

Review

Postharvest softening of apple (*Malus domestica*) fruit: a review

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Abstract Postharvest softening of apple (*Malus domestica* (Borkh.)) fruit is a serious problem for growers in many countries, including New Zealand. To reduce this problem considerable research has been undertaken to determine the biological causes of softening so that this process can be managed or controlled more effectively. This review describes the pattern of softening for harvested apple fruit, and how it is influenced by different preharvest, at-harvest, and postharvest factors. Information is also given on the likely physiological and biochemical causes of apple softening, such as fruit anatomy and cell packing, modification of the cell wall and membranes, changes in cell turgor, and the role of ethylene and other growth regulators. Despite many softening studies, there is still a poor understanding of what causes firmness variation in the marketplace. Until this understanding is improved, apple producers will continue to struggle to meet market requirements for texture.

Keywords *Malus domestica*; firmness; ethylene; quality; preharvest factors; at harvest factors;

postharvest factors; growth regulators; calcium; cell wall

INTRODUCTION

The apple industry is one of the largest producers and exporters of fresh produce in New Zealand, with an annual export value of NZ\$404.5 million (f.o.b.) in 2000 (HortResearch 2000). The New Zealand apple (*Malus domestica* (Borkh.)) industry is export driven, and has been rated the most competitive international producer of apples for the last 5 years (Anon. 2001). However, markets are imposing more stringent quality standards for apples, making it increasingly difficult for growers to meet market requirements.

A major quality problem of apples in the marketplace is soft fruit. Softening is generally considered an undesirable ripening process in apple fruit, as firmer apples tend to be juicier, crisper, crunchier, and less mealy than softer fruit (Abbott et al. 1984; Harker et al. 1997, 2002). Fruit softening is typically assessed using a puncture test, also known as a flesh firmness, fruit firmness, or fruit pressure test. The ability of this test to accurately predict sensory perception of texture in apples is cultivar and sensory-attribute dependent (Abbott et al. 1984; Harker et al. 1997, 2002). Many markets are increasingly using fruit firmness as a guide to ensure that apples are delivered to markets with textural characteristics demanded by consumers, and to ensure year-round supplier consistency in textural quality. Failure of growers to meet these standards can result in shipment rejections, a damaged reputation as a supplier of quality apples, and reduced financial returns.

Before informed commercial decisions can be made to manage the softening process, detailed knowledge on its biology, and the relative influences of preharvest, at-harvest, and postharvest factors on this process is required. This review will outline current knowledge on these aspects of apple softening, and highlight research that is required to improve firmness of apples in the marketplace.

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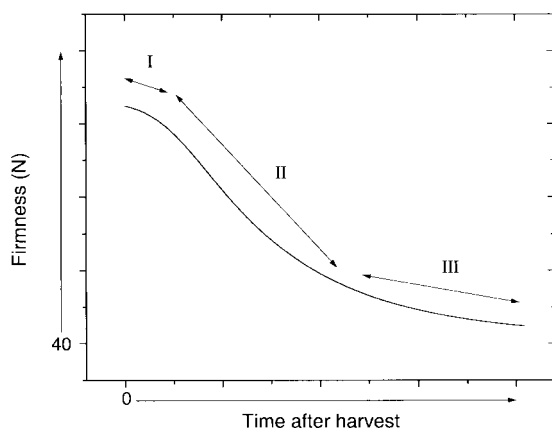


Fig. 1 Typical softening profile for apple (*Malus domestica*) fruit at 0–5°C (redrawn from Johnston et al. 2001a).

WHAT IS THE NATURE OF THE POSTHARVEST SOFTENING CURVE FOR APPLE FRUIT?

The decrease in firmness for many apple cultivars can be characterised by a non-linear curve consisting of three distinct phases. Fruit soften slowly during the first phase (I), more rapidly during the second phase (II), and then again slowly in the final phase (III) (Fig. 1; Johnston et al. 2001a, 2002a). This softening curve is not unique to apple, and is similar to that from fruits of several melon cultivars (Aggelis et al. 1997), tomatoes (Sozzi et al. 1998), and early harvested kiwifruit (MacRae et al. 1989). However, apples only soften by 25–50% to a final firmness of 35–50 N, whereas melons, tomatoes, and kiwifruit soften by 75–100% during ripening to a final firmness of 0–10 N (Bourne 1979; Johnston et al. 2001a).

Research using temperature and controlled atmospheres (CA) indicates that once Phase II softening is induced in apple, subsequent softening is difficult to slow. This suggests that this phase of softening could be considered irrevocable once initiated (Johnston et al. unpubl. data). Thus, Phase I softening needs to be prolonged if firmness is to be maintained long-term in storage. Although several at-harvest and postharvest factors have been found to influence the duration and rate of softening in Phases I and II, little is known about the cellular mechanisms that regulate the onset and rate of

softening of either phase. An understanding of these mechanisms may lead to more effective methods of preventing or controlling softening than conventional storage technologies currently provide.

WHAT IS THE CELLULAR BASIS OF FIRMNESS AND HOW DO APPLES SOFTEN?

