Postharvest Apple Softening: Effects of At-harvest and Post-harvest Factors

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Abstract

'Cox's Orange Pippin' (COP) and 'Royal Gala' (RG) are rapid softening apple cultivars. This makes it difficult for growers to meet minimum firmness standards in the marketplace. Research was undertaken to characterise softening curves of COP and RG in relation to different at- and post-harvest factors, and to compare these cultivars with the slower softening cultivars 'Granny Smith' (GS) and 'Pacific Rose™' (PR). Regular measurement of firmness during low-temperature storage showed that the postharvest softening curve for all cultivars was triphasic with an initial slow softening phase (I), followed by a phase of more rapid softening (II), and then a final slow softening phase (III). Phase I largely determined the fruit market life for firmness, as fruit with a short phase I had less market life than fruit with a longer first phase. Phase I of RG and COP was lengthened by harvesting fruit at an earlier rather than later maturity, by rapidly cooling fruit after harvest to 0.5-3°C, and by placing fruit in controlled atmospheres (CA). Rate of phase II softening was not affected by harvest maturity, but decreased as storage temperature was reduced from 22 to 0°C, and was reduced in CA relative to air. A modified Arrenhius equation described softening rates of COP and RG at different temperatures, where softening rate increased from 0°C to a maximum at 22°C, and then decreased through 35°C. In contrast, this equation could not describe softening rates of PR and GS at different temperatures, as both cultivars softened slowly at similar rates from 0-12°C, and phase II did not occur at 20-35°C. Prior cold or ethylene treatment induced phase II softening at 20°C for GS, but not PR. Internal ethylene concentration (IEC) may have a role in regulating onset of phase II softening in RG and COP at 0-35°C, while for GS and PR fruit sensitivity to ethylene may have a more important regulatory role than IEC. A prototype model was developed for estimating loss of RG and COP firmness through the postharvest handling chain. This model has potential to improve commercial management of the "soft fruit" problem in the marketplace.

Thesis summary

Consumers worldwide are demanding apples that are crisp and crunchy, and not dry or mealy. However, some early season cultivars soften rapidly after harvest making it difficult for growers to meet these consumer requirements. This research was undertaken to obtain information on how different pre- and postharvest factors influence softening rates of the early season apple cultivars 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP). The softening characteristics of these rapid softening cultivars were compared with the slow softening 'Granny Smith' (GS) and 'Pacific Rose' (PR) cultivars, in an attempt to determine why the RG and COP cultivars soften rapidly during postharvest handling. Factors studied in this thesis included harvest maturity, fruit size, orchard, and the temperature and atmosphere during storage.

Regardless of the factor studied, the postharvest softening curves for RG and COP cultivars was triphasic, with an initial slow softening phase immediately after harvest, followed by a phase of more rapid softening, and then a final slow softening phase. COP and RG fruit harvested at a later maturity were softer at harvest, and subsequently had a shorter initial slow softening phase during storage at 0-3°C or 20°C, than fruit picked less mature. Variation in postharvest softening rates of fruit from different orchards in two seasons was largely accounted for by differences in maturity at harvest, where fruit from orchards picked at an earlier maturity generally softened slower in storage than fruit from orchards picked at a later maturity. Fruit size had a minimal effect on all phases of softening when COP and RG were harvested at a early to mid stage of maturity, but at a late stage of maturity smaller fruit softened slower than both medium and large fruit.

Temperature influenced the firmness of apples both physically and physiologically. RG, COP and GS fruit were physically firmer at harvest, and physically softer after storage, when firmness was measured at a fruit temperature of 20°C rather than at 0-3°C. In contrast, firmness of PR was not affected by fruit temperature during measurement, regardless of prior storage duration. The physiological influence of temperature on softening rates differed between cultivars. Softening of RG and COP at different temperatures was described by a modified Arrenhius equation, where rate of softening increased as storage temperature increased from 0 to 22°C, and decreased thereafter as

temperature increased through 35°C. Softening of GS and PR at different temperatures could not be described by a modified Arrenhius equation, as these cultivars softened slowly and at similar rates from 0 to 12°C, and rapid phase softening did not occur at 20 to 35°C. The non-occurrence of rapid phase softening in GS and PR at 20°C was overcome by prior ethylene or cold treatment in GS, but not in PR. Apple cultivars also had different responses to prior temperature treatments, where the softening rate at a given temperature was similar regardless of prior times at 0 to 20°C for both RG and COP, but the longer that GS fruit were stored at 0.5°C the slower the subsequent softening rate was at 20°C. PR was unique in that rapid phase softening did not occur at 20°C, regardless of prior ethylene or cold treatment. It is possible that PR maybe a mutant genotype of apple with reduced capacity for ethylene biosynthesis and action, and hence softening.

Storage of RG and COP in controlled atmospheres (CA) significantly increased the initial slow softening phase and reduced the rate of rapid phase softening when compared to air storage. However for both cultivars, CA was most effective at reducing softening when applied during the initial slow softening phase. Exposure to CA had minimal effects on subsequent softening rates in air at low or shelf life temperatures.

The mechanism by which all these factors influenced softening may be mediated by ethylene, as the initial slow softening phase occurred when internal ethylene concentrations were low (<1.5 $\mu l.l^{-1}$), and the rapid softening phase occurred once IEC's increased from this low basal concentration for all experiments. Thus, it is suggested that the onset of rapid phase softening may be induced by system II ethylene production. However, exceptions to this relationship were observed for GS and PR at 20°C, as the onset of rapid phase softening in GS was delayed relative to the prior cold treatment induced increase in IEC at 20°C, and rapid phase softening was not induced in PR at 20°C despite IEC's being in excess of 100 $\mu l.l^{-1}$. Thus, the fruits sensitivity to ethylene may have a more important role in regulating rapid phase softening than the ethylene concentration *per se* in GS and PR at shelf life temperatures.

Results from temperature studies allowed development of a prototype model (FirmCalc) that describes the influence of temperature on softening of RG and COP through

different phases of postharvest handling. Once harvest maturity, orchard and CA are accounted for in this model, it should be possible to predict softening rates of RG and COP before storage, and therefore provide a tool that can be used commercially to assist with management of the "soft fruit" problem in the marketplace.

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ACC	1-Aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
B	Boron
Ca	Calcium
CA	Controlled atmospheres
CO_2	Carbon dioxide
COP	Cox's Orange Pippin apple cultivar
Cu	Copper
Cs	Caesium
d	Day
DC	Delay between harvest and cooling to 0.5-3°C
DF	Degrees of freedom
DM	Dry matter
DW	Dry weight
$Ea.R^{-1}$	Activation energy • gas constant^{-1} (°K)
f_1	Firmness; fruit stored at 0-3°C and measured without delay at 0-3°C (N)
f_2	Firmness; fruit stored at 0-3°C and measured after 24 hours at 20°C (N)
f_3	Firmness; fruit stored at 0-3°C, transferred to 20°C for 24 hour, and returned to 0-3°C for 24 hour before measurement (N)
$f_{-\infty}$	Initial firmness asymptote for sigmoidal softening equation (N)
$f_{+\infty}$	Final firmness asymptote for sigmoidal softening equation (N)
f_H	Firmness at harvest (N)
Fruc.	Fructose
Δf_{temp}	Physical change in firmness with increasing temperature (N.°C^{-1})
FW	Fresh weight
g	Gram
Gluc.	Glucose
GS	Granny Smith apple cultivar
h	Hour
H1	First harvest date
H2	Second harvest date

H3	Third harvest date
H4	Fourth harvest date
H5	Fifth harvest date
HCl	Hydrochloric acid
$\Delta H.R^{-1}$	Increment of enthalpy • gas constant^{-1} (^{o}K)
hue^{o}	Skin greenness (hue angle)
IEC	Internal ethylene concentration ($\mu l.l^{-1}$)
IW	Temporary period of intermittent warming at 10-20^{o}C during low temperature storage at 0-3^{o}C
K	Potassium
k	Rate of change for sigmoidal softening equation (day^{-1})
k_a	Rate constant for modified Arrenhius equation
kg	Kilograms
l	Litre
LSD	Least significant difference
M	Molarity (mol.l-1)
MCP	1-Methylcyclopropene
Mg	Magnesium
mg	Milligram
min	Minute
ml	Millilitre
mol	Mole
mmol	Millimole
Mn	Manganese
N	Newtons
N_2	Nitrogen
Na	Sodium
NaOH	Sodium hydroxide
nmol	Nanomole
ns	Not significant, P>0.05
NZ	New Zealand
O_2	Oxygen
P	Probability

P	Phosphorus
PG	Polygalacturonase
PME	Pectin methyl esterase
PR	Pacific Rose™ apple cultivar
r	Correlation coefficient
R^2	Coefficient of determination
RA	Regular atmosphere (air)
r_{CO_2}	Respiration rate (nmol.kg.s^{-1})
RG	Royal Gala apple cultivar
RH	Relative humidity (%)
s	Second
SPI	Starch pattern index
Sr	Strontium
$\Delta S.R^{-1}$	Increment of entropy • gas constant^{-1}
Suc.	Sucrose
t	Time (day)
T	Temperature (^{o}C)
TA	Titratable acidity (mmol.l^{-1})
TSS	Total soluble solids (%)
Δt_{temp}	Physical change in tensile strength with increasing temperature (N.^{o}C^{-1})
µl	Microlitre
µmol	Micromole
Zn	Zinc
*	Significant at $P \leq 0.05$
**	Significant at $P \leq 0.01$
***	Significant at $P \leq 0.001$

Chapter 1 Introduction.

The apple industry is one of the largest producers and exporters of fresh produce in New Zealand (HortResearch, 2000). With an annual export value of $404.5 million (f.o.b.) in 2000, apples comprised 24% of total export earnings for New Zealand (NZ) horticulture (HortResearch, 2000). The NZ apple industry has 1,500 growers, 130 packhouses, and a total planted area of 15,500 ha that is predominantly located in the Hawkes Bay (48%) and Nelson (37%) regions (HortResearch, 2000). The remaining 15% of planted area is spread across several other North Island and South Island regions (HortResearch, 2000).

With 61% of the crop being exported in 2000 (HortResearch, 2000), the NZ apple industry is strongly dependent on an increasingly competitive global market in which the NZ industry has been rated most competitive apple producer for the last five years (Anon, 2001). However, markets are imposing increasingly stringent quality standards for apples, making it difficult for producers to meet market requirements. In 1997, it was estimated that failure to comply with market standards cost the NZ industry $64 million in that year alone (Anon, 1997). Thus, one of the key issues identified in an "Industry Strategic Research and Development Report" was the need to be able to predict quality in the market place after prolonged periods of storage and transportation (Anon, 1996).

One such quality problem, and the focus of this thesis, is fruit softening. In particular, 'Royal Gala' and 'Cox's Orange Pippin' are early maturing cultivars in New Zealand that soften rapidly after harvest, making it difficult to meet market specifications for firmness. Both of these cultivars are important export earners for NZ, with 'Royal Gala' comprising 35%, and 'Cox's Orange Pippin' comprising 5% of total export apple earnings in 2000 (HortResearch, 2000). Furthermore, 'Royal Gala' was one of two cultivars that had the highest export earning for NZ apples in 2000 (HortResearch, 2000). Both cultivars are also important in that they are often the first of several NZ cultivars to arrive in the market place, and poor quality in these cultivars can result in bad market signals and a damaged reputation for those cultivars to follow. Before informed commercial decisions can be made to manage or eliminate this softening problem, detailed knowledge on the biology of softening, and the relative influences of

pre- and postharvest factors on softening is required. This review will outline current knowledge on these aspects of apple softening, as well as highlighting knowledge deficiencies, and identifying experiments that are required to improve knowledge on factors that influence the postharvest softening rates of apple fruits.

1.1 Why is apple softening an important issue?

Texture is an important component of quality in most fruits, including apples (Liu and King, 1978). Consumers generally demand apples that are crisp and crunchy, and not dry or mealy. Unfortunately, apples tend to have optimal textural quality during the early stages of ripening, and progressively lose this textural quality as the fruit ripen regardless of whether this occurs at ambient temperatures or during long-term low temperature storage. Thus, flesh firmness (section 1.2) is often used by apple producers to assign quality grades before storage, and by markets as a criterion for accepting or rejecting shipments of fruit after storage and transportation. From the producers' perspective, failure to meet firmness standards can result in shipment rejections, a damaged reputation as a supplier of top quality apples, and consequently reduced returns. From the consumers and retailers perspective, these grades are important to ensure that apples are available in markets with sufficient shelf life, as well as to ensure some year-round consistency in textural quality. Commercial examples of firmness standards used for apples and other fruits are reviewed in Harker et al. (1997a).

The relation of flesh firmness (section 1.2) to sensory perception of ripeness and texture is of both commercial and scientific interest, as this test is used readily to help assess fruit quality before and after storage. For several apple cultivars firmness discriminated between batches of ripe and overripe apples, although discrimination was poor when individual fruit were examined (Blanpied and Blak, 1977). In a study using untrained consumers, 'McIntosh' apples with higher firmness were rated as more crisp and harder than fruit with lower firmness readings (Fig. 1-1) (Liu and King, 1978). Interestingly, some of these consumers rated the fruit as definitely too hard when firmness readings were 6.3 kg (61.8 N) or higher, and perception of fruit being too soft differed for people of different ages (Liu and King, 1978). Wills et al. (1980) found moderate ($r = 0.67$) to strong ($r = 0.83$) associations between flesh firmness and sensory perception of

crispness and mealiness for 'Delicious'. Similarly, strong associations (r = 0.80-0.83) between flesh firmness and ripeness scores from trained industry inspectors occurred for both freshly harvested (Abbott, 1994) and stored (Abbott et al., 1992) 'Delicious' apples. Coefficients of determination between penetrometer firmness and individual sensory attributes (crispness, hardness and toughness) of texture were strong (R^2 = 0.83-0.88) for 'Golden Delicious' and 'Rome' cultivars, moderate (R^2 = 0.52-0.63) for 'Miller Sturdy Spur', and low (R^2 = 0.33-0.50) for 'Redspur' and 'York Imperial' (Abbott et al., 1984). High coefficients of determination for 'Rome' apples also occurred between penetrometer firmness and sensory perception of mealiness (R^2 = 0.77) and juiciness (R^2 = 0.89) (Abbott et al., 1984). Thus, the ability of flesh firmness to predict sensory responses in apples varies between cultivars (Abbott et al., 1984).

