1.8), β -xylanases (EC 3.2.1.37), xyloglucan endotransglycosylase/hydrolases (XTH; EC 2.4.1.207) and expansins. No

pectic transglycosylase activities are known to occur in fruits (García-Romera & Fry, 1994).

During ripening, it was observed a coordinated increase in the mRNA abundance, *de novo* synthesis or activity of a number of putatively cell wall-modifying enzymes that may be responsible or contribute significantly to the polymer modifications observed.

The role of these enzymes in the modifications of the fruit cell wall and in fruit softening has been put forward mainly from correlative evidences, using a “guilty by association” approach (Aravind, 2000). A function is proposed if an increase in mRNA, protein or *in vitro* activity accompanies the course of fruit ripening. Table 2 reviews the literature about the correlative data available between mRNA accumulation or enzyme activity and fruit ripening.

In agreement with the evidences that different fruits soften in response to distinct mechanisms, differences in the pattern of gene expression and activity of cell wall-related enzymes have been reported.

Expression and activity of cell wall-modifying enzymes during fruit softening: differences between species

Pectin modifications and action of PGs differ markedly between fruit species. Some species were reported to lack detectable endo-PG activity, including apples (Abeles & Biles, 1991; Bartley, 1978; Goulao, Santos, de Sousa, & Oliveira, 2007; O’Beirne & Van Buren, 1983; Siddiqui, Brackmann, Streif, & Bangerth, 1996; Yoshioka, Aoba, & Kashimura, 1992), strawberries (Abeles & Takeda, 1990), blackberries (*Rubus fruticosus* L.) (Abeles & Takeda, 1989), cherries (Batisse, Fils-Lycanon, & Buret, 1994), grapes (Nunan, Davies, Robinson, & Fincher, 2001) and muskmelons (Lester & Dunlap, 1985; McCollum, Huber, & Cantliffe, 1989). However, release of polyuronides even in the absence of endo-PG activity was reported in fruits such muskmelon (McCollum *et al.*, 1989). It should be noted that at least one work reports endo-PG activity in apple (Wu, Szakacs-Dobozi, Hemmat, & Hrazdina, 1993) and in strawberry (Redondo-Nevaldo, Moyano, Medina-Escobar, Caballero, & Munoz-Blanco, 2001). These differences in detecting endo-PG activity remain to be conclusively elucidated, but may reside in different methodologies or different cultivars used in the distinct experiments.

In persimmon, although solubilisation and depolymerisation of pectins occur, neither endo- nor exo-PG activities were detected during ripening (Cutillas-Iturralde *et al.*, 1993). No endo-PG activity (Nunan *et al.*, 2001) or changes in the amount of pectins (Nunan, Sims, Bacic, Robinson, & Fincher, 1998) was detected in late softening grapes, although there seems to be depolymerisation at the ‘veraison’ stage (Yakushiji, Sakurai, & Morinaga, 2001). In contrast, pectin solubilisation and depolymerisation together occur in ripening tomato (Ali, Chin, & Lazan, 2004; DellaPenna *et al.*, 1990; Huber, 1983; Smith *et al.*, 1990), European pear (Ali *et al.*, 2004; Yoshioka *et al.*, 1992), or avocado (Huber & O’Donoghue, 1993; Kutsanai, Lin, Percival,

Laties, & Christoffersen, 1993), concomitantly with an increase of endo-PG activity and fruit softening. At least in tomato, European pear (Pressey & Avants, 1976) and peach (Pressey & Avants, 1973), exo-PG was also detected with increased activity during ripening and concomitantly with endo-PG activity. On the other hand, Ali *et al.* (2004) report only exo-PG activity in a variety of tropical fruits, namely banana (*Musa acuminata* Colla), papaya (*C. papaya* L.), mango (*Mangifera indica* L.) and carambola (*Averrhoa carimbola* L.).

The importance given to PME action in fruit ripening derives from its high expression and activity in ripening tomatoes. However, contradictory reports state that PME activity increases during development through the mature green stage and then decreases (Gaffe, Tieman, & Handa, 1994), increases during ripening (Tucker, Robertson, & Grierson, 1982) or increases through the turning stage and decrease thereafter (Harriman, Tieman, & Handa, 1991; Pressey & Avants, 1982). This may reflect different genotypes used or differences in methodology. Also in other fruit species, PME activity is reported to increase (e.g. banana (Ali *et al.*, 2004)) or to decrease during ripening (e.g. avocado (Awad & Young, 1980), mango and papaya (Ali *et al.*, 2004; Paull, Gross, & Qui, 1999)). Absence or low levels of PME activity were detected in ripening strawberries (Abeles & Takeda, 1990), European pear (Ahmed & Labavitch, 1980b) and grapes (Nunan *et al.*, 2001).

In tomato cv. 'Ailsa Craig', a high AFase activity is detected during early fruit development but no increase in total activity was discernible during ripening (Itai, Ishihara, & Bewley, 2003). However, closer examination reveals that, while the activity of two isoforms decreases during ripening, the activity of a third isoform increases (Sozzi, Fraschina, *et al.*, 2002). On the other hand, in Japanese pear (Tateishi & Inoue, 2000; Tateishi, Kanayama, & Yamaki, 1996), and apple (Goulao *et al.*, 2007), fruits that maintain a crispy texture throughout ripening, AFase activity increases extensively with ripening, suggesting a role of this enzyme in fruit softening for some species.