Considerable research has been undertaken to understand the physiological and physical mechanisms involved in fruit softening. Studied mechanisms include the influence of cell shape, cell size, cell packing, and overall fruit anatomy, as well as chemistry of the cell wall and membrane, and the role of cell turgor.

Fruit anatomy and cell packing

The mature apple fruit contains several tissue types including the epidermis, hypodermis, cortex, vascular bundles, and a central core region containing pith and associated seed bearing tissues (Tukey & Young 1942). Of these tissues, the parenchymous cortex tissue is most readily consumed, with tissues in the core region largely avoided. For that reason, destructive assessments of texture are predominantly undertaken on parenchymous tissue excised from the cortex, or on intact cortical tissue with the epidermal and hypodermal cell layers removed.

A number of studies have concluded that apple softening is mediated by loss of cell-to-cell adhesion. Microscopic examination of fracture faces following tensile tests on excised tissue showed tissue failure in soft fruit occurs between cells, whereas failure in firm fruit predominantly occurs through cells (Harker & Hallett 1992; De Smedt et al. 1998). Examination of intact tissue blocks also showed that tissue from soft fruit had rounder cells, more cell separation, and larger intercellular spaces, than tissue from firm or freshly harvested fruit (Lapsley et al. 1992; De Smedt et al. 1998).

There is some evidence indicating that earlier maturing apple cultivars have larger cells, larger intercellular spaces, and are less dense than later maturing cultivars (Kahn & Vincent 1990). This may explain why early season cultivars tend to soften more rapidly in storage than later season cultivars. Fruits with larger cells and more intercellular spaces are generally considered to have weaker tissue than fruits with smaller cells and less intercellular spaces (Harker et al. 1997).

Cell wall

The cell wall is a complex structure important for imposing cell shape and rigidity in many plant tissues. Primary cell walls of plants are considered a network of cellulose microfibrils embedded in a matrix of hemicellulose and pectin (Rose & Bennett 1999; Cosgrove 2001). Pectic polymers are the main constituents of the middle lamella, a region considered important for maintaining cell-to-cell adhesion and cell packing in fruit tissues (Harker et al. 1997; Wakabayashi 2000; Redgwell & Fischer 2002).

Analyses of pectin fractions have shown that apple softening is usually associated with increased content of water-soluble pectin, and reduced galactose and arabinose residues (Knee 1973), with little depolymerisation occurring in any pectin fraction during ripening (Yoshioka et al. 1992). However, it is still not known if the processes of pectin solubilisation and loss of galactose are causal, coincidental, or a consequence of fruit softening (Redgwell et al. 1997a).

A number of cell wall modifying enzymes that have been found in ripening apples may cause softening (Table 1). The enzyme originally considered responsible for pectin solubilisation and therefore softening in most fruits was polygalacturonase (PG), with activities of both exo-PG and endo-PG detected in ripening apples (Bartley 1978; Wu et al. 1993; Atkinson et al. 1998). Electron microscopy revealed that treatment of unripe apple discs with PG caused similar disruption in the middle lamella to that observed in ripe apple tissue (Ben-Arie et al. 1979). However, transgenic tomato experiments with wild-type and ripening mutants produced evidence that PG is not required for initiating fruit softening (Giovannoni et al. 1989; Smith et al. 1990). The importance of exo-PG and endo-PG in the apple softening process is yet to be established.

Apple softening is associated with an increase in pectin methyl esterase (PME) activity and reduced esterification of certain pectin fractions (Klein et al. 1995). Yoshioka et al. (1992) suggested that

Table 1 Summary of cell wall modifying enzymes identified in ripening apple (*Malus domestica*) fruit thus far.

Cell wall enzyme	Function	Δ Activity during ripening	References
Exo-polygalacturonase EC 3.2.1.67	Removal of terminal galacturonosyl residues from pectin	Not measured	Bartley (1978)
Endo-polygalacturonase EC 3.2.1.15	Hydrolytic cleavage of α-1,4-galacturonosyl linkages in unesterified pectin	Increased	Wu et al. (1993); Atkinson et al. (1998)
Pectin methyl esterase EC 3.1.1.11	Removal of methyl groups from esterified pectin	Increased	Klein et al. (1995)
Glycosidases (i.e., β-galactosidase EC 3.2.1.23)	Terminal removal of galactosyl residues from pectin and xyloglucan*	Increased [†]	Wallner (1978); Dick et al. (1990); Yoshioka et al. (1995)
α-L-arabinofuranosidase EC 3.2.1.55	Removal of arabinosyl and some other residues from pectin	Increased	Yoshioka et al. (1995)
Rhamnogalacturonase A	Hydrolyse α-1,2 linkages between galacturonosyl and rhamnosyl residues in pectin	Not measured	Gross et al. (1995)
Xyloglucan- endotransglycosylase EC 2.4.1.207	Hydrolyse and/or transglycosylase xyloglucan	Decreased	Percy et al. (1996)
Endo-glucanases (cellulase) EC 3.2.1.4	Hydrolyse β-1,4 glucan linkages in cellulose and xyloglucan [‡]	Decreased	Abeles & Biles (1991)

**In vivo* substrates and sites of action are yet to be confirmed, as there is some suggestion that β-galactosidase may also have associated α-L-arabinopyranosidase and β-D-fucosidase activities (Dick et al. 1990).