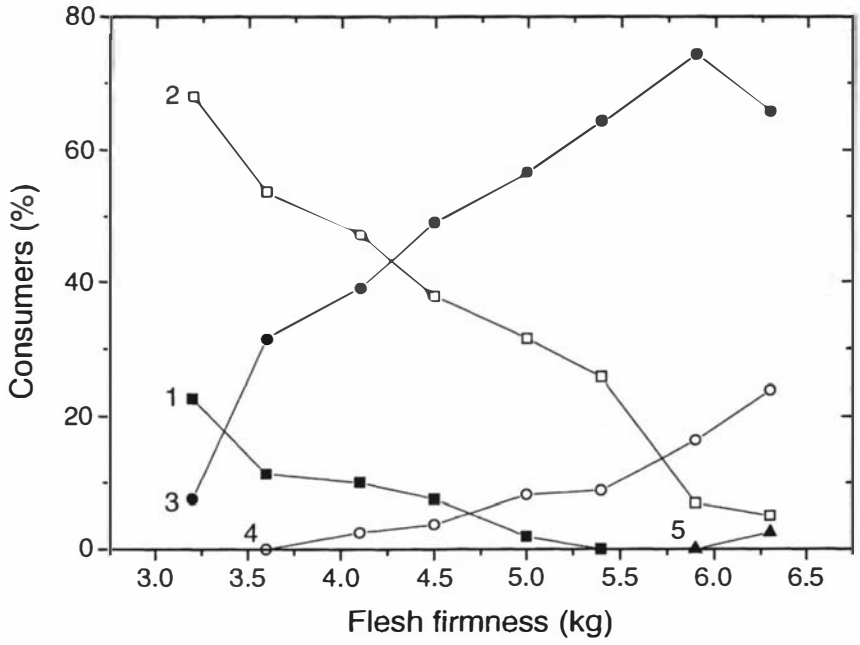

Fig. 1-1 Sensory ratings from 1034 untrained consumers for 'McIntosh' apples with different flesh firmness readings (adapted from Liu and King, 1978). Ratings were definitely too soft (1), slightly too soft (2), crisp and neither too hard nor too soft (3), slightly too hard (4) and definitely too hard (5).

Despite firmness being an important predictor of ripeness and textural quality in apples, it is surprising that the softening curve for harvested apples has not been accurately characterised. Most fruits are considered to have biphasic and/or triphasic softening

curves (MacRae et al., 1990; Harker et al., 1997a). Fruits with a triphasic softening curve have an initial slow softening phase or lag phase, followed by a phase of more rapid softening, and then a final slow softening phase until the tissue collapses from rots and disorders associated with late stages of senescence. Fruits with biphasic softening curves often have no discernable initial slow softening phase and have a rapid softening phase immediately after harvest, followed by a final slow softening phase (Harker et al., 1997a). The softening curve for kiwifruit has been well characterised, and is considered triphasic for fruit harvested at an early maturity, and a biphasic for fruit harvested more mature (MacRae et al., 1989; 1990). For other fruits such as nectarines (King et al., 1989) and pears (Bourne, 1968), the softening curves appeared biphasic. In most apple studies, firmness was usually only measured 1-5 times during storage, making it difficult to accurately ascertain the shape of the softening curve for this fruit. As discussed in following sections, this lack of knowledge on the softening curve for apples makes it difficult to:

- associate changes in softening rates with changes in concentrations of regulatory compounds (such as ethylene; section 1.4.1), and with changes in activities of those enzymes proposed to have roles in mediating tissue softening (such as polygalacturonase; section 1.3.2)
- compare and contrast the softening rates of harvested apples with apples attached to the tree
- determine if different pre-, at- and post-harvest factors affect different parts of the softening curve (section 1.5)
- accurately quantify the relative influence of different pre- and postharvest factors on the softening rates of apples through storage (section 1.5).

1.2 How is apple firmness measured?

Several instrument-based tests have been developed to objectively measure the textural quality of fruits, and such techniques have been reviewed by Harker et al. (1997a). The most common measurement used to assess apple texture is the puncture test, which is often performed with a penetrometer (Harker et al., 1997a). The penetrometer measures the maximum force required to puncture pared fruit tissue with a cylindrical probe to a constant depth, with the results expressed as firmness, fruit firmness, flesh firmness or

fruit pressure. Throughout this review firmness will be used in this sense, and not in the engineering sense where firmness is regarded as the slope of the force-deformation curve (Fig. 1-2) before the bioyield or rupture point (Abbott, 1999).

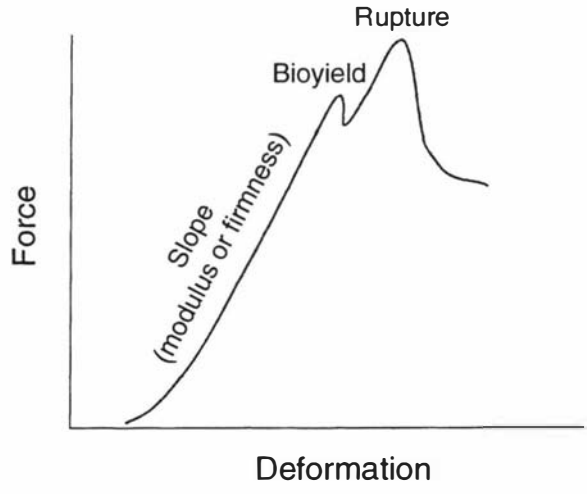

Fig. 1-2 Typical force deformation curves for products with a bioyield point (Adapted from Mohsenin and Mittal (1977) and Abbott (1999)).

Apples typically have three types of force-deformation curves depending on the stage of ripeness (Bourne, 1965) (Fig. 1-3). For freshly harvested apples, the bioyield point generally occurred well before the maximum force recorded by the penetrometer, while the bioyield point occurred immediately prior to the maximum penetrometer force for apples stored at $-1^{\circ}C$, and occurred simultaneously with the maximum penetrometer force for riper apples stored at $15^{\circ}C$ (Bourne, 1965). It should be noted that some commonly used puncture testing devices, such as the electronic pressure tester, measure the force at the bioyield point rather than the maximum force as in most hand-held penetrometers (Lehman-Salada, 1996).

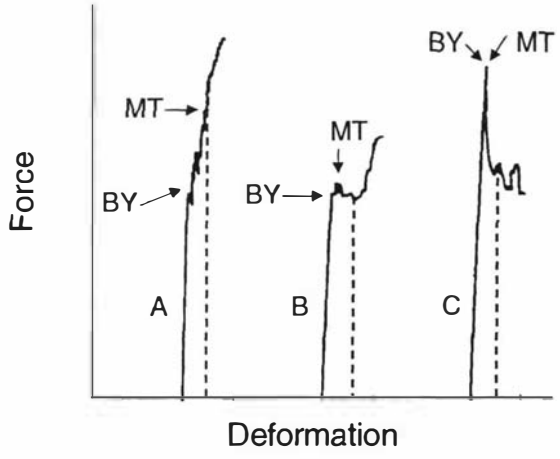

Fig. 1-3 Force deformation curves from puncture testing of apples that were measured immediately after harvest (A), after storage at –1°C (B) and after storage at 15°C (C) (Bourne, 1965). Bioyield (BY) and force recorded by a Magness-Taylor (MT) penetrometer are shown. The dashed line denotes the distance when the penetrometer probe reached a puncture depth of 7.9 mm.

Many types of penetrometer have been developed since the inception of the Magness-Taylor penetrometer in 1925. These include the hand-held Ballauf, Chatillon and Effegi penetrometers; the characteristics of which have been reviewed by Bourne (1982b). Although these instruments were designed to be hand-held, it is often recommended that they be mounted in a drill press to improve operator control and hence reduce variation in firmness readings (Blanpied et al., 1978). However, despite these attempts to improve the control of hand-held penetrometers, firmness readings are still prone to operator variation (Harker et al., 1996). To overcome this problem mechanised devices have been utilised that drive the probe into the fruit (i.e. Instron and motorised presses), or have been developed with speed control devices to ensure that tests are performed at acceptable and consistent speeds (i.e. electronic pressure tester and fruit quality tester). Unfortunately the different testers tend to generate different firmness readings for both firm and soft apples, making it difficult to compare the firmness readings from different testers (Abbott et al., 1976; Voisey, 1977; Harker et al., 1996; Lehman-Salada, 1996; DeLong et al., 2000). Thus, to accurately compare firmness between treatments and to follow softening in storage, it is recommended that a single operator be used to measure firmness when utilising a hand-held device (Harker et al., 1996), and that the same type of device is used throughout each experiment (Abbott et al., 1976).

One of the more obvious disadvantages of the puncture test is that this form of test is destructive. The ability to evaluate firmness nondestructively would enable measurement of individual fruit through time, removing the problem of fruit variation within batches. Furthermore, nondestructive firmness testers could be used on-line in packhouses to segregate fruits with different firmness before packing, removing the problem of firmness variation within batches of fruit before storage. Further information on progress towards development of nondestructive devices for measuring texture can be obtained in reviews by Abbott et al. (1997), Harker et al. (1997a), and Abbott (1999).

Despite the disadvantages of using hand-held puncture testers to measure apple firmness, these devices have the practical advantages that they are relatively inexpensive, readily available, and extremely mobile. These devices are also simple to use when the operator is trained appropriately. It is likely that hand-held penetrometers will continue to be the preferred method of measuring firmness in apple industries worldwide, until new devices can be developed that have similar practical advantages.

1.3 What is the cellular basis of firmness and how do apples soften?

Most fruits can be classified as having either a melting or a non-melting texture once ripe, with apples having non-melting texture (Bourne, 1979). This non-melting or partial softening trait of apples was associated with a "plateau phase", where there became a point in storage when firmness failed to decrease any further (Blanpied, 1975). Non-melting fruits are generally eaten when firm and have only softened slightly, while melting type fruits are generally eaten soft after considerable softening has occurred. Unfortunately, both types of fruit tend to soften past the optimum texture for eating quality, and enter an over-ripe state. Thus, considerable research has been undertaken to understand the physiological and physical mechanisms involved in fruit softening for both types of fruit. Studied mechanisms include cell shape, cell size, cell packing, overall fruit anatomy, cell wall chemistry, membrane permeability, and cell turgor. These characteristics will be reviewed for apples, with references made where appropriate to others fruits.

1.3.1 Fruit anatomy and cell packing.

The anatomical characteristics of apple fruit during growth, maturation and senescence have been well characterised for several cultivars, including 'Cox's Orange Pippin' (Tetley, 1930; 1931; Smith, 1940; Tukey and Young, 1942; Smith, 1950; Bain and Robertson, 1951; Robertson and Turner, 1951; Reeve, 1953; Denne, 1963; Pratt, 1988). 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 (Fig. 1-4) (Tukey and Young, 1942). Of these tissues, the parenchymous cortex tissue is most readily consumed, with tissues in the core region largely avoided. Thus, destructive assessments of texture are predominantly undertaken on tissue excised from the cortex, or on intact cortical tissue with the epidermal and hypodermal cells layer removed.

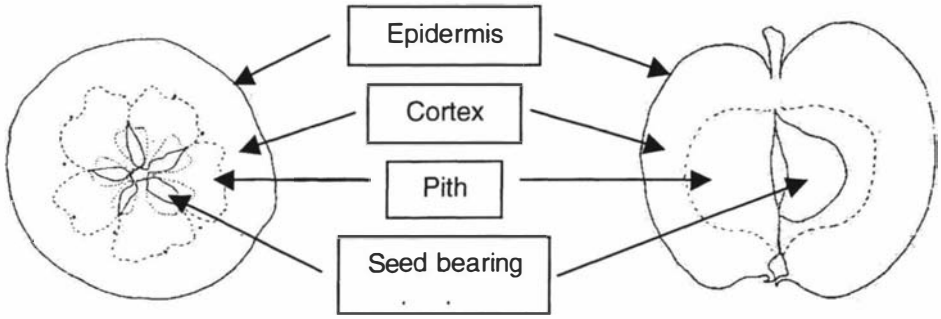

Fig. 1-4 Transverse (left) and longitudinal (right) sections of mature apple fruit (Tukey and Young, 1942).

The hypodermis in apple fruit is located immediately below the epidermis, and contains a thin layer of cells that are small (~50 μm) and radially flattened (Fig. 1-5) (Kahn and Vincent, 1990). Cell size then gradually increases to a maximum of 200-300 μm once 5-10 mm in from the epidermis (Kahn and Vincent, 1990). Cells in the outer cortex were more spherical in shape, while those in the inner cortex were radially elongated, and joined end-to-end to form radial columns that extend from the inner to the outer cortex (Kahn and Vincent, 1990). The amount of cellular material (cell wall and cell

contents) and tissue density were also greater in the inner cortex than in the outer cortex (Kahn and Vincent, 1990).

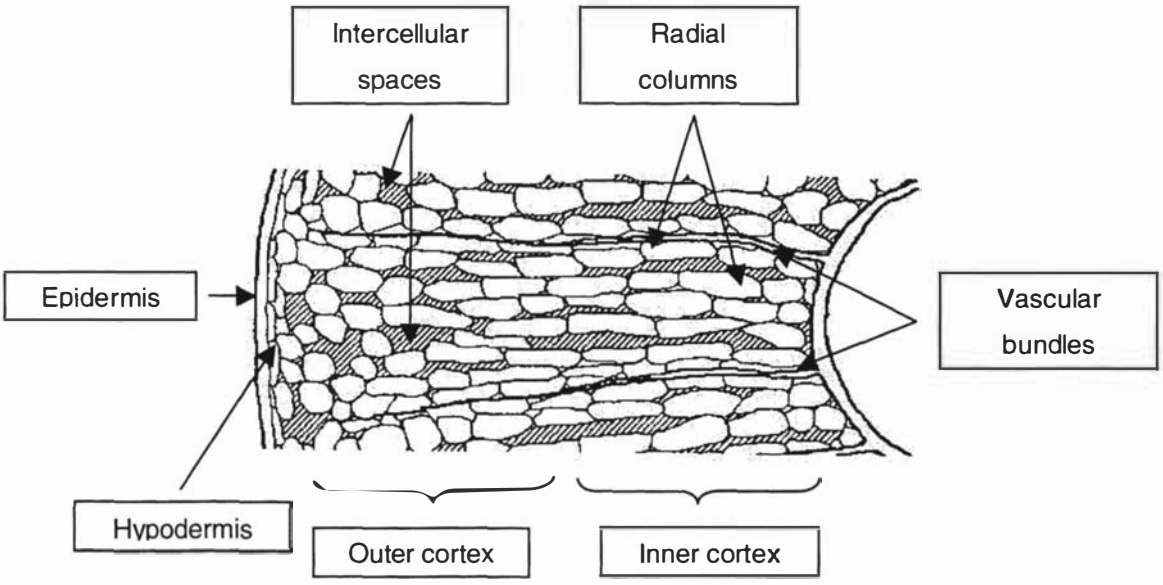

Fig. 1-5 Schematic radial diagram of cell packing in the epidermis, hypodermis and cortex tissues of apple fruit (Kahn and Vincent, 1990).