EGases are thought to contribute substantially to fruit softening in some species (Hatfield & Nevins, 1986; Rose & Bennett, 1999; Hiwasa, Kinugasa, *et al.*, 2003), while in others their role is deemphasised. EGase activity and gene expression increases with ripening of fruits like tomato (Babbitt, Powers, & Patterson, 1973; Brummell, Catalá, Lashbrook, & Bennett, 1997; Campbell *et al.*, 1990; del Campillo & Bennett, 1996; Catalá, Rose, & Bennett, 1997; Hobson, 1968; Huber, 1985; Lashbrook, Gonzalez-Bosch, & Bennett, 1994; Maclachlan & Brady, 1994; Milligan & Gasser, 1995) pear (Hiwasa, Kinugasa, *et al.*, 2003; Yamaki & Matsuda, 1977), blackberry (Abeles & Takeda, 1989), strawberry (Abeles & Takeda, 1990; Harpster, Brummell, & Dunsmuir, 1998; Llop-Tous, Dominguez-Puigjaner, Palomer, & Vendrell, 1999; Manning, 1998; Spolaore, Trainotti, Pavanello, & Casadoro, 2003; Trainotti, Spolaore, Pavanello, Baldan, & Casadoro, 1999), peach (Bonghi,

Ferrarese, Ruperti, Tonutti, & Ramina, 1998; Hinton & Pressey, 1974), papaya (Paull & Chen, 1983) and avocado (Awad & Young, 1979; Cass, Kirven, & Christoffersen, 1990; Christoffersen, Tucker, & Laties, 1984; Kanellis & Kalaitzis, 1992; O'Donoghue & Huber, 1992; Pesis, Fuchs, & Zauberman, 1978; Tucker, Durbin, Clegg, & Lewis, 1987).

However, in nectarines, EGase transcription is low at harvest and remains at low levels of transcription after harvest (Zhou *et al.*, 2000). EGase activity in apple is present in young expanding fruits and decreases as fruits reach full size and ripen (Abeles & Biles, 1991; Goulao *et al.*, 2007) and no activity was detected in 'Shiraz' grapes (Nunan *et al.*, 2001) or muskmelons (Lester & Dunlap, 1985).

The variation of β -xylosidase activity and the expression of putative genes encoding β -xylosidases also does not follow the same pattern in all fruits. It increases with ripening in Japanese pear (Itai, Yoshida, Tanabe, & Tamura, 1999), increases and then decreases in avocado (Ronen *et al.*, 1991), is detected but decreases in tomatoes (Itai *et al.*, 2003) and it was undetected in apples during ripening (Dick, Opoku-Gyamfua, & deMarco, 1990).

Detection of XET activity during the development and ripening of persimmon (Cutillas-Iturralde, Zarra, Fry, & Lorences, 1994), tomato (Maclachlan & Brady, 1994) and kiwifruit (Percy *et al.*, 1996; Redgwell & Fry, 1993; Redgwell *et al.*, 1992; Vincken, Zabolina, Beldman, & Voragen, 1998) is not unexpected due to the known modifications that occur in xyloglucans accompanying fruit softening in these species. However, the information regarding XET activity or expression of XTH transcripts and xyloglucan metabolism contrasts with the one reported for EGases. In fact, increased XET activity is detected in apple fruits after the growth had stopped and throughout softening (Goulao *et al.*, 2007), but in this species, the molecular weight profile of xyloglucan presents a high molecular weight peak which does not change during fruit development and ripening (Percy, Melton, & Jameson, 1997). Similarly, XTH gene expression was also detected in grapes during ripening (Nunan *et al.*, 2001) although no significant loss or degradation of xyloglucans occurs in this fruit after 'veraison' (Nunan *et al.*, 1998; Yakushiji *et al.*, 2001).

In summary, it has become evident that the significance of each candidate cell wall-modifying enzyme family is different in distinct fruit species and this significance can be or cannot be related with known biochemical changes of the cell wall.

It should be noticed that the literature does not refer differences between species in respect to pectate lyase, β -galactosidase and expansin action associated with softening during ripening. This fact, together with the results from genetically modified fruits, may suggest that these enzymes may play a critical role in the common softening mechanism. However, due to the large number of gene members in these families, the occurrence of differences

concerning the expression of distinct isoforms, such as the ones occurring between cultivars from the same fruit (see below), is expected. A comprehensive study involving a larger number of genes, enzyme families and fruit species is needed to confirm or discard these assumptions.

Expression and activity of cell wall-modifying enzymes during fruit softening: differences between cultivars and varieties

As previously stated, the relative extent and timing of polysaccharide solubilisation and/or degradation and the gene expression or enzyme activities of ripening-related enzymes also varies among cultivars of a given species, which has been suggested to entail different softening rates. This sub-chapter presents some examples of differences detected between cultivars or varieties from the same fruit.

In non-melting flesh peaches, the final melting phase of softening is absent and pectins undergo little solubilisation or depolymerisation (Fishman, Levaj, Gillespie, & Scorza, 1993; Pressey & Avants, 1978). Ripening-related exo-PG activity is found in both melting and non-melting peach, but endo-PG activity accumulates only in ripening melting varieties, coincident with the melting phase (Callahan, Scorza, Bassett, Nickerson, & Abeles, 2004; Lester, Speirs, Orr, & Brady, 1994; Manganaris, Vasilakakis, Diamantidis, & Mignani, 2006; Orr & Brady, 1993; Pressey & Avants, 1978). Considerable differences in PME activity occur between two raspberry cultivars with different patterns of fruit softening. While in 'Glen Prosen' (firm cultivar), PME activity is maintained at lower levels throughout ripening, in 'Glen Clova' (soft cultivar) an increase in PME activity occurs as the fruit softens (Iannetta *et al.*, 1998).