[†]This is isozyme dependent, as one isozyme increased, while three isozymes decreased, during ripening in apples (Yoshioka et al. 1995).

[‡]*In vivo* substrates of endo-glucanases are yet to be confirmed (Rose & Bennett 1999).

deesterification of highly methoxylated pectin regions may result in the swelling and solubilisation of pectin during softening. However the role of PME in softening, if any, is likely to be indirect, as tomato softening mutants contain similar PME activity to wild-type fruit (Harriman et al. 1991). It is possible that PME may modify pectin to facilitate pectin solubilisation or depolymerisation by other enzymes such as PG (Wakabayashi 2000).

Glycosidases, such as β -galactosidase, could facilitate both pectin solubilisation and removal of galactose and arabinose residues from arabinogalactan side-chains of pectin during apple softening (Yoshioka et al. 1995). β -galactosidase activity has been found to increase markedly in several apple cultivars during storage, although cultivar differences in softening rates were not explained by differences in activity of this enzyme (Wallner 1978). The role of β -galactosidase in apple softening is further complicated by the finding of four isozymes in ripening apples, of which only one had increased activity during apple ripening (Yoshioka et al. 1995). Another glycoside, α -L-arabinofuranosidase, which may facilitate release of arabinose residues during softening, increased activity during apple ripening (Yoshioka et al. 1995).

Rhamnogalacturonase activity has also been found in apple fruit (Gross et al. 1995), but changes in activity during ripening have not been characterised. More research is required to determine if this enzyme can facilitate pectin solubilisation and softening of apples during ripening. It should also be considered that enzymes other than those in Table 1 could be responsible for mediating the disruption of the pectin-rich middle lamella that may be required for softening to occur (Redgwell & Fischer 2002).

The non-cellulose and cellulose glucose concentrations in the cell wall remain relatively constant during ripening of apples, suggesting that little cellulose and hemicellulose degradation occurs (Bartley 1976). Siddiqui et al. (1996) found little degradation of hemicellulose and cellulose in apples during 6 months of storage. This is reflected in studies where the molecular weight of xyloglucan (predominant hemicellulose polymer in fruits) remained constant, and the activities of xyloglucan modifying enzymes such as xyloglucan endo-transglycosylase (XET) and cellulase decreased during softening (Abeles & Biles 1991; Percy et al. 1996, 1997). It is possible that minimal depolymerisation of hemicellulose during apple ripening may impose the partial softening trait of this fruit.

Without transgenic experiments such as those performed for PG in tomato (Giovannoni et al. 1989; Smith et al. 1990), it is difficult to determine which cell wall modifying enzymes are important for causing apple softening.

Cell membranes

Membranes may have a number of important roles in fruit texture, including: export of compounds and enzymes required for cell wall modification; modulation of solute concentrations and pH in the apoplast; modulation of solutes in the cytoplasm for maintenance of cell turgor; regulation of cytoplasmic concentrations of specific ions that influence signal transduction pathways and gene expression (e.g., calcium (Ca)); and control of the release of water into the apoplast for cell wall swelling (Harker et al. 1997). This last role is important to the perception of juiciness when cell-to-cell adhesion is low (Harker et al. 1997). Apples typically do not undergo a large degree of cell wall swelling as compared to other fruits (Redgwell et al. 1997b), nor is there an increased sensory perception of juiciness (Plocharski & Konopacka 1999) during softening. However, the other membrane roles identified above may influence apple softening. Membrane changes in ripening apples have been characterised (Lurie et al. 1987), but no information appears available relating these changes to softening.

Cell turgor

Water status is an important determinant of fruit texture. It physically influences texture by influencing cell turgor (Harker et al. 1997), and physiologically influences texture by affecting ripening rates (Littmann 1972). A study using a series of solutions with different mannitol concentrations to modify the turgor of excised apple tissue showed that the failure force under compression was reduced when tissue was placed in solutions that were extremely hypotonic or hypertonic (Lin & Pitt 1986). Tissue under high turgor failed by cell wall rupture, whereas tissue under low turgor failed by cell separation. These results suggest a reduction in turgor may reduce firmness.

Direct measurements of turgor showed that cell turgor decreased in four apple cultivars during storage (Tong et al. 1999). There was a positive association between cell turgor and firmness after 6 months at 0–2°C for cultivars with different softening rates. However, cell turgor alone could not explain all the variation in poststorage firmness, and

it was concluded that other cellular factors also influence firmness in storage (Tong et al. 1999). Thus, although a reduction in cell turgor may contribute to loss of firmness during storage, it is unlikely that this process alone causes apples to soften.

WHAT REGULATES SOFTENING?