Radially elongated intercellular spaces also occurred between the radial columns of cells (Reeve, 1953), with intercellular spaces being up to 3 mm long and 100-200 μm wide (Kahn and Vincent, 1990). Like the cells in the outer cortex, the intercellular spaces were smaller and more spherical in shape than intercellular spaces in the inner cortex (Fig. 1-5) (Kahn and Vincent, 1990). The volume of intercellular spaces increased from about 16-17% at harvest to 17-21% during ripening (Harker and Hallett, 1992; Tu et al., 1996), with intercellular space volumes being as high as 27% in some ripe cultivars (Reeve, 1953).

A number of studies have concluded that apple softening and mealiness are mediated by loss of cell to cell adhesion. This was evident from microscopic observations of tissue fracture faces from tensile tests, where failure of soft fruit occurred between cells, whereas failure of firm fruit occurred through cells (Harker and Hallett, 1992; Tu et al., 1996; 1997; Harker et al., 1997b; De Smedt et al., 1998). Furthermore, microscopic

examination of intact tissue blocks showed that tissue from mealy or 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 that earlier maturing apple cultivars have larger cells, larger intercellular spaces, and are less dense than later maturing cultivars (Vincent, 1989; Kahn and Vincent, 1990). This phenomenon 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 as having weaker tissue than fruits with smaller cells and less intercellular spaces (Harker et al., 1997a).

1.3.2 Cell walls.

The cell wall is a complex structure important for imposing cell shape and rigidity in many plant tissues. Primary cell walls in plants are considered a network of cellulose microfibrils embedded in a matrix of hemicellulose and pectin (Rose and Bennett, 1999; Cosgrove, 2001). Pectic polymers are the main constituents of the middle lamella, a region important for maintaining cell to cell adhesion and cell packing in fruit tissues (Bartley and Knee, 1982). The exact nature by which these cell wall components bind or interact with each other to form a rigid structure is not known, although several models have been proposed and are reviewed by Cosgrove (2001).

Electron microscopy studies showed that apples had extensive disruption of the middle lamella once ripe (Ben-Arie et al., 1979). Treatment of unripe apple discs with polygalacturonase (PG) caused similar disruption of the middle lamella to that observed in ripe apple tissue (Ben-Arie et al., 1979). These results suggested that disruption of the pectin-rich middle lamella played an important role in softening of apple fruit.

Apple pectin has been characterised as having "hairy regions" consisting of highly branched arabinogalacturonan side-chains on a backbone of rhamnogalacturonan, and "smooth regions" consisting of highly esterified (70-80%) homogalacturonan (de Vries et al., 1986). The water-soluble fraction of pectin increased during apple ripening (Knee, 1973; Bartley, 1974; 1977; Yoshioka et al., 1992), while galactose residues

decreased markedly, and arabinose residues from the cell wall decreased slightly during softening (Knee, 1973; Bartley, 1974; 1977). While the proportion of water-soluble pectin increased, the proportions of chelator, carbonate and HCl fractions of pectin, and total pectin content, all decreased during ripening (Knee, 1978a; Yoshioka et al., 1992). Furthermore, the proportion of covalently bound and cellulose bound pectin decreased, and the proportion of pectin ionically associated with hemicellulose remained constant during ripening (Siddiqui et al., 1996). Yoshioka et al. (1992) found no depolymerisation of any pectin fraction during ripening, while Knee (1978b) found that the cell wall bound fraction was slightly depolymerised.

Despite extensive chemical analysis of apple pectin during ripening, it is still not clear what roles pectin solubilisation and loss of galactose residues play in the softening of apple fruit. A recent study found that pectin solubilisation was associated with cell wall swelling and softening in several fruit species (Redgwell et al., 1997b). Relative to melting type fruits, apples and other non-melting fruits had a small degree of pectin solubilisation and had minimal cell wall swelling (Redgwell et al., 1997b). Redgwell et al. (1997a) presented evidence that the "hairy regions" of pectin were probably bound or closely associated with cellulose, and were the predominant source for loss of galactose and arabinose residues during ripening. In contrast, the "smooth regions" appeared less intimately associated with cellulose, and maybe the components of pectin that are more susceptible to solubilisation during ripening (Redgwell et al., 1997a). While loss of galactose may modify the physicochemical properties of pectin, there is no evidence to date that suggests that loss of galactose is required for pectin solubilisation or softening to occur (Redgwell et al., 1997a). However, there is some correlative evidence that the extent of the release of terminal arabinose and increase in terminal galactose residues from pectin during softening may influence cultivar sensitivity to development of mealiness during storage (Nara et al., 2001).

Despite a number of cell wall modifying enzymes being found in apples (Table 1-1), the role that each enzyme plays in coordinating in vivo pectin solubilisation and loss of galactose and arabinose residues during softening of apples is not known. The enzyme originally considered responsible for pectin solubilisation and therefore softening in most fruits was PG, with activities of both exo- and endo-PG detected in ripening apples

(Bartley, 1978; Wu et al., 1993; Atkinson, 1994; Atkinson et al., 1998). However, transgenic experiments with a slow softening, low PG activity tomato ripening mutant (ripening inhibitor) showed that PG complementation could not restore the softening rate to wild-type rates (Giovannoni et al., 1989). Likewise, reduced PG expression could not reduce softening in wild-type tomatoes (Smith et al., 1990). These experiments also showed that PG was not required for pectin solubilisation, but was required for pectin depolymerisation (Giovannoni et al., 1989; Smith et al., 1990). Thus, the potential role of exo- and endo-PG in apple softening is not clear.

A potential role for pectin methyl esterase (PME) in apple softening is also unclear, especially when some studies report increased esterification of water-soluble pectin during ripening (Knee, 1978a; Yoshioka et al., 1992), while others reported decreased esterification for the same fraction (Klein et al., 1995; Ben-Shalom et al., 1996). However, these studies did agree in that the chelator soluble pectin fraction became less esterified during ripening. Reduced esterification can be attributed to increased PME activity during softening (Klein et al., 1995). The role of PME in softening, if any, is likely to be indirect, as tomato softening mutants (*nor* and *nr*) contained 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). Furthermore, it has been found that highly methoxylated pectin regions were preferentially lost from the chelator and HCl soluble pectin fractions during apple ripening (Yoshioka et al., 1992). It was suggested that deesterification of highly methoxylated pectin regions may result in swelling and solubilisation of these regions of pectin (Yoshioka et al., 1992).

Table 1-1 Summary of cell wall modifying enzymes identified in ripening apple 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[1]	Increased[2]	Bartley, 1974; 1977; Wallner, 1978; Berard et al., 1982; Dick et al., 1990; Ross et al., 1994; 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[3]	Decreased	Abeles and Takeda, 1990; Abeles and Biles, 1991
Expansins	Induce stress relaxation and extension of cell walls	Yet to be measured in apple	Brummell et al., 1999a; Rose and Bennett, 1999; Cosgrove, 2001

[1]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).
[2]This is isozyme dependent, as one isozyme increased, while three isozymes decreased, during ripening in apples (Yoshioka et al., 1995).
[3]In vivo substrates of endo-glucanases are yet to be confirmed (Rose and Bennett, 1999).

It is possible that glycosidases, such as β-galactosidase, could facilitate both pectin solubilisation and removal of galactose and arabinose residues from arabinogalactan side-chains during apple softening (Yoshioka et al., 1995). β-galactosidase activity has been found to increase markedly in several apples cultivars during storage at different temperatures (Bartley, 1974; Wallner, 1978) and atmospheres (Bartley, 1977; Berard et al., 1982). However, the level of β-galactosidase activity did not relate to softening rate differences between cultivars, as 'York Imperial' apples had higher β-galactosidase activity at harvest and through storage, and softened more slowly in storage, than 'Golden Delicious', 'Lodi' and 'McIntosh' apple cultivars that had lower β-galactosidase activity (Wallner, 1978). Ross et al. (1994) showed that β-galactosidase was active against native cell wall extracts from apple, although the level of galactose released during incubation was significantly less than that measured during fruit ripening. The role of β-galactosidase in apple softening is further complicated by the finding of four isozymes in apples (Yoshioka et al., 1995), and that this enzyme may have α-L-arabinopyranosidase and β-D-fucosidase activity (Dick et al., 1990). However, only one isozyme was shown to have increased activity during apple ripening (Yoshioka et al., 1995). Another glycoside, α-L-arabinofuranosidase, has also been shown to have increased activity during apple ripening, which may facilitate the release of arabinose residues during softening (Yoshioka et al., 1995).

Rhamnogalacturonase activity has only recently been found in apples (Gross et al., 1995), but changes in activity during softening have not been characterised. More research is required to determine if this enzyme can facilitate solubilisation of pectin and therefore softening of apples during ripening. It should also be considered that enzymes other than those in Table 1-1 could be responsible for mediating softening of apple fruit. Without transgenic experiments such as those performed for PG (Giovannoni et al., 1989; Smith et al., 1990), it is difficult to establish roles for the different cell wall modifying enzymes in the softening of apple fruit.

The non-cellulose and cellulose glucose concentrations in the cell wall were relatively constant during ripening of apples, suggesting that little cellulose and hemicellulose degradation occurred (Bartley, 1976). Furthermore, the molecular weight of xyloglucan

(predominant hemicellulose polymer in fruits) did not change during apple ripening (Percy et al., 1997), and activities of xyloglucan endotransglycosylase (XET) (Percy et al., 1996) and cellulase (Abeles and Takeda, 1990; Abeles and Biles, 1991) both decreased during softening. In contrast, Siddiqui et al. (1996) found that hemicellulose proportion of the cell wall decreased by 5%, and the cellulose proportion decreased by 0.5%, after 6 months of storage. Melting type fruits such as kiwifruit had both increased XET activity (Percy et al., 1996) and extensive xyloglucan depolymerisation during early stages of softening (MacRae and Redgwell, 1992). Depolymerisation of xyloglucan in the early stages of softening has also been reported for other melting fruits such as melon (Rose et al., 1998), tomato (Maclachlan and Brady, 1994) and avocado (O'Donoghue and Huber, 1992). XET, expansins, endo-glucanases, and glycosidases (Table 1-1) have all been implicated as having roles in depolymerisation of xyloglucan and therefore softening in melting type fruits (Brummell et al., 1999a, b; Rose and Bennett, 1999). It is possible that minimal depolymerisation of hemicellulose during apple ripening may impose the non-melting softening trait of this fruit.

1.3.3 Cell membranes.

Cell membranes provide important barriers to the movement of compounds within cells, and between the symplast and apoplast. Membranes are considered a fluid bilayer of phospholipids, containing sterols and proteins (Marangoni et al., 1996). Changes in membrane properties during ripening and senescence have been studied in a number of plant tissues, the details of which have been reviewed by Marangoni et al. (1996) and Thompson et al. (1997). Despite postharvest membranes changes being quantified in apples in relation to ripening (Lurie and Ben-Arie, 1983; Bartley, 1985; Lurie et al., 1987), no information appears to be available relating these changes to the softening of apples.

The main physical changes in membranes that occurred during apple ripening at low temperatures (0°C) were a decrease in leakiness and microviscosity (Lurie et al., 1987). In contrast, both membrane leakiness and microviscosity increased during ripening at 20°C, with the largest increase occurring between mid-climacteric and climacteric stages of ripening (Lurie and Ben-Arie, 1983). It has been suggested that the decrease in

leakiness at 0°C was a form of adaptation to low temperatures (Lurie et al., 1987), while the increase in leakiness at 20°C may be due to irreversible loss of membrane functionality through ripening and senescence. Information relating membrane chemistry to physical changes in membranes during apple ripening is given in Lurie and Ben-Arie (1983), Bartley (1985), and Lurie et al. (1987).

It has been suggested that membranes 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 (i.e. calcium)
- influencing release of water into the apoplast for cell wall swelling, and therefore perception of juiciness when cell to cell adhesion is low (Harker et al., 1997a).

Apples typically do not undergo cell wall swelling (Redgwell et al., 1997b), nor is there an increased sensory perception of juiciness (Plocharski and Konopacka, 1999) during softening. However, the other membrane roles identified above may influence apple softening.

1.3.4 Calcium.

Calcium (Ca) has a strong influence on the textural quality of most fruits (Poovaiah et al., 1988). Thus, extensive research has been undertaken to determine the effect of Ca supplementation before and after harvest on softening. In addition, considerable research has been undertaken to determine the physical and physiological mechanisms by which Ca influences the cell walls and cell membranes, and hence softening.

Apples were firmer after storage when Ca was applied to fruit as a preharvest spray (Watkins et al., 1989; Raese and Drake, 1993), or postharvest dip (Mason et al., 1974). Puncture tested Ca treated apples had a force-deformation curve with a steeper initial slope than non-treated apples, suggesting that supplementary Ca treatment increased the rigidity of apple tissue (Sams et al., 1993). Furthermore, the effect of Ca dips on

different textural attributes was more pronounced than the effect of time in storage, which led to the suggestion that Ca treatment induced a different pattern of texture change to that observed in non-dipped fruit (Abbott 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 from Stow (1993) could have been interpreted differently, in that Ca supplementation physically increased the firmness of the tissue, which then softened at a similar rate to control fruit. In other studies, Ca treatment reduced softening, and the associated increase in soluble pectin and decrease in arabinose and galactose residues from the wall, relative to control fruit (Sams and Conway, 1984; Glenn and Poovaiah, 1990). Postharvest Ca treatment also reduced ethylene production (Sams and Conway, 1984). Thus, it appears that Ca influences the texture of apples by reducing ripening associated softening rates and by physically increasing tissue rigidity.

The ability of Ca to reduce ethylene production and improve firmness retention in apples was also partially achieved by application of other divalent ions such as magnesium (Mg) and strontium (Sr) (Conway and Sams, 1987). However, Ca was the most effective ion at reducing softening and ethylene production, especially when higher concentrations were used (Conway and Sams, 1987). Similar results were also seen when using a tensile test in apples, with Ca along with Sr and barium being the most effective ions at increasing tissue strength, and Mg, cerium, lanthanum and samarium being less effective (Stow, 1989a).

Calcium treatment of apple fruit increased both cell wall bound and soluble Ca concentrations in the tissue (Abbott et al., 1989; Saftner et al., 1998). Furthermore, the cell wall bound Ca component became saturated, while the soluble Ca component continued to increase as the concentration of Ca treatment increased, suggesting a limited number of binding sites for Ca in the cell wall (Saftner et al., 1998). Interestingly, the saturation concentration for Ca binding in the cell wall increased during storage, which suggests increased availability of binding sites during ripening (Saftner et al., 1998). These binding sites have been proposed to be non-esterified galacturonic acid residues in pectin, and that Ca may increase tissue rigidity by cross-linking pectin chains at these sites (Grant et al., 1973; Conway et al., 1993). This

hypothesis was strengthened following the finding that Ca treatment increased the amount of Ca bound to pectin, but did not influence the amount of Ca bound to hemicellulose or cellulose (Siddiqui and Bangerth, 1996).