A xylanase isoform developmentally expressed during fruit ripening in papaya correlates with the variation in softening patterns of different varieties (Chen & Paull, 2003) and in strawberry, β -xylosidase activity, mRNA accumulation of an isolated gene and protein accumulation correlates with softening in two cultivars with contrasting firmness (Bustamante, Rosli, Añón, Civello, & Martínez, 2006). In strawberry, softest varieties display highest PME and PG activities than those that remain firmer during ripening (Lefever *et al.*, 2004) and softer raspberry (*R. idaeus* L.) cultivars have lower PG and higher β -Gal activities (Iannetta, van den Berg, Wheatley, McNicol, & Davies, 1999).

The mRNA for an expansin specific isoform, *PpExp3*, is detectable in 'Akatsuki' peaches (a rapidly soften cultivar) but hardly detectable in the 'Manami' cultivar (a cultivar which remains firm during postharvest storage) (Hayama, Ito, Moriguchi, & Kashimura, 2003). Wakasa *et al.* (2006) analysed the mRNA accumulation patterns of a ripening-related mRNA in 'Golden Delicious' apples in a set of other apple cultivars and the results show that this gene is transcribed during ripening at relative high levels in some cultivars, including 'Golden Delicious' or 'Kitarou', while in others, like 'Fuji' and 'Ralls Janet' it was below the experimental detection level, and in others it increases until

commercial maturity and decreases thereafter, during ripening (e.g. 'Kotaro'). The same association of expression of expansins and differences in firmness was reported in the non-climacteric fruit, strawberry. Protein abundance (Dotto, Martínez, & Civello, 2006) and the expression of three out of five expansin mRNAs were correlated with fruit firmness in three cultivars which differ in fruit firmness during ripening (Dotto *et al.*, 2006; Salentijn, Aharoni, Achaart, Boone, & Frenks, 2003).

In apple, it has been accepted that loss of arabinose during fruit ripening is low (Gross & Sams, 1984). However, a comparison of several cultivars including 'Honeycrisp', a cultivar that maintains its crispness through long storage, was performed and the only consistent difference between crisp apple and its parents, 'Macoun' and 'Honeygold', which soften over the same time period, was showed to be the arabinose content (Tong *et al.*, 1999). Interestingly, AFase activity increases dramatically from commercial maturity to the overripe stages in 'Royal Gala'; a cultivar that soften considerably during postharvest (Goulao *et al.*, 2007).

Finally, another interesting aspect is that, cell wall-modifying enzymes might be differentially expressed in localised regions of the same fruit. In peaches, expansins were reported to be abundant in the parts of the fruit with a high free water content (juicy), but absent in low free water parts (mealy) of the same fruit, and the reduction of expansin abundance precedes the appearance of mealiness (Obenland, Crisosto, & Rose, 2003).

Probing the function of ripening-related cell wall-modifying enzymes using altered genetic backgrounds

Results from genetically modified lines in which fruit ripening-associated cell wall genes have been suppressed or overexpressed and analysis of gene expression in known ripening-impaired mutants, have provided more direct information about the possible function of each gene family and isoform in ripening. This sub-section reviews and discusses the literature concerning the results of the manipulation of individual cell wall-modifying genes in fruits, and its outcome in fruit softening and biochemical characteristics of the cell wall.

Polygalacturonase

Studies using antisense and overexpression technologies revealed that, although necessary for polyuronide degradation, PG is not determinant for softening since it is neither sufficient nor necessary to promote fruit softening. Antisense tomato expressing 1% of wild-type PG activity reduced mRNA abundance and enzymatic activity (Sheehy, Kramer, & Hiatt, 1988; Smith *et al.*, 1988), and inhibited depolymerisation in tissue homogenates, but sustained almost normal pectin solubilisation and did not prevent fruit softening or the fruits become just slightly firmer than controls (Brummell & Labavitch, 1997; Smith *et al.*, 1990). Moreover, a transposon-tagged tomato line was reported with an insertion in PG, inactivating the gene and massively

reducing polygalacturonase expression, but resulting in fruits with normal softening behaviour (Cooley & Yoder, 1998). In mutant *rin* (ripening-inhibitor) overexpressing PG to 60% of normal activity, solubilisation and depolymerisation of polygalacturonan occur *in vivo*, but do not restore softening (DellaPenna *et al.*, 1990; Giovannoni, DellaPenna, Bennett, & Fisher, 1989). These results deemphasise the previously established concept of the role of pectin depolymerisation in fruit softening and the PG as key enzyme but can indicate that PG is the primary determinant of cell wall polyuronide degradation. Tomato fruit transformed with a PG antisense gene exhibited no reduction in chelator-soluble polyuronides, indicating a mechanism for PG-independent solubilisation (Smith *et al.*, 1990). A PG-independent mechanism is also proposed by Huber and O'Donoghue (1993) since polyuronide solubilises before the increase of PG activity is detected. It should be noted that transformed fruit exhibited a substantial, but not total, reduction in PG mRNA and enzyme accumulation.

Nevertheless, inhibition of PG in tomatoes results in the improvement of some important physical properties that have implications in postharvest characteristics, including extended fruit shelf-life, reduction of the susceptibility to postharvest pathogens and increased viscosity of juice and paste (Kramer *et al.*, 1992; Langley *et al.*, 1994), without affecting other quality attributes like colour, pH, or Brix values (Powell, Kalamaki, Kurien, Gurrieri, & Bennett, 2003). Huber and O'Donoghue (1993) suggest that the divergence in quality between the normal and transgenic tomato fruits during late development or during transport and handling is a consequence of developmental or damage induced modulations in the apoplastic environment. Cellular leakage results from impact or compression bruising as occurs normally due to membrane dysfunction during late fruit development. This would alter the apoplast ionic composition, and possibly provide conditions for maximum potential of polyuronide depolymerisation and, thus, rapid deterioration of fruit with normal levels of PG. Similarly, the juice from transgenic tomatoes is more viscous because of less PG after release of endogenous cellular fluids (Huber & O'Donoghue, 1993).