Ethylene

Ethylene is a major plant growth regulator and ripening hormone in climacteric fruits such as apple. Its role in apple softening has been studied using several approaches. These include the use of preharvest ethylene releasing sprays (Watkins et al. 1989), the addition and scrubbing of ethylene from storage atmospheres (Gerhardt & Siegelman 1955; Stow et al. 2000), preharvest application of an inhibitor of ethylene biosynthesis (amino-ethoxyvinylglycine; Halder-Doll & Bangerth 1987), postharvest inhibitors of ethylene action (Fan et al. 1999; Mir et al. 2001), and relating softening with endogenous changes in ethylene concentration through maturation (Lau et al. 1986; Blankenship & Unrath 1988).

The most conclusive evidence that ethylene promotes apple softening has resulted from experiments using inhibitors of ethylene action. Apples treated at harvest with 1-methylcyclopropene (MCP) softened slower and had reduced internal ethylene concentrations (IECs) relative to untreated fruit in storage (Fan et al. 1999; Watkins et al. 2000). Re-treatment of apples with MCP during storage showed that apples treated more regularly were firmer through storage than apples only treated at harvest (Mir et al. 2001). It is suggested that the loss of MCP inhibition over time is through a slow release of the inhibitor from the active site, or that the tissue generates new active sites (Sisler & Serek 1999).

Studies seeking to identify relationships between IEC and softening have resulted in an unclear role for ethylene in the softening process of apple fruit. Lau et al. (1986) and Blankenship & Unrath (1988) found that firmness declined before the IEC increased during on-tree maturation, and suggested that ethylene may not be required for initiation of on-tree softening. A low basal rate of ethylene production may have been sufficient to promote on-tree softening, as has been suggested for the early phases of kiwifruit softening when ethylene

production is low (Kim et al. 1999). However, on-tree fruit softening may have been the result of cell expansion and increased fruit size that ordinarily occurs during maturation. Once harvested, the firmness of apples generally declines as ethylene production or the IEC increases (Yoshioka et al. 1995; Watkins et al. 2000). Anecdotal evidence indicates that softening is reduced in CA storage when ethylene is maintained below internal and external concentrations of $0.1 \mu\text{l litre}^{-1}$ (Stow et al. 2000) and $1 \mu\text{l litre}^{-1}$ (Liu 1977), respectively. Johnston et al. (2001a, 2002a,b) found that onset of rapid softening was consistently associated with IEC exceeding $1.5 \mu\text{l litre}^{-1}$ in some cultivars, whereas in other cultivars the fruit sensitivity to ethylene appeared to have more of a regulatory role in determining the occurrence of rapid softening than IEC.

Ethylene may induce softening in apples by regulating expression of cell wall modifying enzymes. Expression of PG was shown to increase during the early phases of softening for several apple cultivars, with the increase occurring as IEC increased from 0.7 to $2.1 \mu\text{l litre}^{-1}$ for 'Royal Gala', 0.2 – $2.7 \mu\text{l litre}^{-1}$ for 'Granny Smith', and 11 – $46 \mu\text{l litre}^{-1}$ for 'Braeburn' (Atkinson et al. 1998). Analysis of cell wall enzymes in transgenic melons with suppressed ethylene biosynthesis and reduced softening showed that the activities of endo-PG, β -galactanase, α -arabinosidase, and β -galactosidase were ethylene-dependent, whereas activities of exo-PG and PME were ethylene independent (Pech et al. 1999).

Other growth regulators?

The role of growth regulators, other than ethylene, in regulating the ripening and softening of fruit is not well known. Auxins and gibberellins appear to reduce apple firmness, although auxin may promote softening by inducing ethylene production (Looney 1971; Wang & Steffens 1987; Curry & Greene 1993). In contrast the use of cytokinin and cytokinin-like compounds produces firmer apples before and after storage (Curry & Greene 1993; Elfving & Lougheed 1994). Abscisic acid is thought to have an important role in promoting climacteric ethylene production in apple (Lara & Vendrell 2000), and therefore may induce apple softening indirectly. Nitric oxide is known to delay ripening and senescence in many fruits and vegetables by influencing ethylene production (Leshem et al. 1998). Thus, nitric oxide may decrease apple softening indirectly by reducing ethylene production.

Endogenous polyamines may also affect apple firmness. 'Golden Delicious' apples were firmer immediately after polyamine treatment, but then softened at similar rates to non-treated fruit (Kramer et al. 1991). The effectiveness of the exogenous polyamine treatment in increasing firmness was similar to that obtained with a Ca treatment (Kramer et al. 1991). However, unlike Ca, polyamine treatments did not reduce softening of fruit during storage. Polyamines may temporarily firm apple fruit by binding to and improving the strength of the cell wall, and may compete with Ca for binding sites (Wang et al. 1993).

Apoplastic pH

The role of apoplastic pH in regulating apple softening is not known. Pear discs were firmer when infiltrated with a pH buffer of 8.0 than a pH buffer of 3.2 (Knee 1982). During the softening of tomato, apoplastic pH decreased from 6.7 to 4.4, and the ionic concentration of the apoplast increased (Almeida & Huber 1999). Apoplastic changes in pH and ionic concentration may regulate softening by modifying activity of cell wall degrading enzymes (Almeida & Huber 1999). Also, pH interacts with the degree of esterification of pectin to affect the strength of pectin gels (Crandall & Wicker 1986). It is possible that changes in apoplastic pH and degree of pectin esterification affect the strength of pectin in apple, and thus softening.