The most compelling evidence to date that Ca improves the textural quality of apples by strengthening pectin was observed in microscopy studies, as Ca treated fruit had less disruption and degradation of the pectin-rich middle lamella, and had more cell to cell contact than non-treated fruit (Glenn and Poovaiah, 1990; Siddiqui and Bangerth, 1996). Furthermore, microscopic examination of fracture faces from tensile tests of stored apples showed that Ca treated tissue were fractured across and through cells, while tissue from non-treated apples were separated by loss of cell to cell adhesion (Glenn and Poovaiah, 1990).

Ca treatment 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. Picchioni et al. (1995) suggested that Ca infiltration affects membrane functionality by delaying galactolipid degradation and increasing the level of sterol conjugation. Microscopic observation of stored apple tissue suggested that fruit with low Ca had greater membrane disorganisation than fruit with higher Ca concentrations (Fuller, 1980).

1.3.5 Cell turgor and water loss.

Water status is an important determinant of fruit texture, as water status physically influences texture through cell turgor (Harker et al., 1997a) and physiologically influences texture by affecting ripening rates (Littmann, 1972). The relative importance of physical and ripening associated changes in firmness from changes in water status is currently not known.

The physical influence of cell turgor on fruit texture is often difficult to quantify, as cell turgor is notoriously difficult to measure (Harker et al., 1997a). 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 (Fig. 1-6) (Lin and Pitt, 1986). The mode of tissue failure was dependent on cell turgor, as tissue under high turgor failed by cell wall rupture, while tissue under low turgor failed by cell separation (Lin and Pitt, 1986). In contrast, the mode of failure for pear tissue during a tensile test was not influenced by cell turgor (De Belie et al., 2000). However, cell turgor did influence the tensile failure force in unripe pears when tissue failure occurred by cell wall rupture, but not when tissue failure occurred by cell separation as in riper pears (De Belie et al., 2000). Further research is required to determine if the influence of cell turgor on textural characteristics of apple fruit is also influenced by stage of ripening.

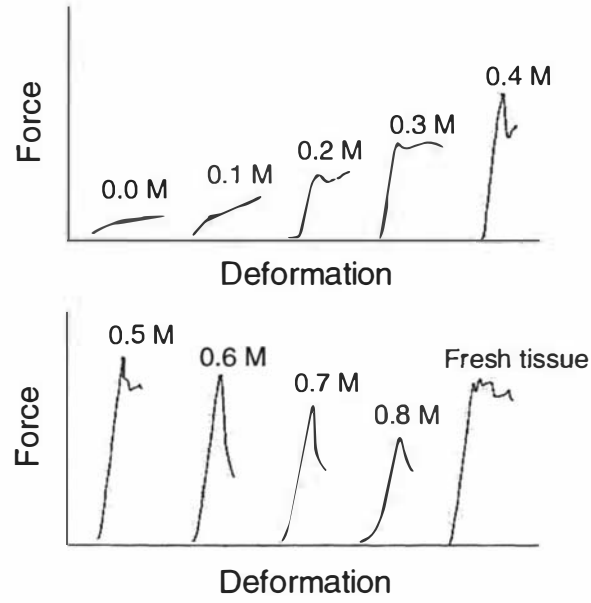

Fig. 1-6 Force-deformation curve from compression of 'Ida Red' apple tissues incubated in different concentrations of mannitol (Lin and Pitt, 1986).

Measurement of cell turgor has provided some correlative evidence that cell turgor may have an important role in apple softening, as cell turgor declined during the softening of four apple cultivars (Tong et al., 1999). Furthermore, there was positive association between cell turgor and firmness of fruit after 6 months at 0-2°C for cultivars with different softening rates (Tong et al., 1999). However, cell turgor alone could not

explain all the variation in poststorage firmness between cultivars, and it was concluded that some other cellular factor also influences softening of apples (Tong et al., 1999).

Studies using different storage humidities have also provided some 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 and Knee, 1988). A sensory panel also rated the fruit that lost more weight as being firmer, tougher and less mealy than fruit that lost less weight (Hatfield and Knee, 1988). Poststorage firmness of 'Spartan' apples was inconsistently affected by storage humidity, with fruit stored in 80% relative humidity (RH) being about 0.6 kg firmer than fruit stored in 92-94% RH in one season, while poststorage firmness was not influenced by storage RH in another season (Porritt and Meheriuk, 1973). In contrast to these studies, the poststorage firmness of 'McIntosh' apples was progressively reduced as the storage RH decreased from 96-100% to 75% (Blanpied, 1981; Lidster, 1990).

The mechanism by which RH influenced poststorage firmness is not clear. Hatfield and Knee (1988) found that high-weight loss treatment reduced the increase in intercellular airspace volume during storage, and consequently suggested that these fruit had greater cell to cell contact and therefore greater firmness. Microscopy studies showed that water loss influenced the shape of apple cells, where cells from fruit that lost less water were rounder than cells from fruit with more water loss (Bolin and Huxsoll, 1987), reducing cell to cell contact. There is some evidence that storage RH influenced cell to cell adhesion, as the tensile strength of apple tissue after 10-15 days at 20°C 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 these tensile tests indicated that fruit stored at high RH tended to separate between cells, rather than across or through cells as in fresh tissue (Tu et al., 2000).

Water loss enhanced the ripening rates of several fruits, including banana, avocado and pear fruit (Littmann, 1972). However, limited information is available for apple fruit, especially with regards 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 from the reviewed studies that both direction and extent of the effect of water loss on apple softening is still not clear, making it difficult to predict the textural consequences of using postharvest technologies that influence water loss.

1.4 What regulates softening?

1.4.1 Ethylene.

In climacteric fruits such as apples, ethylene is often regarded as the ripening hormone. Considerable research has been undertaken to understand the biochemical pathways involved in ethylene biosynthesis, and how plant cells perceive and act on ethylene to initiate ripening processes such as softening.

1.4.1.1 Ethylene biosynthesis and action.

The biosynthetic pathway for ethylene has three main steps: 1) generation of S-adenosyl-L-methionine (SAM) from 5'-methylthioadenosine (MTA) via the methionine salvage pathway (Yang cycle); 2) formation of 1-aminocyclopropane-1-carboxylic acid (ACC) and MTA from SAM by ACC synthase; and 3) formation of ethylene from ACC by ACC oxidase (Yang and Hoffman, 1984). SAM is also a precursor for polyamine (section 1.4.2) synthesis (Miyazaki and Yang, 1987), and ACC can be conjugated to form *N*-malonyl-ACC (Liu et al., 1985). Detailed information on the genetic control and biochemistry of ethylene biosynthesis can be found in reviews by Yang and Hoffman (1984), Zarembinski and Theologis (1994), and Lelievre et al. (1997).

Pathways involved in ethylene perception and signal transduction are complex, and yet to be fully resolved. Five ethylene receptors (ETR family) have been identified in Arabidopsis and tomato, with physiological roles for all receptors yet to be determined (Klee et al., 1999; Tieman et al., 2000). Evidence to date indicates that the ethylene receptors are membrane bound, have homology with the two component histidine-kinase receptors found in bacteria (Bleecker et al., 1999), and require oxygen to bind ethylene (Burg and Burg, 1967). Components identified in the signal transduction pathway include: CTR1 which has homology to serine/threonine protein kinases that

induce MAP (mitogen-activated) kinase cascades in eukaryotes; followed by EIN2 which is related to a family of metal transporters in eukaryotes; and finally EIN3 which represents a family of transcription factors unique to plants (Bleecker et al., 1999).

During growth and development, preclimacteric fruits often have a low basal level of ethylene production (system I), which then increases autocatalytically (system II) during the climacteric in association with ripening and increased respiration rates (McMurchie et al., 1972; Oetiker and Yang, 1995). The ability for fruits to develop system II ethylene production depends on the physiological maturity at harvest, as fruits harvested too immature often fail to gain the competence to induce system II ethylene production and consequently ripen abnormally (Pech et al., 1994).

The mechanism by which fruits gain competency to initiate system II ethylene production and ripening has not been fully elucidated. It is generally considered that the formation of ACC by ACC synthase is the rate-limiting step for ethylene biosynthesis, although ACC synthase and ACC oxidase are both induced during ripening and both have been implicated as having important regulatory roles in ethylene biosynthesis (Oetiker and Yang, 1995). Current models for explaining the transition from system I to system II ethylene production are based on tightly controlled changes in expression of specific isozymes of ACC synthase during development and ripening (Barry et al., 2000), and changes in expression of specific ethylene receptors (Payton et al., 1996).

1.4.1.2 Role of ethylene in apple softening.

The biosynthesis and action of ethylene has been proposed to have an important role in regulating the softening of apples. Approaches used to investigate the role of ethylene in apple softening have included the softening response to exogenous ethylene concentration, preharvest or postharvest application of inhibitors of ethylene biosynthesis and action, and relating firmness with endogenous changes in ethylene concentration through ripening.

Preharvest ethephon (ethylene releasing compound) sprays caused softer 'McIntosh' (Pollard, 1974), 'Delicious' (Brohier and Faragher, 1984) and 'Cox's Orange Pippin'

(Watkins et al., 1989) fruit, but had no effect on the firmness of 'Jonathan' apples (Brohier and Faragher, 1984). Ethylene scrubbing in controlled atmospheres (CA) has been reported to maintain firmness in several studies (Forsyth et al., 1969; Blanpied et al., 1972; Liu, 1985; Stow et al., 2000). However, these benefits were cultivar dependent and were often only achieved when:

- external ethylene concentrations were maintained below 1 $\mu l.l^{-1}$ (Liu, 1977; 1978a)
- fruit were harvested at a preclimacteric stage of development, were sprayed with diaminozide before harvest (Liu, 1985), or were treated with a CO_2 shock treatment at harvest (Stow, 1990)
- fruit were rapid cooled and placed into CA immediately after harvest (Liu, 1985).

No reproducible softening benefits have been reported for ethylene scrubbing in air storage (Fidler, 1950; Gerhardt and Siegelman, 1955; Fidler and North, 1969; Knee, 1976). Furthermore, addition of ethylene to apples stored in air did not reduce firmness unless the ethylene concentration exceeded 1000 $\mu l.l^{-1}$ (Gerhardt and Siegelman, 1955; Knee, 1976).

Inhibitors of ethylene biosynthesis have been extensively used to study the role of ethylene in apple ripening processes. Preharvest application of aminoethoxyvinylglycine (AVG) to 'Golden Delicious' and 'King of the Pippin' apples resulted in firmer fruit at harvest and through storage (Bangerth, 1978; Halder-Doll and Bangerth, 1987). In contrast, AVG treatment of 'Cox's Orange Pippin' had no effect on firmness at harvest, but improved firmness after storage (Child et al., 1984). AVG treated fruits softened as rapidly as untreated fruit when challenged with exogenous ethylene in storage (Autio and Bramlage, 1982), or when stored with ethylene producing untreated fruits (Bramlage et al., 1980). These studies confirmed that AVG reduced softening by reducing ethylene production, and not by affecting the fruits sensitivity to exogenous ethylene.

Inhibitors of ethylene action have also been used to study the role of ethylene in apple ripening. Apple softening and ethylene production were reduced in several cultivars when treated with either diazocyclopentadiene (DCAP), 2,5-norbornadiene (NBD), or 1-methylcyclopropene (MCP) at harvest (Blankenship and Sisler, 1989; 1993; Fan et al., 1999; Rupasinghe et al., 2000; Watkins et al., 2000). It has been suggested that these

inhibitors block ethylene action by binding to the ethylene receptor (Sisler and Serek, 1999). A common aspect of all these inhibitors was that the effectiveness was progressively reduced during storage, indicating that these chemicals may diffuse from the active site, or that the tissue generated new active sites (Blankenship and Sisler, 1989; 1993). Interestingly a repeat application of DCAP during storage reinstated low ethylene production, but could not reduce softening in actively softening apples (Blankenship and Sisler, 1993). These results suggest that ethylene has a role in initiating apple softening, but may not be required to sustain softening once initiated.

In the section on cell walls (1.3.2), a number of cell wall degrading enzymes were identified that may mediate the softening of apples during ripening. It is possible that ethylene induces softening in apples by regulating expression of these enzymes. Expression of PG increased during the early phases of apple softening for several cultivars, with increased expression coinciding with the time when internal ethylene concentration (IEC) increased from 0.7 to 2.1 $\mu l.l^{-1}$ for Royal Gala, from 0.2 to 2.7 $\mu l.l^{-1}$ for Granny Smith, and from 11 to 46 $\mu l.l^{-1}$ 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, while activities of exo-PG and PME were ethylene independent (Pech et al., 1999). Furthermore, the softening rate of transgenic melons was restored to the wild-type rate when treated with 2.5 $\mu l.l^{-1}$ ethylene (Pech et al., 1999; Botondi et al., 2000). Transgenic tomatoes with reduced ethylene biosynthesis and slightly reduced softening rates also had activities of PG (Sirit and Bennett, 1998) and galactosidase (Sozzi et al., 1998) that were ethylene dependent. In contrast for kiwifruit and avocados, increased expression and activity of PG (Fuchs et al., 1986; Wang et al., 2000) and cellulase (Fuchs et al., 1986) during softening preceded the rapid increase in ethylene production. However, PG expression was induced by very low ethylene concentrations (0.1 $\mu l.l^{-1}$) in transgenic tomatoes with reduced ethylene biosynthesis. This suggests that despite ethylene concentrations being low in kiwifruit and avocado fruit during early stages of ripening, ethylene may still have a role in regulating activity of PG and cellulase in these fruits.

The reviewed literature indicates that ethylene has an important role in promoting softening of unripe apples. However, it is possible that softening could regulate the rate of ethylene biosynthesis in ripe apples. Cell wall fragments and PG extracted from tomato stimulated ethylene biosynthesis once infiltrated into unripe tomato fruit (Gross, 1985; Baldwin and Pressey, 1988; Brecht and Huber, 1988; Tong and Gross, 1990). Likewise, cell wall fragments extracted from pears also stimulated ethylene production once applied to pear cell cultures (Tong et al., 1986).

Studies identifying relationships between endogenous ethylene and softening have resulted in an unclear exact role for ethylene in the softening process of apple fruit. Lau et al. (1986) and Blankenship and 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. However, it is possible that 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). Once harvested, the firmness of apples generally declines as the IEC or rate of ethylene production increases (Yoshioka et al., 1995; Watkins et al., 2000). Anecdotal evidence indicates that softening is reduced in CA storage when ethylene is maintained below the internal and external concentrations of 0.1 $\mu l.l^{-1}$ (Stow et al., 2000) and 1 $\mu l.l^{-1}$ (Liu, 1977) respectively. However, no study has been published for apples, or many other fruits, relating ethylene production to rates of softening through storage. This form of research may more accurately identify ethylene concentrations required to trigger fruit softening both on and off the tree.

1.4.2 Other growth regulators?

The role of growth regulators, other than ethylene (section 1.4.1), in regulating the ripening and softening of climacteric fruits is not well known. Potential roles of abscisic acid, auxins, gibberellins, cytokinins, polyamines and nitric oxide will be discussed in relation to fruit ripening and softening.