The overall results obtained with genetic transformation approaches suggest that the previously conceived idea that softening derives primarily from endo-PG action deserves further investigation.

Pectin methylesterase

Antisense suppression of PME mRNA abundance and activity by 90% in tomato (Gaffe *et al.*, 1994; Tieman, Harriman, Ramamohan, & Handa, 1992) has little effect on fruit firmness (Tieman *et al.*, 1992). Nevertheless, a marked influence on fruit pectin metabolism was evident in antisense fruits since pectin fragments extracted from cell walls of these fruits revealed decreased pectin degradation, and an increase in methylesterification (Gaffe *et al.*, 1994; Hall *et al.*, 1993; Tieman *et al.*, 1992; Watson, Zheng, &

DellaPenna, 1994). Low PME activity in the antisense fruit pericarp modified both accumulation and partitioning of cations between soluble and bound forms and selectively impaired accumulation of calcium and magnesium over the major cations (Tieman & Handa, 1994). This effect helps explaining the absence of phenotype during tomato ripening. Conceivably, the loss of bound calcium and the ability to form cross-bridges might have annulled any effects of reduced depolymerisation of pectins on ripening-associated softening of transgenic fruits. Under low calcium levels, polygalacturonans have been suggested to form primary units of two chains in antiparallel configuration with about 50% of the carboxyl groups neutralized with calcium. The excess calcium is weakly bound to sheet-like aggregates formed with several primary units which add little strength to the polygalacturonate gels (Morris, Powell, Gidley, & Rees, 1982; Powell, Morris, Gidley, & Rees, 1982; Walkinshaw & Arnott, 1981). On the other hand, some favourable changes also occurred in the juice and paste viscosity of transformed fruits, including higher levels of soluble solids and increased viscosity of the paste. This fact can be explained since methylated pectins are not accessible to PG resulting in increased molecular weight. However, tissue disintegration associated with senescence was affected and antisense PME fruits possess reduced shelf-life (Thakur, Singh, & Handa, 1996; Tieman & Handa, 1994; Tieman *et al.*, 1992; Tieman, Kausch, Serra, & Handa, 1995). It should be considered that these antisense lines only suppressed the activity of type 1 and had no effect on type 2, that are also present in fruit tissues (Gaffe *et al.*, 1994), so they might represent an incomplete picture.

The role of PME in fruit softening has also been questioned since absence of softening has been reported in tomato mutants *nor* (non-ripening) and *Nr* (never-ripe), which display PME activity similar to that of normally ripening genotypes (Harriman *et al.*, 1991). However, the middle lamella of ripe fruit of the tomato mutant *Cnr* (colourless non-ripening) – a non-ripening phenotype with firm fruits that exhibit reduced cell-to-cell adhesion – lack long un-methylesterified homogalacturonan (HGA) blocks (Orfila *et al.*, 2002). Moreover, unesterified HGA-containing pectic regions in *Cnr* cell walls appeared to have a different de-esterified block structure that may account for its ineffectiveness to bind calcium and consequent calcium-mediated chain association and cell adhesion (Orfila *et al.*, 2002). The *Cnr* mutation appears to have effects on the solubility of HGA and *Cnr* cell walls contain less chelator-soluble HGA that is more susceptible to endo-PG degradation (Orfila *et al.*, 2002). Moreover, the ripening-associated PME isoform is not active in *Cnr* fruits (Orfila *et al.*, 2002).

Pectate lyase

No biochemical function has been experimentally shown for any plant PL genes and the function has been assumed based on its sequence conservation in relation to pathogen

PLs (Benítez-Burraco *et al.*, 2003). Its significance in fruit ripening only recently started to receive scientific attention, after a PL gene has been isolated from ripe strawberry (Medina-Escobar, Cardenas, Moyano, Caballero, & Munoz-Blanco, 1997), and banana (Dominguez-Puigjaner, Llop, Vendrell, & Prat, 1997), with expression restricted to ripening fruits.

In contrast with antisense studies in tomato for PG, PME, or β -Gal, strawberry fruits suppressed in PL mRNA expression were significantly firmer than controls and their cell wall material showed a lower degree of *in vitro* swelling and a lower amount of ionically bound pectins, indicating a higher integrity of cell wall structure than control fruits (Jiménez-Bermúdez *et al.*, 2002). At the stage of full ripen, no differences in colour, size, shape and weight were observed between antisense strawberry lines and controls. For this reason, this enzyme family became a strong candidate to be investigated in other species. However, it should be noted that no data concerning enzyme activity or pectin molecular size is given by the authors.

β -Galactosidase

Three isoforms of β -Gal were identified from ripening tomato fruits (Pressey, 1983) and at least seven tomato β -Gal genes are expressed during fruit development, of which, six express during ripening (Smith & Gross, 2000). The role of each individual gene in tomato softening has been accessed by antisense technology experiments. Antisense tomato plants suppressed for TBG1 showed a reduction in mRNA accumulation to 10% of normal levels but total β -Gal and exo-galactanase activities and fruit softening were unaffected (Carey *et al.*, 2001). Probably TBG1 mRNA levels are a minor constituent of the total mRNA of all β -Gal genes expressed and would not be noticed in crude cell wall preparations. Alternatively, other isoforms of β -Gal may be up-regulated to compensate for the reduction of TBG1, or TBG1 can act on a restricted, but specific set of galactan-rich polymers in the wall. TBG1,2,3 and 5 are present in *rin*, *nor* (non-ripening) and *Nr* (never-ripe) mutant fruits in a chronological pattern similar to that of wild-type accumulation (Smith & Gross, 2000). Therefore, it is unlikely that these TBGs could be solely responsible for cell wall modifications that result in fruit softening during ripening (Smith & Gross, 2000). However, reduced levels of TBG3 did result in lower levels of enzyme activity, altered cell wall composition and decreased rate of deterioration of fruits. TBG4 transcript accumulation is significantly impaired in *rin* and *nor* mutants relative to wild-type, suggesting that TBG4 may be involved in softening (Smith & Gross, 2000). TBG6 persisted in mutants whereas it is not detected in wild fruit (Smith & Gross, 2000).