WHAT EFFECTS DO PREHARVEST, AT-HARVEST, AND POSTHARVEST FACTORS HAVE ON APPLE SOFTENING?

Preharvest factors

Fruit from different orchards often differ in firmness after storage, despite being stored in similar conditions. This variation in quality is the result of differences in storage potential at harvest, that in turn are determined by the collective influence of several preharvest and at-harvest factors (Bramlage 1993). Two main approaches have been undertaken to determine the influence of preharvest factors on the firmness of apples. The first is the systematic process of changing one variable in the orchard and assessing the consequent quality at harvest and after storage (Johnson 1994). The second is by collecting fruit from orchards with a range of preharvest practices and analysing attributes of the fruit at harvest that indicate storage potential (Bramlage 1993; Johnson

& Ridout 1998). The problem with the first approach is that it is often difficult to change one factor without inadvertently changing another factor, whereas the second approach does not necessarily identify the relative importance of individual preharvest factors.

Preharvest factors that influence apple quality before and after storage include: climatic factors such as light intensity, temperature, and rainfall; cultural factors such as mineral nutrition, timing, and extent of thinning that affects crop load, orchard floor management, irrigation, tree management, and use of growth regulators; and genetic factors that involve choice of cultivar or clone, rootstocks, and interstocks (Bramlage 1993; Harker et al. 1997; Sams 1999). The individual influence of each of these factors on firmness has been reviewed by Harker et al. (1997) and Sams (1999), and therefore will not be reviewed here. Notwithstanding this, there is limited information available on the influence of preharvest factors on softening rates of apples through storage.

Several studies have attempted to predict the poststorage firmness of apples before storage using preharvest or at-harvest measurements of meteorological variables, harvest indices, and mineral concentrations (Bramlage et al. 1985; Fallahi et al. 1985; Marmo et al. 1985; Johnson et al. 1987; Ingle & Morris 1989; Knee & Farman 1989; Knee & Smith 1989; Blankenship et al. 1997; Johnson & Ridout 1998; de Jager & de Putter 1999; Ingle et al. 2000; Johnson 2000). In general, the best predictions came from prestorage assessments of firmness, where fruit with higher firmness at harvest were firmer after storage than fruit with lower firmness. However, predictive accuracy of prestorage firmness measurements varied substantially between seasons and cultivars. Knee & Farman (1989) found predictive accuracy of models based on firmness were improved by including estimates of time between harvest and an internal ethylene concentration of $0.1 \mu\text{l litre}^{-1}$, or inclusion of harvest dates. However, these multifactor models could still only account for c. 60% of the variation in firmness after CA storage (Knee & Farman 1989). Inclusion of leaf boron, skin greenness, fruit nitrogen, and certain meteorological variables into a firmness model explained 76% of the variation in poststorage apple firmness as compared with only 55% for at-harvest firmness alone (Johnson & Ridout 1998). In general, minerals alone have been poor predictors of poststorage firmness in apples (Bramlage et al. 1985; Fallahi et al. 1985; de Jager & de Putter 1999). However, there have been reported instances of

associations between poststorage firmness and concentrations of phosphorus, zinc, manganese, boron, potassium, sodium, copper, magnesium, and Ca concentrations in some cultivars (Johnson et al. 1987; Johnson 2000).

At harvest factors

Two major factors that influence postharvest softening of apples at harvest are maturity and fruit size. Horticultural maturity is defined as the stage of development at which horticultural crops are harvested to meet consumer requirements (Watada et al. 1984). Of the different stages of development (growth, maturation, ripening, and senescence), apple fruits are considered horticulturally mature during maturation and early stages of ripening (Watada et al. 1984). With regards to texture, apples harvested at a later stage of maturity are often softer at harvest and after storage than apples picked less mature (Ingle et al. 2000). However, a few exceptions to this trend have been observed, where apples harvested at a later maturity were firmer than earlier harvested fruit after storage (Ingle & Morris 1989). Analysis of softening curves showed that less mature fruit were firmer at harvest and had a longer initial slow softening phase than fruit harvested more mature (Johnston et al. 2002a). However, harvest date did not influence the subsequent rate of rapid phase softening.

It is generally accepted that larger fruits are softer than smaller fruits (Harker et al. 1997), as smaller fruits generally have more cell wall material per unit volume, and therefore should have stronger tissue than larger fruits. Larger apples have been reported to be softer than smaller fruit both at harvest and after storage (Blanpied et al. 1978; Marmo et al. 1985; Siddiqui & Bangerth 1995). However, it is not known if these effects of fruit size were because of physical differences in tissue strength as a result of differences in cell size and cell number, and/or physiological differences in firmness as a result of the different sized fruit being picked at different maturity. Fruit size did not affect any aspect of the softening curve at 0.5–3°C when fruit were harvested at an early stage of maturity, but when fruits were harvested at a later stage of maturity, smaller fruit tended to soften slower than larger fruit (Johnston et al. 2002a).

Postharvest factors

The main postharvest factors that influence apple softening include temperature, relative humidity (RH), Ca treatment, atmosphere, and ethylene.