The influence of abscisic acid (ABA) on softening of apples is not known. However in bananas and kiwifruit, ABA treated fruit softened more rapidly than non-treated fruit

(KunSong et al., 1999; Jiang et al., 2000). It was also found that MCP inhibited the stimulatory effect of ABA on banana ripening, indicating that ABA may influence ripening through ethylene (Jiang et al., 2000). Research in apples has shown that ABA accumulates during maturation (Lara and Vendrell, 1998), and that ABA may have an important role in regulating the onset of system II ethylene production (Lara and Vendrell, 2000). Thus, it is likely that ABA may also promote softening in apples through stimulating system II ethylene production, although this remains to be tested.

Application of auxin in the forms of either 2,4,5-T or 1-naphthaleneacetic acid (NAA) had inconsistent effects on apple firmness. Preharvest application of 2,4,5-T reduced firmness at harvest and after storage, although diaminozide (known as Alar and B9), a chemical considered to interfere with ethylene production, was shown to reverse the effect of 2,4,5-T (Looney, 1971). Thus, auxin may accelerate softening through stimulating ethylene production. However, in another study using NAA, auxin had no effect on firmness at harvest or after storage (Elfving and Lougheed, 1994). Thus, the role of auxin in apple softening is inconclusive.

Paclobutrazol (also known as Cultar or PP333), considered an antagonist of gibberellins, is used to manage vegetative growth of apple trees (Wang and Steffens, 1987). Apples treated with this chemical before storage were slightly firmer than non-treated fruit at harvest (Luo et al., 1989; Khurshid et al., 1997) and after storage (Wang and Steffens, 1987; Luo et al., 1989). These results suggest that gibberellins reduce apple firmness, which is contrary to findings that gibberellins generally retard fruit ripening (Vendrell and Palomer, 1998).

Preharvest application of cytokinin slightly increased the firmness of apples at harvest and after storage, although this effect was not consistent across seasons (Elfving and Lougheed, 1994). Treatment of apples with cytokinin like compounds such as thidiazuron (*N*-phenyl-*N*'-1,2,3-thiadiazol-5-ylurea) and CPPU (*N*-(2-chloro-4-pyridyl)-*N*'-phenylurea) had a more pronounced influence on apples firmness, where preharvest treatment with either compound increased firmness at harvest and after storage (Curry and Greene, 1993; Greene, 1995). Interestingly, gibberellin application reversed the

effect of CPPU on firmness (Curry and Greene, 1993), providing further evidence that gibberellins promote, rather than reduce apple softening.

Endogenous polyamines may also have a role in determining apple firmness. 'Golden Delicious' apples were firmer immediately after polyamine treatment, but then softened at similar rates to non-treated fruit at 0°C (Kramer et al., 1991). Interestingly, the effectiveness of the exogenous polyamine treatment in increasing firmness was similar to that obtained with a calcium treatment (Kramer et al., 1991). However, unlike calcium, polyamine treatments were not able to reduce the softening rate of fruit in storage (Kramer et al., 1991). It has been shown that calcium treatment before polyamine treatment reduced the amount of polyamine bound to the wall, and polyamine treatment before calcium treatment reduced the amount of calcium bound to the wall (Wang et al., 1993). These results suggest that polyamines may improve the textural quality of apples by binding to and improving the strength of the cell wall, and that they may compete with calcium for binding sites (Wang et al., 1993). Polyamines, like calcium, were able to reduce in vitro polygalacturonase activity (Kramer et al., 1989), but unlike calcium, were not able to reduce ethylene production (Wang et al., 1993).

Nitric oxide (NO) has recently been identified as a potential endogenous regulator of maturation and senescence in plants (Leshem et al., 1998). Endogenous NO concentrations decreased in several fruits during maturation and senescence, and exogenous treatment of fruits with NO slowed ripening (Leshem et al., 1998). It is suggested that NO may slow ripening by reducing ethylene production (Leshem et al., 1998). The role of NO in ripening and softening of apple fruits is yet to be determined. However, if NO reduces ethylene production in apples as in other climacteric fruits, then it is likely that NO will also reduce apple softening.

Apart from polyamines, the effects of growth regulators on the softening rates of apples through storage are not well known. Characterisation of the postharvest softening curve for apples may allow more accurate associations between softening rates and endogenous changes in concentrations of these growth regulators during ripening, that in turn may clarify the roles of these compounds in regulating apple softening. Further

research is also required to investigate the interactions between these growth regulators, and determine if these interactions have additive or synergistic effects on softening.

1.4.3 Apoplastic pH.

It is clear from sections above that plant growth regulators have an important role in modulating softening of apples. However, the role of apoplastic pH in regulating softening is not so clear. Pear discs infiltrated with different pH buffers became increasingly firmer as pH of the buffer increased from 3.2 to 8.0 (Knee, 1982). During maturation and ripening in tomatoes the apoplastic pH decreased from 6.7 to 4.4, and the ionic concentration of the apoplast increased (Almeida and Huber, 1999). Apoplastic changes in pH and ionic concentration may regulate softening by modifying the *in vivo* activity of cell wall degrading enzymes (Chun and Huber, 1998; Almeida and Huber, 1999). *In vitro* enzyme assays for exo-polygalacturonase and β-galactosidase extracts from apples showed a pH optima of between 4 and 5 (Bartley, 1974; 1978), although these pH optima's may not be a true reflection of those *in vivo*, as the ionic conditions *in vitro* were most likely different to those found *in vivo* (Almeida and Huber, 1999). It should also be noted that pH interacts with the degree of esterification of pectin to affect the strength of pectin gels (Crandall and Wicker, 1986). This could mean that changes in apoplastic pH and degree of pectin esterification may affect the strength of pectin and hence the middle lamella during ripening.

1.5 What effect do pre-, at, and postharvest factors have on apple softening?

1.5.1 Preharvest factors.

Fruit from different orchards often differ in firmness after storage, despite being stored in similar conditions. This variation in firmness is induced by differences in storage potential at harvest, that in turn are determined by the collective influence of several pre- and at-harvest factors. Two main approaches have been undertaken to determine the influence of preharvest factors on the firmness of apples:

1) the systematic process of changing one variable in the orchard and assessing the consequent quality at harvest and after storage (Johnson, 1994)

2) collecting fruit from orchards with a range of preharvest practices and analysing attributes of the fruit at harvest that may indicate the storage potential of the fruit (Johnson and Ridout, 1998).

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

Preharvest factors that influence apple firmness 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
- genetic factors that involve choice of cultivar or clone, rootstocks and interstocks (Harker et al., 1997a; DeEll et al., 1999; Sams, 1999).

The individual influence of each of these preharvest factors on firmness has been reviewed by Harker et al. (1997a), DeEll et al. (1999), 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 pre- or at-harvest measurements of meteorological variables, maturity indices and mineral concentrations (Bramlage et al., 1985; Fallahi et al., 1985; Marmo et al., 1985; Johnson et al., 1987; Ingle and Morris, 1989; Knee and Farman, 1989; Knee and Smith, 1989; Blankenship et al., 1997; Johnson and Ridout, 1998; de Jager and 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 at harvest. However, the predictive accuracy of prestorage firmness measurements varied substantially between seasons and cultivars. Knee and Farman (1989) found that the predictive accuracy of models based on firmness was improved by including estimates of time between harvest and an internal ethylene concentration of 0.1 $\mu l.l^{-1}$, or inclusion of harvest dates. However, these multifactor models could still only account for about 60% of the variation in firmness after CA storage (Knee and 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 and Ridout, 1998). In general, minerals alone were poor predictors of poststorage firmness (Bramlage et al., 1985; Fallahi et al., 1985; de Jager and de Putter, 1999). However, there have been some reported instances of associations between poststorage firmness and concentrations of phosphorus, zinc, manganese, boron, potassium, sodium, copper, magnesium and calcium concentrations in some apple cultivars (Johnson et al., 1987; Johnson, 2000).

1.5.2 At-harvest factors.

The two main 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 later stage of maturity are often softer at harvest and after storage than apples picked less mature (Liu, 1978a; Olsen and Martin, 1980; Blanpied, 1986; Wang et al., 1990; Ingle et al., 2000; Stow and Genge, 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 (Lidster and Porritt, 1978; Ingle and Morris, 1989). Despite these few exceptions, it is often recommended that rapid softening cultivars be harvested earlier, rather than later, during the commercial harvest period to maximise firmness after storage. While the effect of harvest maturity on pre- and poststorage firmness has been studied extensively, there is a lack of detailed information as to the effect of this factor on postharvest softening rates. Thus, there is a need to characterise the softening curves for apples harvested at different stages of maturity.

It is generally accepted that larger fruits are softer than smaller fruits (Harker et al., 1997a), as smaller fruits generally have more cell wall material per unit volume, and therefore should have stronger tissue than larger fruits. Results with apples support this

contention, as larger fruit have been reported to be softer than smaller fruit both at harvest and after storage (Blanpied et al., 1978; Marmo et al., 1985; Siddiqui and Bangerth, 1995). However, it is not known if these effects of fruit size were due to: physical differences in tissue strength as a result of differences in cell size and cell number; and/or physiological differences in firmness due to the different sized fruit being picked at different maturity. It should be noted that firmness declines in parallel with increased fruit size during maturation on the tree, making it difficult to differentiate the physical effects of fruit size from the physiological effects of maturation. Furthermore, there is little published information as to the effects of fruit size on the postharvest softening rates of apples. Thus, like harvest maturity, the postharvest softening curves for different sized apples needs to be determined.

1.5.3 Postharvest factors.

Postharvest factors that influence the softening of apples include temperature, atmosphere, ethylene, Ca drenches, and water loss (relative humidity). The effects of temperature and atmosphere on apple softening will be reviewed in this section, while the effects of ethylene (1.4.1), calcium drenches (1.3.4) and water loss (1.3.5) were reviewed in the indicated previous sections.

1.5.3.1 Temperature.

Temperature can induce both physical and physiological changes in apple texture. The physiological change in texture is irreversible and is developmentally regulated, while the physical change in texture is reversible and not developmentally regulated. The influence of temperature on both types of texture change will be discussed.

Surprisingly, there is a lack of published information on the effect of temperature on the ripening-associated softening rates of apple fruit. Studies by Magness and Diehl (1924) and Landfald (1966) showed that several apple cultivars softened faster as the storage temperature increased from 0 to 21°C. However in both of these studies, fruit were only stored at 2-4 temperatures, and firmness was only measured 5 times at each temperature, making it difficult to accurately characterise the softening curve for apples at different temperatures. There is also a lack of information on softening rates of apples between

20°C and those temperatures used in heat treatment studies (section 1.5.3.2). A study on postharvest changes in skin colour of apples showed that the rate of yellowing could be described by a modified Arrenhius equation at temperatures between 0 and 35°C, with the rate of yellowing increasing from 0 to 20-28°C, and decreasing thereafter through 35°C (Dixon and Hewett, 1998). It is yet to be determined if softening at different temperatures can also be described by a modified Arrenhius equation. Except for heat treatments, there is also limited published information as to whether the softening rate at a given temperature is influenced by prior exposure to other temperatures. Detailed information on softening rates of apples at different temperatures could be used commercially to estimate the consequences of holding fruit at non-optimal temperatures during postharvest handling.

Knowledge on 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 (1982a) 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, 1982a). In contrast, studies by Haller (1941) and Saltveit (1984) found that fruit temperature had no influence on the firmness of several cultivars. Thus, the physical influence of fruit temperature on apple firmness is not clear. The firmness temperature coefficient was found to change during kiwifruit ripening (Jeffery and Banks, 1994), which could explain the inconsistent results in apples. Further research is required to clarify if temperature physically affects apple firmness, and to determine if the magnitude of physical change in firmness is dependent on the stage of ripeness.

1.5.3.2 Heat treatments.

Short-term heat treatments are an effective treatment for disinfestation and for improving the postharvest quality of several horticultural crops (Lurie, 1998). With regards to apples, short-term heat treatments (2-6 days at 38-40°C) slowed the softening rates during storage at both low (Porritt and Lidster, 1978) and shelf-life (Liu, 1978b) temperatures. Subsequent studies have been done to determine the temperature and the

duration of the heat treatment needed to optimise postharvest quality (Klein and Lurie, 1990; Klein and Lurie, 1992). Analysis of force-deformation curves from compression tests showed that heat-treated apples had a "harder" or "tougher" texture than non-treated apples (Conway et al., 1994). Furthermore, a sensory panel rated heat-treated apples as being crisper than non-treated apples (Lurie and Nussinovitch, 1996). Heat treatments have also been shown to act synergistically with calcium dipping, and additively with vacuum calcium infiltration, to reduce softening (Lurie and Klein, 1992; Conway et al., 1994; Klein and Lurie, 1994).

Several biochemical differences have been identified between heat-treated and non-treated fruit, that in combination may explain the mechanism by which heat treatment reduces softening. Heat-treated fruit generally had a slower increase in membrane microviscosity and leakiness than non-treated fruit during ripening (Lurie et al., 1995). Heat treated fruit also temporarily possessed heat shock proteins that may play a role in slowing softening, although the nature of this role is currently not known (Lurie and Klein, 1990). Analysis of the cell walls showed that heat treated fruit had less soluble pectin and more insoluble pectin (Klein and Lurie, 1990; Ben-Shalom et al., 1993), had similar degrees of pectin esterification (Klein et al., 1995; Ben-Shalom et al., 1996), and lost more arabinose residues and the same amount of galactose residues (Ben-Shalom et al., 1996), than non heated fruit after ripening. Ethylene production was initially lower in heat-treated fruit than in non-heated fruit, that may explain reduced softening rates that occurred early in storage in the former fruit (Klein and Lurie, 1990). However, heat-treated apples produced more ethylene and continued to soften slower than non-treated apples once placed at 20°C after different times at 0°C (Klein and Lurie, 1990). Furthermore, slower softening of heat-treated fruit than non-treated fruit could not be reversed by treating the heat-treated fruit with ethylene (Klein et al., 1990). Thus, the role of ethylene in reducing softening rates in heat-treated fruit has not been fully elucidated.

1.5.3.3 Controlled atmospheres.

Studies by Kidd and West (1933; 1936) first showed the commercial potential of storing apples in controlled atmospheres (CA) to slow loss of quality, including softening.

However like most other factors reviewed thus far, the softening curve for apples in CA has not been accurately characterised. The effectiveness of CA in reducing softening of apples is cultivar dependent (Dilley et al., 1989), and influenced by:

- time in air before CA storage
- rate of establishment of CA conditions (Smock and Blanpied, 1963; Sharples and Munoz, 1974; Fica et al., 1985)
- duration of CA storage (Lidster, 1982)
- O_2 and CO_2 concentrations (Magness and Diehl, 1924; Kidd and West, 1939; Knee, 1980; Stow, 1989b)
- temperature (Kidd and West, 1933)
- exogenous ethylene concentration (section 1.4.1.2).