Suppression of transcription of TBG4 by antisense technology (Smith, Abbott, & Gross, 2002) was correlated with a reduction in extractable exo-galactanase activity. However, total β -Gal activity and total cell wall galactosyl

contents in the antisense fruit were not significantly different from control, (TBG4 codes for β -Gal II, an enzyme known to have both β -Gal and exo-galactanase activity (Carey *et al.*, 1995)) but some lines were significantly firmer than untransformed fruits. Even though TBG4 does not contribute significantly for the total detectable β -Gal activity during fruit ripening (Smith *et al.*, 2002), these studies suggest an involvement of this gene product in cell wall modifications leading to softening. The reason why fruits remain firmer, but neither total activity nor biochemical composition was affected may be attributable to alteration in the combination of β -Gal gene family member mRNA levels. Other justification has been suggested concerning the correlation between reduced levels of exo-galactanase activity during early stages of ripening and fruit firmness during later stages of ripening, which may suggest that galactosyl-containing side-chains in the wall result in decreasing wall porosity, thereby obstructing access to wall components by other wall hydrolases (Brummell & Harpster, 2001). Finally, suppression of TBG6, which is highly abundant during early stages of tomato development (Moctezuma, Smith, & Gross, 2003) resulted in increased fruit cracking, reduced locular space and doubling of thickness of cuticle. This isoform is important in early fruit growth and development, in the most rapid period of cell elongation and fruit expansion. Postharvest texture was not affected, so TBG6 product may not play a role in fruit loss of texture.

The combined results suggest that in some cases, suppression of TBG may enhance the total activity in some developmental stages. This can be the result of compensation provided via other members of the gene family (Moctezuma *et al.*, 2003).

Endo-1,4- β -glucanase

In tomato, at least two EGases, *LeCell* and *LeCel2* mRNAs increase in abundance coincidentally with ripening (Gonzalez-Bosch, Brummell, & Bennett, 1996; Lashbrook *et al.*, 1994). However, antisense suppression of each one of these genes did not result in detectable differences on fruit softening (Brummell, Hall, & Bennett, 1999; Lashbrook, Giovannoni, Hall, Fisher, & Bennett, 1998). Antisense suppression of *LeCell* transcripts in transgenic tomato fruit to values lower than 1% of the control did not affect neither fruit firmness (Lashbrook *et al.*, 1998) nor EGase activity nor fruit softening probably because of the presence of *LeCel2* transcript (Brummell, Harpster, Civello, *et al.*, 1999). Suppression of *LeCel2* to levels lower than 5% of controls did not alter fruit firmness but did enhance the break strength of abscission zone (Brummell, Hall, *et al.*, 1999). However, no work reports the simultaneous suppression of the two putative ripening-related isoforms. In pepper, suppression of a ripening-related endo-1,4-beta-glucanase did not prevent depolymerisation of cell wall polysaccharides during ripening (Harpster, Brummell, & Dunsmuir, 2002) and its overexpression in

transgenic tomatoes did not increase xyloglucan depolymerisation nor fruit softening (Harpster, Dawson, *et al.*, 2002). Similarly, a ripening-related EGase, *FaCell*, from strawberry was down-regulated to express less than 0.5% mRNA of wild-type fruits and, as with tomato, no significant effect on fruit firmness was detected (Palomer *et al.*, 2006; Woolley, James, & Manning, 2001). However, a second isoform, *FaCel2* is known to be present but no significant reduction of the accumulation of this protein was found, even in double-transgenic lines (Palomer *et al.*, 2006).

Xyloglucan endotransglycosylase/hydrolase

Two review papers have referred to an experiment in which the expression of a ripening-related tomato XTH gene was down-regulated in antisense fruits (Brummell & Harpster, 2001; Da Silva, Arrowsmith, Hellyer, Whiteman, & Robinson, 1994). According to these reports, no differences were detected in the transformed lines regarding softening or postharvest behaviour. However, no detailed data have been published describing this experiment, so it is not clear if the suppression of XTH lead to any effect on the structure of the polysaccharides or architecture of the cell wall. However, endotransglycosylase activity measured from extracts of ripening tomato fruits was reported to be lower in the non-softening *rin* mutant (Maclachlan & Brady, 1992, 1994).

Expansin

The identification of a fruit-specific and ripening-related expansin (therefore, expressing in the absence of cell expansion) in tomato suggested that these proteins might be involved in fruit softening (Rose, Lee, & Bennett, 1997). At least seven expansin isoforms are expressing with different and characteristic expression patterns during tomato growing and ripening but only *LeExp1* is fruit specific and ripening related (Brummell, Harpster, & Dunsmuir, 1999; Rose *et al.*, 1997). In tomato fruits suppressed in *LeExp1*, fruit softening still occurred, although at lower rate than in controls, particularly in the late ripening stage. Conversely, in *LeExp1* overexpressing lines, fruits soften more rapidly, particularly in the early ripening stages (Brummell, Harpster, Civello, *et al.*, 1999; Brummell, Howie, Ma, & Dunsmuir, 2002). Moreover, postharvest and processing characteristics, such as paste viscosity, were modified in antisense lines (Brummell *et al.*, 2002).