Temperature

Temperature strongly influences the postharvest life of apple fruit. Most apples are stored at 0–3°C to slow loss of quality after harvest, with the temperature used depending on cultivar sensitivity to chilling injury. Studies by Magness & Diehl (1924) and Landfald (1966) first showed that fruit from several apple cultivars softened slower as temperature decreased from 21 to 0°C. Johnston et al. (2001a, 2002b) found three softening responses to temperature existing among apple cultivars, particularly when exposed to shelf-life temperatures (e.g., 20°C). The first response occurred for ‘Royal Gala’ and ‘Cox’s Orange Pippin’, where softening rate increased with temperature from 0°C to a maximum rate at 22°C, before decreasing thereafter through 35°C (Johnston et al. 2001a). This response was described by a modified Arrhenius equation, which suggested that these cultivars exhibited a typical biological response to temperature (Johnson & Thornley 1985). The second response occurred for ‘Granny Smith’, where fruit softened slowly and at similar rates from 0 to 12°C, and rapid softening only occurred at 20°C once fruit were exposed to ethylene or 0.5°C (Johnston et al. 2001a, 2002b). The third response occurred for ‘Pacific Rose’™, which had a similar response to ‘Granny Smith’ when continuously stored at a range of temperatures from 0 to 35°C, except that rapid softening did not occur at 20°C when exposed to ethylene or cold temperatures.

Despite 0–3°C being the optimum postharvest temperature for slowing loss of firmness and many other aspects of quality loss, it is difficult to maintain fruit at these optimum temperatures through the entire postharvest handling chain. Fruit are often exposed to non-optimal temperatures during grading, packing, distribution, ship loading and unloading, and in retail outlets while on display. In addition, fruit can be accidentally exposed to non-optimal temperatures through coolstore or container malfunction, and incorrect operation of facilities for rapid cooling. For this reason considerable research has been undertaken to determine the consequences of exposing apple fruit to non-optimal temperatures at different stages of postharvest handling. In general the longer the delay before cooling after harvest, the softer the fruit through storage (Magness & Diehl 1924; Blanpied 1975; Johnston et al. unpubl. data). In terms of the softening curve, this cooling delay reduces the initial slow softening phase, and shortens the time before onset of the irrevocable rapid softening phase for cultivars such as ‘Royal Gala’

and 'Cox's Orange Pippin' at 0.5–3°C (Johnston et al. unpubl. data). These cultivars lost 10–30% of marketable life with each additional day at 20°C before cooling to 0.5–3°C. However, the effectiveness of rapid cooling in retaining firmness is often dependent on cultivar, and time in storage before assessment of firmness (Magness & Diehl 1924; Blanpied 1975).

Knowledge of the extent of physical change in firmness with temperature is important, as this will determine whether fruit should be equilibrated to a standard temperature before firmness is measured. Studies by Blanpied et al. (1978) and Bourne (1982) have shown that apples of similar ripeness were firmer when measured at a fruit temperature of 0–2°C, than at a fruit temperature of 21–45°C. Furthermore, the firmness temperature coefficients (Δ firmness/ Δ °C) of apples varied between cultivars (Bourne 1982). In contrast, Saltveit (1984) found that fruit temperature had no influence on the firmness of several cultivars. These inconsistent results could be attributed to a change in firmness-temperature coefficient with storage time and cultivar (Johnston et al. 2001b). For cultivars such as 'Cox's Orange Pippin', 'Royal Gala', and 'Granny Smith', apples were physically firmer at harvest and physically softer after 100 days of storage when measured at a fruit temperature of 20°C as compared to 0.5–3°C. In contrast, firmness of 'Pacific Rose'TM was not physically affected by fruit temperature regardless of time in storage.

Relative humidity

Studies using different storage humidities have provided evidence, albeit contradictory in some instances, that water status influences apple firmness. 'Cox's Orange Pippin' apples with greater weight loss had higher maximum and bioyield forces from puncture and shear tests, than fruit that lost less weight during storage (Hatfield & Knee 1988). Poststorage firmness of 'Spartan' apples was inconsistently affected by storage humidity; in one season fruit stored in 80% RH were c. 0.6 kg firmer than fruit stored in 92–94% RH, whereas in another season firmness was not affected by storage RH (Porritt & Meheriuk 1973). In contrast to these studies, the poststorage firmness of 'McIntosh' apples was reduced as storage RH decreased (Lidster 1990).

The mechanism by which RH influences poststorage firmness is not clear. Hatfield & Knee (1988) found that high-weight loss treatment reduced the increase in intercellular airspace during storage, and consequently suggested that these fruit had

greater cell-to-cell contact and therefore greater firmness. Cells from fruit with more water were rounder than cells from fruit that had lost more water (Bolin & Huxsoll 1987), suggesting reduced cell-to-cell contact. Further evidence that storage RH influences cell-to-cell adhesion was that tensile strength of apple tissue was generally lower in 'Braeburn' and 'Jonagold' when stored at 95% RH, relative to storage at 65% and 30% RH (Tu et al. 2000). Microscopic examination of tissue fracture faces from tensile tests indicated that fruit stored at high RH tended to separate between cells, rather than across or through cells as in fresh tissue.