Current CA conditions used for different cultivars, and for fruit grown in different countries and regions, are reviewed by Kupferman (1997).

Incremental reduction of O_2 concentrations from 21% (RA) to 1-2.5% improved the poststorage firmness of several apple cultivars (Kidd and West, 1933; 1936; 1939; Knee, 1980; Dewey and Bourne, 1982; Fan et al., 1997). Furthermore, softening was completely inhibited in 'McIntosh' apples at 0.75-1% O_2 (Dewey and Bourne, 1982). However at O_2 concentrations less than 0.75-1%, softening tended to increase (Stow, 1989b; Fan et al., 1997), presumably through the deleterious effects of low-O_2 disorders on texture. Progressive increases in CO_2 concentration from 0 to 50% also slowed softening in several cultivars, regardless of O_2 concentration (Magness and Diehl, 1924; Kidd and West, 1933; 1936; 1939; Ben-Arie et al., 1993). The mechanism by which CA reduces softening is not known, although correlative evidence suggests that reduced softening can be partially attributed to the ability of CA to reduce ethylene production and action (Burg and Burg, 1967; Fica et al., 1985; Dilley et al., 1989).

1.5.3.4 High-CO_2 and low-O_2 shock treatments.

The utilisation of prestorage CO_2 shock treatment has showed some potential for retaining firmness in air (Brooks, 1939) and CA (Couey and Olsen, 1975), although the effectiveness of this treatment tended to be inconsistent across seasons (Meheriuk et al., 1977). Furthermore, any firmness benefits accrued from the CO_2 shock treatment were

often not sufficient to compensate for increased incidence of disorders associated with CO_2 injury (Bramlage et al., 1977; Lau and Looney, 1978).

Like high CO_2 shock treatments, low O_2 (<1%) shock treatments have inconsistently improved firmness retention in different apple cultivars. For cultivars such as 'McIntosh', 'Spartan', 'Golden Delicious' and 'Granny Smith', low O_2 shock treatments reduced subsequent softening and incidence of some disorders in CA, but increased the incidence of low O_2 disorders when duration of the shock treatment increased (Little et al., 1982; Lister et al., 1985; 1987). In other cultivars such as 'Cox's Orange Pippin', these low O_2 shock treatments did not improve firmness retention, and increased the incidence of rots and some physiological disorders in storage (Fidler and North, 1971). Thus, the commercial quality benefits of using either high CO_2 or low O_2 treatments remains inconclusive.

1.6 A schematic model of apple softening.

From the information given in previous sections on the biology of apple softening (1.3 and 1.4), and influences of pre-, at- and post-harvest factors on firmness (1.5), a simple schematic model of apple softening was developed (Fig. 1-7). This model is based on the principle that apple fruits soften by loss of cell to cell adhesion, and that increased ethylene production has an important role in promoting this process. However, the pathways by which increased ethylene production facilitates cell to cell debonding is not clear, and could be due to changes in expression of any number of enzymes that alter the physical properties of both cell walls and cell membranes (1.3.2 and 1.3.3). This model also summarises the influence of different pre-, at- and post-harvest factors on the firmness of apples after storage; these effects may be directly or indirectly mediated by increased ethylene production.

A problem with this model is that it does not incorporate the influence of storage time, and therefore does not reflect the influence of the different factors on softening rates. However, given that the postharvest softening curve for apples has not been characterised, it is not possible to unequivocally ascertain the effects of different factors on softening rates of apples. Clarification of the nature of the softening curve for apples

should allow determination of softening rates through storage, as well as providing a basis for associating changes in softening rates with changes in concentrations of regulatory compounds and activities of enzymes involved in softening. The usefulness of characterising the softening curve of fruits is evident from kiwifruit research, as associations between the softening curve and changes in activities of cell wall degrading enzymes, cell wall chemistry and other ripening related phenomena, has facilitated development of conceptual models explaining the mechanisms involved in softening of this fruit (MacRae et al., 1990; MacRae and Redgwell, 1992).

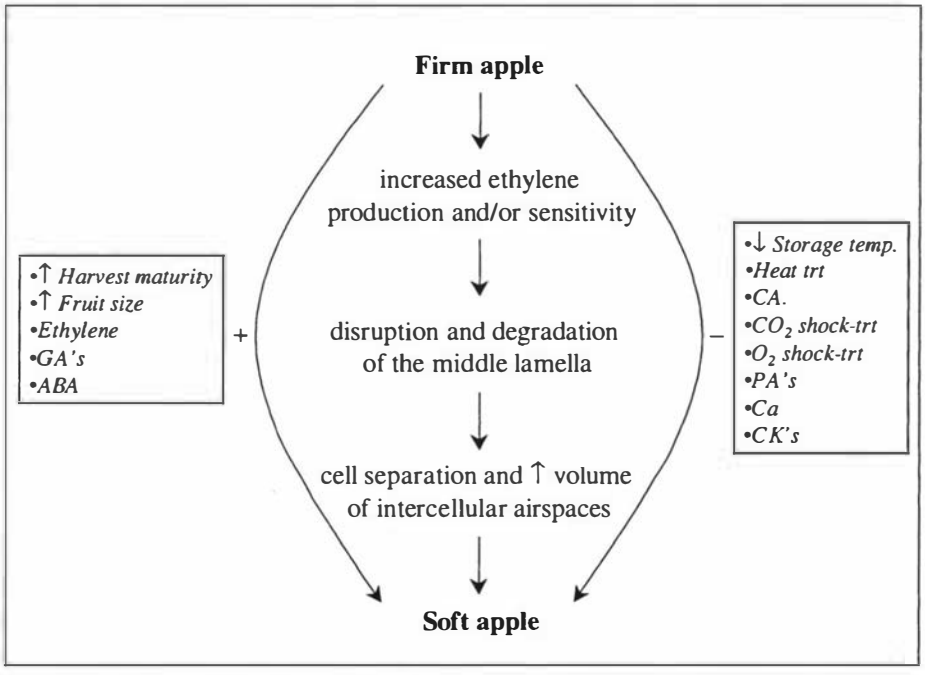

Fig. 1-7 Schematic diagram summarising the mechanism for apple softening, and effects of different pre-, at- and post-harvest factors on loss of apple firmness. Factors that accelerate development of soft fruit are denoted as + factors, and factors that slow development of soft fruit are denoted as – factors. Abbreviations: increased (↑); decreased (↓); gibberellins (GA's); abscisic acid (ABA); temperature (temp.); treatment (trt); controlled atmospheres (CA); carbon dioxide (CO_2); oxygen (O_2); polyamines (PA's); calcium (Ca); and cytokinins and cytokinin-like compounds (CK's).

1.7 Can softening rates of apples be predicted before storage?

The firmness of apples is influenced by a number of pre-, at- and post-harvest factors (section 1.5). Thus, the rate at which apples soften in storage is often unpredictable,

making it difficult to estimate the market life of fruit before storage. While the combination of low temperatures and controlled atmospheres are effective at reducing softening of apples in storage, rapid softening cultivars still soften sufficiently rapidly to severely limit their market life. The use of genetic modification, or growth regulators such as MCP (section 1.4.1.2), could also be used commercially to control softening. However, growing consumer opposition may limit the immediate commercially applicability of these technologies. An alternative approach would be to predict softening rates before storage. The ability to predict storage rates before storage would allow:

- segregation of fruit before storage for differences in market life
- estimation of market life for different batches of fruit before storage
- quantification of the consequences of exposing fruit to non-optimal temperatures during postharvest handling
- assessment of the relative influence of different at- and post-harvest factors on softening
- optimisation of the use of temperature and controlled atmospheres to reduce softening during postharvest handling.

Before predictive models can be developed, the postharvest softening curve for apples needs to be characterised in relation to harvest maturity, fruit size, and storage temperature and atmosphere. Furthermore, softening curve differences for fruit from different orchards needs to be predicted prior to, or at harvest, from specific fruit or orchard characteristics. While some of these pre-, at- and post-harvest factors have been extensively studied in relation to firmness at harvest and after storage (section 1.5), these factors were often studied separately rather than collectively, and were often studied on different cultivars making it difficult to compare the relative importance of each factor. Furthermore, most of these studies only measured firmness 2-5 times during storage, making it difficult to accurately characterise the softening curve, and therefore the change in softening rates through storage.

1.8 Thesis objectives.

Despite the firmness of apples being measured in numerous studies, little is known about the change in softening rates of apples through time. Until the softening curve for apples has been characterised, the influence of different pre-, at- and post-harvest factors on softening cannot be accurately determined. Furthermore, the ability to accurately predict postharvest softening rates before storage will be impeded without prior knowledge about the shape of the softening curve. From a mechanistic perspective, characterisation of the softening curve should improve the ability to associate the timing of changes in concentrations of regulatory compounds (such as ethylene) and changes in enzyme activities (such as those involved in cell wall disassembly), with changes in softening rates. Thus, the objectives of this thesis were to:

- Quantify the physical change in apple firmness with change in fruit temperature, and determine whether this physical effect is affected by cultivar and storage time.
- Characterise the softening curves for rapid and slow ripening apple cultivars.
- Compare and contrast the softening and ethylene biosynthetic responses of rapid and slow softening apple cultivars at a range of temperatures.
- Determine if the rate of softening of apples at a given temperature is influenced by prior exposure to another temperature between 0°C and 20°C.
- Characterise the softening curve for apples harvested at different stages of maturity.
- Determine if fruit size affects the softening profile of harvested apples, and if so determine if this effect was mediated by maturity differences at harvest.
- Identify the extent of variation in softening rates of fruit from different orchards, and if this variation can be predicted before storage by measuring specific fruit attributes at harvest.
- Characterise the softening curve for apples stored in controlled atmospheres.
- Quantify the effect of time in air storage on subsequent softening rates of apples in controlled atmospheres.
- Determine the effect of time in controlled atmospheres on subsequent softening rates of apples in air at 0.5-3°C and 20°C.
- Determine if changes in endogenous ethylene concentrations through storage relate to the softening profile for harvested apples.

1.9 References

Abbott, J.A., 1994. Firmness measurement of freshly harvested 'Delicious' apples by sensory methods, sonic transmission, Magness-Taylor, and compression. J. Am. Soc. Hortic. Sci. 119, 510-515.

Abbott, J.A., 1999. Quality measurement of fruits and vegetables. Postharvest Biol. Technol. 15, 207-225.

Abbott, J.A., Affeldt, H.A., Liljedahl, L.A., 1992. Firmness measurement of stored 'Delicious' apples by sensory methods, Magness-Taylor, and sonic transmission. J. Am. Soc. Hortic. Sci. 117, 590-595.

Abbott, J.A., Conway, W.S., Sams, C.E., 1989. Postharvest calcium chloride infiltration affects textural attributes of apples. J. Am. Soc. Hortic. Sci. 114, 932-936.

Abbott, J.A., Lu, R., Upchurch, B.L., Stroshine, R.L., 1997. Technologies for nondestructive quality evaluation of fruits and vegetables. Hortic. Rev. 20, 1-120.

Abbott, J.A., Watada, A.E., Massie, D.R., 1976. Effe-gi, Magness-Taylor, and Instron fruit pressure testing devices for apples, peaches, and nectarines. J. Am. Soc. Hortic. Sci. 101, 698-700.

Abbott, J.A., Watada, A.E., Massie, D.R., 1984. Sensory and instrument measurement of apple texture. J. Am. Soc. Hortic. Sci. 109, 221-228.

Abeles, F.B., Biles, C.L., 1991. Cellulase activity in developing apple fruits. Sci. Hortic. 47, 77-87.

Abeles, F.B., Takeda, F., 1990. Cellulase activity and ethylene in ripening strawberry and apple fruits. Sci. Hortic. 42, 269-275.

Almeida, D.P.F., Huber, D.J., 1999. Apoplastic pH and inorganic ion levels in tomato fruit: a potential means for regulation of cell wall metabolism during ripening. Physiol. Plant. 105, 506-512.

Anon., 1996. A strategic research and development plan for the New Zealand Pipfruit Industry. ENZAFRUIT New Zealand (International), Hastings, New Zealand.

Anon., 1997. Poor quality a heavy cost. Orchardist New Zeal. 70, 36.

Anon., 2001. Measuring international competitiveness. Sixth annual comparison of 27 major apple producing countries. World Apple Rep. February, 4-5 and 11.

Atkinson, R.G., 1994. A cDNA clone for endopolygalacturonase from apple. Plant Physiol. 105, 1437-1438.

Atkinson, R.G., Bolitho, K.M., Wright, M.A., Iturriagagoitia-Bueno, T., Reid, S.J., Ross, G.S., 1998. Apple ACC-oxidase and polygalacturonase: ripening specific gene expression and promoter analysis in transgenic tomato. Plant Mol. Biol. 38, 449-460.

Autio, W.R., Bramlage, W.J., 1982. Effects of AVG on maturation, ripening, and storage of apples. J. Am. Soc. Hortic. Sci. 107, 1074-1077.

Bain, J.M., Robertson, R.N., 1951. The physiology of growth in apple fruits. I Cell size, cell number, and fruit development. Australian J. Sci. Res. B4, 75-91.

Baldwin, E.A., Pressey, R., 1988. Tomato polygalacturonase elicits ethylene production in tomato fruit. J. Am. Soc. Hortic. Sci. 113, 92-95.

Bangerth, F., 1978. The effect of a substituted amino acid on ethylene biosynthesis, respiration, ripening and preharvest drop of apple fruits. J. Am. Soc. Hortic. Sci. 103, 401-404.

Barry, C., S, Llop-Tous, I., Grierson, D., 2000. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system 1 to system 2 ethylene synthesis in tomato. Plant Physiol. 123, 979-986.

Bartley, I.M., 1974. β-Galactosidase activity in ripening apples. Phytochem. 13, 2107-2111.

Bartley, I.M., 1976. Changes in the glucans of ripening apples. Phytochem. 15, 625-626.

Bartley, I.M., 1977. A further study of β-galactosidase activity in apples ripening in store. J. Exp. Bot. 28, 943-948.

Bartley, I.M., 1978. Exo-polygalacturonase of apple. Phytochem. 17, 213-216.

Bartley, I.M., 1985. Lipid metabolism of ripening apples. Phytochem. 24, 2857-2859.

Bartley, I.M., Knee, M., 1982. The chemistry of textural changes in fruit during storage. Food Chem. 9, 47-58.

Ben-Arie, R., Kislev, N., Frenkel, C., 1979. Ultrastructural changes in the cell walls of ripening apple and pear fruit. Plant Physiol. 64, 197-202.

Ben-Arie, R., Levin, A., Sonego, L., Zutkhi, Y., 1993. Differential effects of CO_2 at low and high O_2 on the storage quality of two apple cultivars. Acta Hortic. 326, 165-174.