In fruits suppressed in *Exp1* protein accumulation, polyuronide depolymerisation was substantially arrested later in ripening, probably due to reduced pectin breakdown (Brummell, Harpster, Civello, *et al.*, 1999). Overexpression did not produce any significant difference on polyuronide depolymerisation, as compared with controls (Brummell, Harpster, Civello, *et al.*, 1999). The reduction of polyuronide depolymerisation by suppression of *Exp1* was greater than the one caused by suppression of PG activity (Brummell & Labavitch, 1997; Brummell, Harpster, Civello,

et al., 1999), probably by indirect effect because overexpression of *Exp1* did not alter pectin depolymerisation (Brummell, Harpster, Civello, *et al.*, 1999). An inter-relationship between *Exp* and PG has been suggested, perhaps by controlling access of PG to its substrate promoted by expansins. Suppression of *Exp1* protein accumulation did not detectably affect cell wall hemicellulose—molecular mass profiles during ripening, suggesting that the increased firmness of *Exp1*-suppressed fruit is not due to the depolymerisation degree of the hemicellulosic fraction (Brummell, Harpster, Civello, *et al.*, 1999). On the other hand, the overexpression of *Exp1* is correlated with cell wall hemicellulose depolymerisation, and occurs even in mature green fruits before ripening (Brummell, Harpster, Civello, *et al.*, 1999). The same authors suggest that, although depolymerisation of hemicelluloses occurs according to a pathway independent of the expansin activity, the overexpression of expansins in tissues rich in hydrolases, namely EGases and XTHs, may increase the accessibility of the substrates to those enzymes. Suppression of *LeExp1* in tomato fruits increased shelf-life to 5 days in clam shells to 10 days on boxes, and increased viscosity of paste in about 19%, without affecting either the final size of ripe fruits nor fruit set (Brummell *et al.*, 2002). Disease resistance did not change, or was slightly reduced (Brummell *et al.*, 2002). Reduction of both direct effect of *LeExp1* on wall loosening and indirect effect on pectin disassembly may together be responsible for the substantial improved shelf-life of antisense fruits (Brummell *et al.*, 2002).

The overall results from gene manipulation of members of the candidate enzyme families in transgenic fruits suggest that no single cell wall enzyme appears to be sufficient nor necessary to account for the textural changes that occur during fruit ripening. This suggests a cooperative action between various enzymes, the action on very specific bonds within the cell wall polysaccharide networks, which can be necessary for subsequent and generalized degradation by other enzymes, or generation of oligogalacturonides or oligosaccharides that will induce cell signalling pathways (John, Rohrig, Schmidt, Walden, & Schell, 1997; Moscatiello, Mariani, Sanders, & Maathuis, 2006).

However, since the experiments are mainly focused on the changes in fruit firmness and not on the changes in cell wall metabolism, the absence of a fruit phenotype does not clearly indicate that the studied enzyme is not related to softening. The presence of several isoforms from a given family also mean that the subtle balance in expression or compensation should be considered when discussing the results obtained.

Methodological constrains to the study of cell wall-related activities

The role of each enzyme cannot be explained by studying a single specific isoform since the presence of several isoforms, with distinct patterns of expression, may mask

the total activity in a given developmental stage. Hence, assays for monitoring the changes in the activity during the development of the fruit are still informative and needed to complement the studies of genetic expression. Examination of the gene expression and enzyme activity data in the literature often shows a poor correlation between them and between its patterns of expression and their effect in fruit softening. Despite this is often due to posttranslational regulation, there are methodological concerns about measurement of enzymatic activities.

It is important to recognize that although *in vitro* quantitative studies of enzyme activity in a crude protein extract sampled at different stages of development can suggest possible changes in the enzyme action, no *in vitro* assay of enzyme activity can show that the enzyme really acts on the cell wall *in vivo*, and the activity levels may not necessarily be equivalent to the enzyme action *in vivo*. For instances, the extent of PG and β -Gal activities during ripening accessed *in vitro* is not enough to produce the observed *in vivo* losses of galactose (De Veau *et al.*, 1993; Ross, Redgwell, & MacRae, 1993; Seymour, Lasslett, & Tucker, 1987) and in tomato, although PG is known to catalyse massive degradation of polyuronides *in vitro*, the activity of the enzyme *in mero* appears to be limited by some chemical or physical restriction (Huber & O'Donoghue, 1993; Seymour *et al.*, 1987). On the other hand, application of purified tomato PG preparations to mature green fruit tissue duplicates the changes in the cell wall noted during normal ripening (Crookes & Grierson, 1983). Similarly, the *in vitro* activity of a purified apple β -Gal against native substrates (Ross, Wegrzyn, MacRae, & Redgwell, 1994; Yoshioka, Kashimura, & Kaneko, 1995) is insufficient to account for the observed *in vivo* loss of galactose from apple cells during ripening (Ross *et al.*, 1994). These situations can be due to the fact that the enzyme may be spatially separated from its primary substrate (Bourquin *et al.*, 2002), the secondary substrate may not be present in the cell apoplast (Lin & Kao, 2001), or the *in vivo* assay conditions concerning the optimal pH, ionic strength or redox-potential (Almeida & Huber, 1999; Chun & Huber, 1998; Payasi & Sanwal, 2003; Takeda & Fry, 2004), presence of specific promoters, essential cofactors or inhibitors, may not be matching the *in vitro* assay mixtures. Incomplete extraction, enzyme lability, inhibition or enzyme multiplicity also can influence the results of the activity assays. Moreover, during homogenisation of the tissue, cellular compartmentation is disrupted, so it is unclear how much of a given extract activity is located in the apoplast, and in a position to act on cell wall polymers.