Water loss enhances the ripening rates of several fruits, including banana, avocado, and pear (Littmann 1972). However, limited information is available for apple fruit, especially with respect to softening rates. In the RH studies outlined above, firmness was usually only measured 1–4 times during storage, making it difficult to accurately determine the ripening and softening rates of fruit with different water status. It is also notable that both the direction and extent of the effect of water loss on apple softening is not clear, making it difficult to predict the textural consequences of using post-harvest technologies that influence water loss.

Calcium treatments

Calcium improves the storage performance of many fruits (Poovaiah et al. 1988). Ca-treated apples were firmer after storage than non-treated apples (Watkins et al. 1989). Stow (1993) suggested that Ca supplementation was able to reverse softening, and that apple softening was mediated by loss of Ca from the pectin-rich middle lamella. However, data of Stow (1993) could be interpreted as showing that Ca supplementation physically increased firmness of the tissue, which then softened at a similar rate to control fruit. In other studies, Ca treatment reduced the softening rate and associated increase in soluble pectin relative to control fruit (Sams & Conway 1984; Glenn & Poovaiah 1990). Thus, Ca appears to maintain the texture of apples by reducing ripening associated softening rates and physically increasing tissue rigidity.

Calcium treatment of apple fruit increased both cell wall bound and soluble concentrations of Ca in the tissue (Saftner et al. 1998). As concentration of Ca treatment increased, the cell wall bound Ca component became saturated, while the soluble Ca component continued to increase, suggesting that the number of Ca binding sites in the cell wall is limited. The saturation concentration for Ca binding in the

cell wall increased during storage, which suggests increased availability of binding sites during ripening. These binding sites may be non-esterified galacturonic acid residues in pectin, at which Ca may increase tissue rigidity by cross-linking pectin chains (Conway et al. 1993). Ca treatment increases the amount of Ca bound to pectin, but not that bound to hemicellulose or cellulose (Siddiqui & Bangerth 1996).

Calcium-treated fruit have less disruption and degradation of the pectin-rich middle lamella, and have more cell-to-cell contact than non-treated fruit (Glenn & Poovaiah 1990; Siddiqui & Bangerth 1996). Furthermore, Ca-treated tissues fractured across and through cells, whereas tissues from non-treated apples were separated by loss of cell-to-cell adhesion (Glenn & Poovaiah 1990).

Calcium appears to improve the texture of apples by maintaining cell wall integrity but Ca may also influence apple softening by changing the physical properties of the cell membrane. Legge et al. (1982) found that Ca stabilised and increased the rigidity of membranes at the surface, and Paliyath et al. (1984) found that Ca treatment slowed the increase in membrane microviscosity that normally occurs during ripening of apples. Fruit with low Ca had greater membrane disorganisation than fruit with higher Ca concentrations (Fuller 1980).

Controlled atmospheres

Studies by Kidd & West (1933) first showed the commercial potential of placing apples in CA storage to slow loss of quality, including softening. CA storage was shown to extend the initial slow softening phase and reduce rate of rapid phase softening when applied without delay after harvest (Johnston et al. unpubl. data). In terms of market life for firmness, CA conditions of 2% O₂:1.8% CO₂ extended the life of 'Royal Gala' and 'Cox's Orange Pippin' by 100–200% over air storage at the same temperature (Johnston et al. unpubl. data). The effectiveness of CA in reducing softening is cultivar dependent (Dilley et al. 1989), and influenced by: the O₂ and CO₂ concentration (Kidd & West 1933; Hertog et al. 2001); time between harvest and establishment of CA conditions (Dilley et al. 1989); storage temperature (Kidd & West 1933); fruit maturity (Tu et al. 1997); and exogenous ethylene concentration in store (Liu 1985).

Incremental reduction of O₂ concentrations from 21% (RA) to 1–2.5% improves the poststorage firmness of several apple cultivars (Kidd & West 1933; Hertog et al. 2001). At O₂ concentrations less

than 0.75–1%, softening tends to increase (Hertog et al. 2001), presumably through the deleterious effects of low-O₂ disorders on texture. Progressive increases in CO₂ concentration from 0 to 50% also slowed softening in several cultivars (Magness & Diehl 1924; Kidd & West 1933), whereas Hertog et al. (2001) found that CO₂ was more effective at reducing softening at O₂ concentrations greater than 1–1.5%.

For most cultivars, firmness retention is improved in CA when it is established earlier, rather than later, after harvest (Dilley et al. 1989). The longer the delay before transferring fruit from air to CA, the faster the subsequent rate of softening when fruit is placed in CA. Johnston et al. (unpubl. data) found the softening rate in CA was similar to that in regular storage, if CA was delayed until fruit were in the rapid phase of softening. This suggests firmness benefits may only be achieved from CA if it is established while fruit are in the initial slow softening phase. There is some evidence that the same firmness benefits are not achieved from rapid establishment of CA when fruit are destined for ultra-low O₂ (<1.5% O₂) storage (Stow 1986). This could be the result of ultra-low O₂ CA being more effective at retarding softening than CA with O₂ concentrations greater than 1.5% (Stow 1986).