Ben-Shalom, N., Hanzon, J., Klein, J.D., Lurie, S., 1993. A postharvest heat treatment inhibits cell wall degradation in apples during storage. Phytochem. 34, 955-958.

Ben-Shalom, N., Hanzon, J., Pinto, R., Lurie, S., 1996. Cell wall changes and partial prevention of fruit softening in prestorage heat treated 'Anna' apples. J. Sci. Food Agric. 72, 231-234.

Berard, L.S., Lougheed, E.C., Murr, D.P., 1982. β-Galactosidase activity of 'McIntosh' apples in storage. HortScience 17, 660-661.

Blankenship, S.M., Parker, M., Unrath, C.R., 1997. Use of maturity indices for predicting poststorage firmness of 'Fuji' apples. HortScience 32, 909-910.

Blankenship, S.M., Sisler, E.C., 1989. 2,5-Norbornadiene retards apple softening. HortScience 2, 313-314.

Blankenship, S.M., Sisler, E.C., 1993. Response of apples to diazocyclopentadiene inhibition of ethylene binding. Postharvest Biol. Technol. 3, 95-101.

Blankenship, S.M., Unrath, C.R., 1988. Internal ethylene levels and maturity of 'Delicious' and 'Golden Delicious' apples destined for prompt consumption. J. Am. Soc. Hortic. Sci. 113, 88-91.

Blanpied, G.D., 1975. The effect of cooling rate on the market quality of 'McIntosh' apples. Proc. Am. Soc. Hortic. Sci. 70, 58-66.

Blanpied, G.D., 1981. A relationship between water loss and storage breakdown of 'McIntosh' apples. HortScience 16, 525-526.

Blanpied, G.D., 1986. A study of the relationship between fruit internal ethylene concentration at harvest and post-storage fruit quality of cv Empire apples. J. Hortic. Sci. 61, 465-470.

Blanpied, G.D., Blak, V.A., 1977. A comparison of pressure tests, acid levels, and sensory evaluations of overripeness in apples. HortScience 12, 73-74.

Blanpied, G.D., Bramlage, W.J., Dewey, D.H., LaBelle, R.L., Massey, L.M., Mattus, G.E., Stiles, W.C., Watada, A.E., 1978. A standardized method for collecting apple pressure test data. N.Y. Food Life Sci. Bull. 74, 8 pp, Cornell University, Ithaca, New York.

Blanpied, G.D., Cadun, O., Tamura, T., 1972. Ethylene in apple and pear experimental CA chambers. J. Am. Soc. Hortic. Sci. 97, 207-209.

Bleecker, A.B., Hall, A.E., Rodriquez, F.I., Esch, J.J., Binder, B., 1999. The ethylene signal transduction pathway. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 51-57.

Bolin, H.R., Huxsoll, C.C., 1987. Scanning electron microscope/image analyzer determination of dimensional postharvest changes in fruit cells. J. Food Sci. 52, 1649-1650.

Botondi, R., Cardarelli, M.T., Mencarelli, F., 2000. The role of ethylene in regulating cell wall degrading enzyme activity using antisense ACC-oxidase in cantaloupe melons. Acta Hortic. 510, 471-477.

Bourne, M.C., 1965. Studies on punch testing of apples. Food Technol. 415, 113-115.

Bourne, M.C., 1968. Texture profile of ripening pears. J. Food Sci. 33, 223-226.

Bourne, M.C., 1979. Texture of temperate fruits. J. Text. Stud. 10, 25-44.

Bourne, M.C., 1982a. Effect of temperature on firmness of raw fruits and vegetables. J. Food Sci. 47, 440-444.

Bourne, M.C., 1982b. Food texture and viscosity: concept and measurement. Academic Press, New York.

Bramlage, W.J., Bareford, P.H., Blanpied, G.D., Dewey, D.H., Taylor, S., Porritt, S.W., Lougheed, E.C., Smith, W.H., McNicholas, F.S., 1977. Carbon dioxide treatments for 'McIntosh' apples before CA storage. J. Am. Soc. Hortic. Sci. 102, 658-662.

Bramlage, W.J., Greene, D.W., Autio, W.R., McLaughlin, J.M., 1980. Effects of aminoethoxyvinylglycine on internal ethylene concentrations and storage of apples. J. Am. Soc. Hortic. Sci. 105, 847-851.

Bramlage, W.J., Weis, S.A., Drake, M., 1985. Predicting the occurrence of poststorage disorders of 'McIntosh' apples from preharvest mineral analyses. J. Am. Soc. Hortic. Sci. 110, 493-498.

Brecht, J.K., Huber, D.J., 1988. Products released from enzymatically active cell wall stimulate ethylene production and ripening in preclimacteric tomato (*Lycopersicon esculentum* Mill.) fruit. Plant Physiol. 88, 1037-1041.

Brohier, R.L., Faragher, J.D., 1984. Effects of ethephon on ripening, red colour, and storage performance of Jonathan and Delicious apples. Australian J. Exp. Agric. Animal Husbandry. 24, 130-136.

Brooks, C., 1939. Effect of carbon dioxide treatment upon the rate of ripening in apples. Proc. Am. Soc. Hortic. Sci. 37, 463-466.

Brummell, D.A., Harpster, M.H., Civello, P.M., Palys, J.M., Bennett, A.B., Dunsmuir, P., 1999a. Modification of expansin protein abundance in tomato fruit alters softening and cell wall polymer metabolism during ripening. Plant Cell 11, 2203-2216.

Brummell, D.A., Harpster, M.H., Dunsmuir, P., 1999b. Differential expression of expansin gene family members during growth and ripening of tomato fruit. Plant Mol. Biol. 39, 161-169.

Burg, S.P., Burg, E.A., 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42, 144-152.

Child, R.D., Williams, A.A., Hoad, G.V., Baines, C.R., 1984. The effects of aminoethyoxyvinylglycine on maturity and post-harvest changes in Cox's Orange Pippin apples. J. Sci. Food Agric. 35, 773-781.

Chun, J.P., Huber, D.J., 1998. Polygalacturonase-mediated solubilisation and depolymerisation of pectic polymers in tomato fruit cell walls. Plant Physiol. 117, 1293-1299.

Conway, W.S., Sams, C.E., 1987. The effects of postharvest infiltration of calcium, magnesium, or strontium on decay, firmness, respiration, and ethylene production in apples. J. Am. Soc. Hortic. Sci. 112, 300-303.

Conway, W.S., Sams, C.E., Tobias, R.B., 1993. Reduction of storage decay in apples by postharvest calcium infiltration. Acta Hortic. 326, 115-121.

Conway, W.S., Sams, C.E., Wang, C.Y., Abbott, J.A., 1994. Additive effects of postharvest calcium and heat treatment on reducing decay and maintaining quality in apples. J. Am. Soc. Hortic. Sci. 119, 49-53.

Cosgrove, D.J., 2001. Wall structure and wall loosening: a look backwards and forwards. Plant Physiol. 125, 131-134.

Couey, H.M., Olsen, K.L., 1975. Storage response of 'Golden Delicious' apples after high carbon dioxide treatment. J. Am. Soc. Hortic. Sci. 100, 148-150.

Crandall, P.G., Wicker, L., 1986. Pectin internal gel strength: theory, measurement, and methodology. In: Fishman, M.L. , Jen, J.J. (eds.), Chemistry and function of pectins. American Chemical Society, Washington, pp. 157-174.

Curry, E.A., Greene, D.W., 1993. CPPU influences fruit quality, fruit set, return bloom, and preharvest drop of apples. HortScience 28, 115-119.

De Belie, N., Hallett, I.C., Harker, F.R., De Baerdemaeker, J., 2000. Influence of ripening and turgor on the tensile properties of pear fruit: a microscopic study of cellular and tissue changes. J. Am. Soc. Hortic. Sci. 125, 350-356.

de Jager, A., de Putter, H., 1999. Preharvest factors and postharvest quality decline of apples. Acta Hortic. 485, 103-110.

De Smedt, V., Pauwels, E., De Baerdemaeker, J., Nicolai, B., 1998. Microscopic observation of mealiness in apples: a quantitative approach. Postharvest Biol. Technol. 14, 151-158.

de Vries, J.A., Voragen, A.G.J., Rombouts, F.M., Pilnik, W., 1986. Structural studies of apple pectins with pectolytic enzymes. In: Fishman, M.L. , Jen, J.J. (eds.), Chemistry and function of pectins. American Chemical Society, Washington, pp. 157-174.

DeEll, J.R., Saad, F., Khanizadeh, S., 1999. Factors influencing apple fruit firmness. Compact Fruit Tree 32, 56-58.

DeLong, J.M., Prange, R.K., Harrison, P.A., McRae, K.B., 2000. Comparison of a new apple firmness penetrometer with three standard instruments. Postharvest Biol. Technol. 19, 201-209.

Denne, M.P., 1963. Fruit development and some tree factors affecting it. New Zeal. J. Bot. 1, 265-294.

Dewey, D.H., Bourne, M.L., 1982. Low oxygen CA storage of McIntosh apples. Proc. Third Nat. CA Res. Conf., 22-24 July, 1981, Oregon State University, pp. 101-107.

Dick, A.J., Opoku-Gyamfua, A., DeMarco, A.C., 1990. Glycosidases of apple fruit: a multi-functional β-galactosidase. Physiol. Plant. 80, 250-256.

Dilley, D.R., Lange, E., Tomala, K., 1989. Optimizing parameters for controlled atmosphere storage of apples. Fifth Nat. CA Res. Conf., 14-16 June 1989, Wenatchee, Washington, USA, pp. 221-236.

Dixon, J., Hewett, E.W., 1998. Temperature affects postharvest color change of apples. J. Am. Soc. Hortic. Sci. 123, 305-310.

Elfving, D.C., Lougheed, E.C., 1994. Storage responses of 'Empire' apples to benzyladenine and other chemical thinners. J. Am. Soc. Hortic. Sci. 119, 253-257.

Fallahi, E., Righetti, T.L., Richardson, D.G., 1985. Predictions of quality by preharvest fruit and leaf mineral analyses in 'Starkspur Golden Delicious' apple. J. Am. Soc. Hortic. Sci. 110, 524-527.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Fan, X., Mattheis, J.P., Patterson, M., Fellman, J.K., 1997. Optimum harvest date and controlled atmosphere storage potential of 'Fuji' apples. Seventh Int. CA Res. Conf., July 13-18, 1997, University of California, Davis, pp. 42-49.

Fica, J., Skrzynski, J., Dilley, D.R., 1985. The effect of delayed cooling and delayed application of CA storage of McIntosh apples under low and high ethylene levels. Proc. Fourth Nat. CA Res. Conf., 23-26 July, 1985, Raleigh, North Carolina, USA, pp. 82-94.

Fidler, J.C., 1950. Studies of the physiologically-active volatile organic compounds produced by fruits: II The rate of production of carbon dioxide and of volatile organic compounds by King Edward. IV Apples in gas storage, and the effect of removal of the volatiles from the atmosphere of the store on the incidence of superficial scald. J. Hortic. Sci. 25, 81-110.

Fidler, J.C., North, C.J., 1969. Production of volatile organic compounds by apples. J. Sci. Food Agric. 20, 521-526.

Fidler, J.C., North, C.J., 1971. The effect of periods of anaerobiosis on the storage of apples. J. Hort. Sci. 46, 213-221.

Forsyth, F.R., Eaves, C.A., Lightfoot, H.J., 1969. Storage quality of McIntosh apples as affected by removal of ethylene from the storage atmosphere. Can. J. Plant Sci. 49, 567-572.

Fuchs, Y., Zauberman, G., Akerman, M., 1986. Polygalacturonase, cellulase and softening, as related to ethylene production in avocado fruit stored at chilling temperature. Acta Hortic. 179, 801-804.

Fuller, M.M., 1980. Cell ultrastructure in apple fruits in relation to calcium concentration and fruit quality. In: Atkinson, D., Jackson, J.E., Sharples, R.O. , Waller, W.M. (eds.), Mineral nutritional of fruit trees. Butterworths, London, pp. 51-55.

Gerhardt, F., Siegelman, H.W., 1955. Storage of pears and apples in the presence of ripened fruit. J. Agric. Food Chem. 3, 428-433.

Giovannoni, J.J., DellaPenna, D., Bennett, A.B., Fischer, R.L., 1989. Expression of a chimeric polygalacturonase gene in transgenic *rin* (ripening inhibitor) tomato fruits results in polyuronide degradation but not fruit softening. Plant Cell 1, 53-63.

Glenn, G.M., Poovaiah, B.W., 1990. Calcium-mediated postharvest changes in texture and cell wall structure and composition in 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 115, 962-968.

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., Thom, D., 1973. Biological interactions between polysaccharides and divalent cations: the egg-box model. FEBS Letters 32, 195-198.

Greene, D.W., 1995. Thidiazuron effects on fruit set, fruit quality, and return bloom of apples. HortScience 30, 1238-1240.

Gross, K.C., 1985. Promotion of ethylene evolution and ripening of tomato fruit by galactose. Plant Physiol. 79, 306-307.

Gross, K.C., Starrett, D.A., Chen, H.J., 1995. Rhamnogalacturonase, α-galactosidase and β-galactosidase: potential roles in fruit softening. Acta Hortic. 398, 121-130.

Halder-Doll, H., Bangerth, F., 1987. Inhibition of autocatalytic C_2H_4-biosynthesis by AVG applications and consequences on the physiological behaviour and quality of apple fruits in cool storage. Sci. Hortic. 33, 87-96.

Haller, M.H., 1941. Fruit pressure testers and their practical applications. USDA Circular 627.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortScience 27, 1291-1294.

Harker, F.R., Maindonald, J.H., Jackson, P.J., 1996. Penetrometer measurement of apple and kiwifruit firmness: operator and instrument differences. J. Am. Soc. Hortic. Sci. 121, 927-936.

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., 1997a. Texture of fresh fruit. Hortic. Rev. 20, 121-224.

Harker, F.R., Stec, M.G.H., Hallett, I.C., Bennett, C.L., 1997b. Texture of parenchymatous plant tissue: a comparison between tensile and other instrumental and sensory measurements of tissue strength and juiciness. Postharvest Biol. Technol. 11, 63-72.

Harriman, R.W., Tieman, D.M., Handa, A.K., 1991. Molecular cloning of tomato pectin methylesterase gene and its expression in Rutgers, ripening inhibitor, nonripening, and never ripe tomato fruits. Plant Physiol. 97, 80-87.

Hatfield, S.G.S., Knee, M., 1988. Effects of water loss on apples in storage. Int. J. Food Sci. Technol. 23, 575-583.

HortResearch., 2000. Horticultural facts and figures. The Horticulture and Food Research Institute of New Zealand, Palmerston North, New Zealand.

Ingle, M., D'Souza, M.C., Townsend, E.C., 2000. Fruit characteristics of 'York' apples during development and after storage. HortScience 35, 95-98.