Together with transcriptional and posttranscriptional regulation, the alterations of the conditions of the apoplast during fruit ripening modulate the activity of enzymes that are known to modify the cell wall, causing fruit softening. The pH of the apoplast from mature green tomato fruits decreases from around 3.0 pH units during ripening, accompanied by a three-fold increase of the apoplast potassium

levels (Almeida & Huber, 1999; Ruan, Mate, Patrick, & Brady, 1995; Ugalde, Jerie, & Chalmers, 1988). Such effect determines and regulates the extent of the activity in a strict coordinated way, and is possibly involved in determining the temporal pattern of action of the cell wall-modifying enzymes. This aspect has particular importance when measuring enzymatic activities. For instances, maximal activity of purified apple PME was obtained at pH 7.5, which is close to the pH used in the routinely assays. However, at pH 4.5, PME activity represents about 1% of optimum, but was sufficient to destabilize the cloud of apple juices (Denès, Baron, Renard, Péan, & Drilleau, 2000).

The cell wall has a net negative charge due to the contribution of AGPs and pectins and the immobilization of an enzyme on a charged surface dramatically alters its kinetic properties and pH response (Ricard, Noat, Crasnier, & Job, 1981). Enzymes with basic isoelectric point (pI) (XTH, PME, PG) are likely to be immobilized in the cell wall, whereas enzymes with acidic pIs (β -Gal and EGase) can be bound to extensins in the pH range of the apoplast. Therefore, the activity of cell wall enzymes can be different in the apoplast, with restricted mobility, than free in solution.

On the other hand, a steady state level of activity determined using artificial substrates is not necessarily an indicator of all potential *in vivo* activity of the enzyme. For instances, it was showed that β -glycosidase isolated from tomato fruits is more active on the *p*-nitrophenyl substrate than on natural glycosides (Pharr, Sox, & Nesbitt, 1976). The standard assay for EGase activity is based on their competence to reduce the viscosity of solutions containing carboxymethyl-cellulose (CMC). Yet, the activity of EGase against CMC may not reflect its *in vivo* function as hydrolase of hemicelluloses. Some EGase (e.g. from avocado) were reported to hydrolyse only CMC showing very limited activity against native xyloglucan (O'Donoghue & Huber, 1992).

The major β -Gal from apple has an associated β -D-fucosidase and α -L-arabinopyranosidase activities, which act synergistically (Dick *et al.*, 1990). The occurrence of such three related activities in a single enzyme may provide an efficient and highly developed hydrolytic mechanism for complex apple cell wall polysaccharide substrates such as arabinofucogalactoxyloglucans (Aspinall & Fanous, 1984), but makes the determination of individual enzymatic activities more difficult. Similarly, β -xylosidases were reported to be active against *p*-nitrophenyl- α -L-arabinofuranoside in addition to their natural substrates containing D-xylose (Bustamante *et al.*, 2006). For these reasons, activity assays against native substrates can provide more realistic indications of the *in vivo* role of the enzymes.

On the other hand, an effect observed *in vivo* may not be ascribed to any enzyme activity detectable *in vitro*. For example, ethylene treatment of watermelon fruit causes extensive depolymerisation of pectic polysaccharides but has no effect on the level of *in vitro* assay endo-PG activity (Karakurt & Huber, 2002).

In order to overcome this constraint and to achieve a clear correlation between enzymatic activities and their role in fruit softening, assays aimed to investigate the activity *in situ*, as outlined by Fry (2004), must be developed and implemented.

Non-enzymatic control of cell wall modifications during ripening

More recently, it was suggested that ripening could occur as the result of non-enzymatic modifications of the cell wall components, both pectins and xyloglucans. Membrane permeabilisation occurring early in ripening, leads to the release of ascorbate into the apoplast, where it may trigger apoplastic hydroxyl production via the Fenton reaction and this radical is potentially involved in non-enzymatic scission of plant cell wall polysaccharides.

During ripening of tomato, excised pieces of living tomato show increased ability to release endogenous ascorbate. Moreover, progressively increasing levels of copper and ascorbate in the fruit apoplast lead to high hydroxyl production, and consequentially lead to non-enzymatic scission of polysaccharides during ripening, contributing to natural softening of the fruit (Dumville & Fry, 2003). Monitoring the cell walls of ripening pear to hydroxyl attack, Fry, Dumville, and Miller (2001) demonstrate evidences that progressive hydroxyl radical attack on polysaccharides occurs during the softening process. Also in this fruit, solubilisation seems to be the result from the action of ascorbate-generated hydroxyl radicals which can cause non-enzymatic scission of polysaccharides (Dumville & Fry, 2003; Fry *et al.*, 2001). De-esterified citrus pectin was found to be more susceptible to ascorbate-induced scission *in vitro* than methylesterified pectin, suggesting a possible new significance for PME activity in fruit ripening (Dumville & Fry, 2003). Oxidative ions are known to occur during tomato ripening, as revealed by increases of hydrogen peroxide content, lipid peroxidation and protein oxidation (Jimenez *et al.*, 2002). Aqueous-phase antioxidants glutathione and ascorbate increase during ripening (Jimenez *et al.*, 2002) and changes in the activities of superoxide dismutase, catalase and enzymes involved in ascorbate-glutathione cycle during ripening indicates that the antioxidative system plays a fundamental role in ripening of tomato fruits (Jimenez *et al.*, 2002).

Interestingly, down-regulation of PL in strawberry has the effect of decreasing both fruit softening and ascorbate production (Agius *et al.*, 2003). This suggests a mechanism in which both enzymatic and non-enzymatic mechanisms may be linked, and should be further exploited in the future.