Despite ethylene biosynthesis and action being reduced in CA (Burg & Burg 1967; Dilley et al. 1989), the presence of exogenous ethylene can affect firmness retention in CA (Liu 1985). However, ethylene scrubbing in CA has produced inconsistent results between cultivars, with firmness benefits often only achieved when external and internal ethylene concentrations are maintained below 1 and 0.1 µl litre⁻¹, respectively (Liu 1985; Stow et al. 2000). Also, firmness benefits from ethylene scrubbing in CA often only occur for pre-climacteric fruit, and when fruit are rapidly cooled and placed in CA without delay after harvest (Liu 1985).

The mechanism by which CA reduces softening is largely unknown, but could be partially explained by reduced ethylene biosynthesis and action, respiration rate, and cell wall disassembly in CA (Burg & Burg 1967; Dilley et al. 1989; Siddiqui et al. 1996; Hertog et al. 2001).

Short-term stress treatments

Short-term stress treatments have shown potential for improving fruit quality in storage, and to pre-condition fruit to the temperature and atmosphere of the intended storage environment. Such treatments include use of heat, low O₂, and high CO₂.

Short-term heat treatments (2–6 days at 38–40°C) slowed the softening rates of apples at low and shelf-life temperatures (Liu 1978; Porritt & Lidster 1978). Subsequent studies have characterised the temperature and the duration of heat treatment needed to optimise quality retention during storage (Klein & Lurie 1992). Force-deformation curves indicated heat-treated apples have a “harder” and “tougher” texture than non-treated apples (Conway et al. 1994). Sensory panels also rated heat-treated fruit as being crisper than non-treated fruit (Lurie & Nussinovitch 1996). Heat treatments act synergistically with Ca dipping, and additively with Ca vacuum infiltration, to further reduce softening (Conway et al. 1994; Klein & Lurie 1994). The mechanisms by which heat treatments improve fruit quality are reviewed in Lurie (1998).

The utilisation of prestorage CO₂ shock treatment has showed some potential for retaining firmness in storage, although the effectiveness of this treatment is inconsistent across seasons (Meheriuk et al. 1977) and growing regions (Bramlage et al. 1977). Increased incidence of CO₂ injury was also associated with such treatments (Bramlage et al. 1977).

Like high CO₂ shock treatments, low O₂ (<1%) shock treatments have inconsistently improved firmness retention in different apple cultivars. Low O₂ shock treatments reduced subsequent softening and incidence of some disorders in ‘McIntosh’, ‘Spartan’, ‘Golden Delicious’, and ‘Granny Smith’ in CA, but also increased incidence of low O₂ disorders (Little et al. 1982; Lister et al. 1987). In other cultivars such as ‘Cox’s Orange Pippin’, low O₂ shock treatments did not improve firmness retention, and increased the incidence of rots and physiological disorders during storage (Fidler & North 1971).

The commercial quality benefits of using either high CO₂ or low O₂ treatments remain inconclusive. In contrast, heat treatments show more potential as a short-term shock treatment, as not only does this technology reduce softening, but it also has considerable potential as a non-chemical method of disinfestation (Lurie 1998).

CONCLUSIONS

Despite the large number of studies dedicated to understanding the process of apple softening, it is still not known what causes firmness variation in the marketplace. This could be expected given that softening is a complex biological process, and it is influenced by several preharvest, at-harvest, and

postharvest factors. Research characterising the postharvest softening patterns of apples in relation to several at-harvest and postharvest factors has shown that the market life is largely determined by the duration of the initial slow softening phase. Once rapid softening is induced, it is irrevocable and difficult to control using conventional storage technologies. It would be advantageous if new technologies or cultivars could be produced that reduce or control this phase of softening.

Ethylene has an important role in promoting apple softening. The ability to reduce ethylene biosynthesis and action could be a viable commercial method for reducing rapid softening. However, ethylene is equally important for promoting ripening processes other than softening, such as aroma and flavour volatile production, which are important attributes of fruit quality. It would be preferable to inhibit only those processes affected by ethylene that contribute to softening.

It is generally accepted that apple softening is largely mediated by disruption of the pectin-rich middle lamella, resulting in loss of cell-to-cell contact. A number of cell wall degrading enzymes have been identified in apple that may cause this. However, the role each enzyme has in apple softening is not yet known, and identification of the enzyme(s) responsible for rapid softening could provide a means to control this process through genetic modification or plant breeding.

There is a lack of knowledge on how different preharvest factors influence softening rates of harvested apples. Research is required that characterises the softening curves for apples in relation to different preharvest factors, so that orchard practises can be identified that maximise the initial slow softening phase in storage. It is also not known how different preharvest factors interact, and influence the storage potential of fruit at harvest. With increasing consumer resistance to use of chemicals and genetic modification for means of improving food quality, it would also be advantageous to continue research in development of models that predict softening rates before storage. Until the process of apple softening is better understood, it is likely softening will continue to be an unpredictable and frustrating problem for apple producers around the world.

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