Taking advantage of natural variation for improving texture in fruits

This review points out important differences concerning the softening behaviour of individual fruits. This aspect means that each fruit cultivar from a given species should be regarded to have putatively some specificities in relation

to softening-related cell wall metabolism. This implies that a substantial amount of work must be undertaken in order to clarify specific aspects of fruit softening, in order to fully understand the mechanism. However, these natural occurring differences can be exploited to facilitate breeding for fruits with improved textural attributes.

In fact, genetic mapping of gene sequences related to a particular biological phenomenon represents a simple but powerful tool for gaining insight into specific gene function. Prediction of texture characteristics and softening behaviour by detection of marker genes before the tree even started to bear fruit would be useful. The identification of chromosomal regions contributing to major attributes of fruit texture is the first stage in developing selectable markers for the early selection of desirable genotypes. One of the major benefits from determining chromosomal positions of ripening-related genes is the ability to hypothesize corresponding gene function based on linkage with characterised ripening mutants (Giovannoni *et al.*, 1999). Functionally linked genes are co-regulated and occur in the proximity to each other in the chromosome, so the occurrence of an uncharacterised gene under the same operon of genes of known function could potentially provide information about its function. Identification of other candidate genes can also be facilitated through studies of co-linearity (conservation of gene order) with other genomes. This could be particularly important to identify downstream events and signalling and coordination mechanisms involved in the induction and regulation of ripening.

Existing fruit cultivars vary considerably in their intrinsic textural properties and it is apparent that there is a considerable cultivar-specific softening behaviour, and therefore a genetic contribution to fruit texture. Populations of introgression lines of plants produced from crosses between cultivars with superior and poor softening rates and storage ability may provide a powerful source of phenotypic variation in a defined genetic background which facilitates the identification of Quantitative Trait Loci (QTL) related to fruit textural attributes, which can be used for marker-assisted selection (MAS). If fully informative markers are mapped and the position of a functional gene coincides with that of a QTL on genetic linkage maps based on segregant populations from crosses in which polymorphisms are found, the biological nature of the QTL could be discussed in respect with fruit ripening or softening behaviour and storage potential, since it is expected that such gene may be a strong candidate to be involved in the process.

Using the model plant, tomato, several genetic linkage maps were developed to study diverse fruit developmental, ripening and quality loci (Bucheli, Lopez, Voirol, Petiard, & Tanksley, 1999; Doganlar, Tanksley, & Mutschler, 2000; Grandillo, Ku, & Tanksley, 1999; Ku, Vision, Liu, & Tanksley, 2000). QTL related with important quality traits, including fruit mass, pH, soluble solid concentration (Paterson *et al.*, 1991), shape, size, ripening time (Doganlar *et al.*, 2000; Grandillo *et al.*, 1999; Ku *et al.*, 2000) and

organoleptic qualities (Causse *et al.*, 2002) are available. In peach, QTLs for several fruit quality traits including acidity and soluble solid content have been located in the same regions of just two linkage groups (Dirlewanger *et al.*, 1999). Relating specific gene functions with these anonymous mapped markers, is needed to increase the knowledge about this physiological event.

In addition to textural and other quality traits, which reflect a resulting phenotype from a complex set of biochemical events, mapping of QTL affecting sugar composition or other differences of the fruit cell walls can also be informative. Such approaches can also take advantage of the existence of natural variation to identify candidate genes involved in cell wall modifications. A similar approach was conducted in maize (*Zea mays* L.) and allowed the identification of glycosyltransferases involved in cell wall biosynthesis (Hazen, Hawley, Davis, Henrissat, & Walton, 2003).

Conclusions and prospects

Dietary guidelines recommending the consumption of fresh fruits will not be succeeded if consumer dissatisfaction with the product quality limits fruit consumption. However, contemporary traditional postharvest approaches for fruits are not sufficient to meet the increasing consumer's demand on quality due to the development of undesirable characteristics. Increasing the storage life of fruits through the development of new techniques aimed to reduce the rate of deterioration while maintaining the favourable characteristics of the fruits, would greatly expand the opportunities for the industry to supply high quality fruits to local and export markets. Development of the most effective handling procedures and innovative technologies to assure the quality without compromising the safety and nutritional value of fruits depends on a better understanding of fruit biology and physiology. Plant genome analysis and technology is rapidly approaching the juncture where the efficiency of isolating, characterising and mapping gene sequences related to a developmental stage or response of interest can be merged with the accurate and efficient mapping of QTLs and single-gene mutation loci to predict candidate gene which can be specifically targeted for confirmation via transgenic or genetic means, in order to both accelerate gene identification and facilitate the characterisation of gene function.

A significant amount of information about the textural changes that occur during ripening of model species like tomato, strawberry or melon has been reported in the last years and a model for fruit ripening has been proposed. However, the research was extended to include other fruit species and cultivars from a given species. The results obtained suggest that softening and textural changes may be the result of different and specific cell wall modifications in different species and emphasise that each one needs to be investigated separately, since observations made using

a particular species cannot necessarily be extended to others (see also Brummell, 2006).

Future work involving determination of enzymatic activity *in muro*, determination of the true substrate and optimal conditions for each enzyme, localised analyses of the cell walls and metabolism of genetically manipulated fruits, and cloning and analyses of expression of every gene from a given family up-regulated with ripening, including identification and analysis of regulatory motifs present in promoters, is need to extent current knowledge about the molecular regulation of fruit ripening. It is advisable to demonstrate the function of each gene and gene product in each fruit, in order to replace homology-based discussions with clear-cut answers.

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