Ingle, M., Morris, J.C., 1989. Predicting firmness changes of 'Rome' apples in refrigerated storage. J. Am. Soc. Hortic. Sci. 114, 90-94.

Jeffery, P.B., Banks, N.H., 1994. Firmness-temperature coefficient of kiwifruit. New Zeal. J. Crop Hortic. Sci. 22, 97-101.

Jiang, Y., Joyce, D.C., Macnish, A.J., 2000. Effect of abscisic acid on banana fruit ripening in relation to the role of ethylene. J. Plant Growth Reg. 19, 106-111.

Johnson, D.S., 1994. Influence of time of flower and fruit thinning on the firmness of 'Cox's Orange Pippin' apples at harvest and after storage. J. Hortic. Sci. 69, 197-203.

Johnson, D.S., 2000. Mineral composition, harvest maturity and storage quality of 'Red Pippin', 'Gala' and 'Jonagold' apples. J. Hortic. Sci. Biotechnol. 75, 697-704.

Johnson, D.S., Marks, M.J., Pearson, K., 1987. Storage quality of Cox's Orange Pippin apples in relation to fruit mineral composition during development. J. Hortic. Sci. 62, 17-25.

Johnson, D.S., Ridout, M.S., 1998. Prediction of storage quality of 'Coxs Orange Pippin' apples from nutritional and meteorological data using multiple regression models selected by cross validation. J. Hortic. Sci. Biotechnol. 73, 622-630.

Kahn, A.A., Vincent, J.F.V., 1990. Anistropy of apple parenchyma. J. Sci. Food Agric. 52, 455-466.

Khurshid, T., McNeil, D.L., Trought, M.C.T., 1997. Effect of foliar-applied gibberellins and soil-applied paclobutrazol on fruit quality at harvest and during storage of 'Braeburn' apples growing under a high-density planting system. New Zeal. J. Crop Hortic. Sci. 25, 59-65.

Kidd, F., West, C., 1933. Gas storage of fruit. III. Lane's Prince Albert apples. J. Pomol. Hortic. Sci. 11, 149-170.

Kidd, F., West, C., 1936. Gas-storage of fruit IV. Cox's Orange Pippin apples. J. Pomol. Hortic. Sci. 14, 276-294.

Kidd, F., West, C., 1939. The controlling influence of carbon dioxide VI. The effect of the tension of oxygen and of carbon dioxide in the atmosphere upon the course of chemical change in stored apples. New Phytol. 38, 105-122.

Kim, H.O., Hewett, E.W., Lallu, N., 1999. The role of ethylene in kiwifruit softening. Acta Hortic. 498, 255-261.

King, G.A., Henderson, K.G., Lill, R.E., 1989. Ultrastructural changes in the nectarine cell wall accompanying ripening and storage in a chilling-resistant and chilling-sensitive cultivar. New Zeal. J. Crop Hortic. Sci. 17, 337-344.

Klee, H., Tieman, D., Lashbrook, C., 1999. Ethylene perception in tomato: lots of genes, lots of functions. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 351-356.

Klein, J.D., Hanzon, J., Irwin, P.L., Ben-Shalom, N., Lurie, S., 1995. Pectin esterase activity and pectin methyl esterification in heated golden delicious apples. Phytochem. 39, 491-494.

Klein, J.D., Lurie, S., 1990. Prestorage heat treatment as a means of improving poststorage quality of apples. J. Am. Soc. Hortic. Sci. 115, 265-269.

Klein, J.D., Lurie, S., 1992. Prestorage heating of apple fruit for enhanced postharvest quality: interaction of time and temperature. HortScience 27, 326-328.

Klein, J.D., Lurie, S., 1994. Time, temperature, and calcium interact in scald reduction and firmness retention in heated apples. HortScience 29, 194-195.

Klein, J.D., Lurie, S., Ben-Arie, R., 1990. Quality and cell wall components of 'Anna' and 'Granny Smith' apples treated with heat, calcium, and ethylene. J. Am. Soc. Hortic. Sci. 115, 954-958.

Knee, M., 1973. Polysaccharide changes in cell walls of ripening apples. Phytochem. 12, 1543-1549.

Knee, M., 1976. Influence of ethylene on the ripening of stored apples. J. Sci. Food Agric. 27, 383-392.

Knee, M., 1978a. Metabolism of polymethylgalacturonate in apple fruit cortical tissue during ripening. Phytochem. 17, 1261-1264.

Knee, M., 1978b. Properties of polygalacturonate and cell cohesion in apple fruit cortical tissue. Phytochem. 17, 1257-1260.

Knee, M., 1980. Physiological responses of apple fruits to oxygen concentrations. Ann. App. Biol. 96, 243-253.

Knee, M., 1982. Fruit softening III. Requirement for oxygen and pH effects. J. Exp. Bot. 33, 1263-1269.

Knee, M., Farman, D., 1989. Sources of variation in the quality of CA stored apples. Proc. Fifth Int. CA Res. Conf., Wenatchee, Washington, USA, pp. 255-261.

Knee, M., Smith, S.M., 1989. Variation in quality of apple fruits stored after harvest on different dates. J. Hortic. Sci. 64, 413-419.

Kramer, G.F., Wang, C.Y., Conway, W.S., 1989. Correlation of reduced softening and increased polyamine levels during low-oxygen storage of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 114, 942-946.

Kramer, G.F., Wang, C.Y., Conway, W.S., 1991. Inhibition of softening by polyamine application in 'Golden Delicious' and 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 116, 813-817.

KunSong, C., Fang, L., ShangLong, Z., Ross, G.S., 1999. Role of abscisic acid and indole-3-acetic acid in kiwifruit ripening. Acta Hortic. Sinica 26, 81-86.

Kupferman, E., 1997. Controlled atmosphere storage of apples. Seventh Int. CA Res. Conf., July 13-18, 1997, University of California, Davis, pp. 1-30.

Landfald, R., 1966. Temperature effects on apples during storage. Bull. Int. Instit. Refrig. Ann. 1966-1, 453-460.

Lapsley, K.G., Escher, F.E., Hoehn, E., 1992. The cellular structure of selected apple varieties. Food Structure 11, 339-349.

Lara, I., Vendrell, M., 1998. Relationships between ethylene, abscisic acid and quality during postharvest storage of 'Granny Smith' apples. Postharvest Biol. Technol. 13, 11-18.

Lara, I., Vendrell, M., 2000. Development of ethylene-synthesizing capacity in preclimacteric apples: interaction between abscisic acid and ethylene. J. Am. Soc. Hortic. Sci. 125, 505-512.

Lau, O.L., Liu, Y., Yang, S.F., 1986. Effects of fruit detachment on ethylene biosynthesis and loss of flesh firmness, skin color, and starch in ripening 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 111, 731-734.

Lau, O.L., Looney, N.E., 1978. Effects of a pre-storage high CO_2 treatment on British Columbia and Washington State 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 103, 341-344.

Legge, R.L., Thompson, J.E., Baker, J.E., Lieberman, M., 1982. The effect of calcium on the fluidity and phase properties of microsomal membranes isolated from postclimacteric Golden Delicious apples. Plant Cell Physiol. 23, 161-169.

Lehman-Salada, L., 1996. Instrument and operator effects on apple firmness readings. HortScience 31, 994-997.

Lelievre, J.-M., Latche, A., Jones, B., Bouzayen, M., Pech, J.C., 1997. Ethylene and fruit ripening. Physiol. Plant. 101, 727-739.

Leshem, Y.A.Y., Ku, V.V., Wills, R.B.H., 1998. Evidence for the function of the free radical gas - nitric oxide (NO) - as an endogenous maturation and senescence regulating factor in higher plants. Plant Physiol. Biochem. 36, 825-833.

Lidster, P.D., 1982. Low oxygen atmospheres to maintain apple quality in storage. Proc. Third Nat. CA Res. Conf., 22-24 July, 1981, Oregon State University, pp. 109-120.

Lidster, P.D., 1990. Storage humidity influences fruit quality and permeability to ethane in 'McIntosh' apples stored in diverse controlled atmospheres. J. Am. Soc. Hortic. Sci. 115, 94-96.

Lidster, P.D., Porritt, S.W., 1978. Influence of maturity and delay of storage on fruit firmness and disorders in 'Spartan' apple. HortScience 13, 253-254.

Lidster, P.D., McRae, K.B., Johnston, E.M., 1985. Retention of apple quality in low-oxygen storage followed by standard controlled atmosphere regimens. J. Am. Soc. Hort. Sci. 110, 755-759.

Lidster, P.D., Lougheed, E.C., McRae, K.B., 1987. Effects of sequential low-oxygen and standard controlled atmosphere storage regimes on apple quality. J. Am. Soc. Hort. Sci. 112, 787-793.

Lin, T-T., Pitt, R.E., 1986. Rheology of apple and potato tissue as affected by cell turgor pressure. J. Text. Stud. 17, 291-313.

Little, C.R., Faragher, J.D., Taylor, H.J., 1982. Effects of initial oxygen stress treatments in low oxygen modified atmosphere storage of 'Granny Smith' apples. J. Am. Soc. Hort. Sci. 107, 320-323.

Littmann, M.D., 1972. Effect of water loss on the ripening of climacteric fruits. Queensland J. Agric. Animal Sci. 29, 103-113.

Liu, F.W., 1977. Varietal and maturity differences of apples in response to ethylene in controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 102, 93-95.

Liu, F.W., 1978a. Effects of harvest date and ethylene concentration in controlled atmosphere storage on the quality of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 103, 388-392.

Liu, F.W., 1978b. Modification of apple quality by high temperature. J. Am. Soc. Hortic. Sci. 103, 730-732.

Liu, F.W., 1985. Conditions for low ethylene CA storage of apples: a review. Proc. Fourth Nat. CA Res. Conf., 23-26 July, 1985, Raleigh, North Carolina, USA, pp. 127-134.

Liu, F.W., King, M.M., 1978. Consumer evaluation of 'McIntosh' apple firmness. HortScience 13, 162-163.

Liu, Y., Su, L.-Y., Yang, S.F., 1985. Ethylene promotes the capability to malonylate 1-aminocyclopropane-1-carboxylic acid and D-amino acids in preclimacteric tomato fruits. Plant Physiol. 77, 891-895.

Looney, N.E., 1971. Interaction of ethylene, auxin, and succinic acid-2,2-dimethylhydrazide in apple fruit ripening control. J. Am. Soc. Hortic. Sci. 96, 350-353.

Luo, Y., Wainwright, H., Moore, K.G., 1989. Effects of orchard applications of paclobutrazol on the composition and firmness of apple fruits. Sci. Hortic. 39, 301-309.

Lurie, S., 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14, 257-269.

Lurie, S., Ben-Arie, R., 1983. Microsomal membrane changes during the ripening of apple fruit. Plant Physiol. 73, 636-638.

Lurie, S., Klein, J.D., 1990. Heat treatment of ripening apples: differential effects on physiology and biochemistry. Physiol. Plant. 78, 181-186.

Lurie, S., Klein, J.D., 1992. Calcium and heat treatments to improve storability of 'Anna' apples. HortScience 27, 36-39.

Lurie, S., Nussinovitch, A., 1996. Compression characteristics, firmness, and texture perception of heat treated and unheated apples. Int. J. Food Sci. Technol. 31, 1-5.

Lurie, S., Othman, S., Borochov, A., 1995. Effects of heat treatment on plasma membrane of apple fruit. Postharvest Biol. Technol. 5, 29-38.

Lurie, S., Sonego, L., Ben-Arie, R., 1987. Permeability, microviscosity and chemical changes in the plasma membrane during storage of apple fruit. Sci. Hortic. 32, 73-83.

Maclachlan, G., Brady, C., 1994. Endo-1,4-b-glucanase, xyloglucanase, and xyloglucan endo-transglycosylase activities versus potential substrates in ripening tomatoes. Plant Physiol. 105, 965-974.

MacRae, E.A., Lallu, N., Searle, A.N., Bowen, J.H., 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49, 413-430.

MacRae, E.A., Redgwell, R.J., 1992. Softening in kiwifruit. Postharvest News Inform. 3, 49N-52N.

MacRae, E.A., Redgwell, R.J., Wegrzyn, T., 1990. The whens and hows of fruit softening. New Zeal. Kiwifruit 64, 15,17.

Magness, J.R., Diehl, H.C., 1924. Physiological studies on apples in storage. J. Agric. Res. 27, 1-38.

Marangoni, A.G., Palma, T., Stanley, D.W., 1996. Membrane effects in postharvest physiology. Postharvest Biol. Technol. 7, 193-217.

Marmo, C.A., Bramlage, W.J., Weis, S.A., 1985. Effects of fruit maturity, size, and mineral concentrations on predicting the storage life of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 110, 499-502.

Mason, J.L., Drought, B.G., McDougald, J.M., 1974. Effect of a calcium chloride dip on senescent breakdown, firmness and calcium concentration in 'Spartan' apple. HortScience 9, 596.

McMurchie, E.J., McGlasson, W.B., Eaks, I.L., 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237, 235-236.

Meheriuk, M., Porritt, S.W., Lidster, P.D., 1977. Effects of carbon dioxide treatment on controlled atmosphere storage behavior of McIntosh apples. Can. J. Plant Sci. 57, 457-460.

Miyazaki, J.H., Yang, S.F., 1987. The methionine salvage pathway in relation to ethylene and polyamine biosynthesis. Physiol. Plant. 69, 366-370.

Mohsenin, N.N., Mittal, J.P., 1977. Use of rheological terms and correlation of compatible measurements in food texture research. J. Text. Stud. 8, 395-408.

Nara, K., Kato, Y., Motomura, Y. 2001. Involvement of terminal-arabinose and -galactose pectic compounds in mealiness of apple fruit during storage. Postharvest Biol. Technol. 22, 141-150.

O'Donoghue, E.M., Huber, D.J., 1992. Modification of matrix polysaccharides during avocado (*Persea americana*) fruit ripening: an assessment of the role of Cx-cellulase. Physiol. Plant. 86, 33-42.

Oetiker, J.H., Yang, S.F., 1995. The role of ethylene in fruit ripening. Acta Hortic. 398, 167-178.

Olsen, K.L., Martin, G.C., 1980. Influence of apple bloom date on maturity and storage quality of 'Starking Delicious' apples. J. Am. Soc. Hortic. Sci. 105, 183-186.

Paliyath, G., Poovaiah, B.W., Munske, G.R., Magnuson, J.A., 1984. Membrane fluidity in senescing apples: effects of temperature and calcium. Plant Cell Physiol. 25, 1083-1087.

Payton, S., Fray, R.G., Brown, S., Grierson, D., 1996. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. Plant Mol. Biol. 31, 1227-1231.

Pech, J.C., Balague, C., Latche, A., Bouzayen, M., 1994. Postharvest physiology of climacteric fruits: recent developments in the biosynthesis and action of ethylene. Sci. Ali. 14, 3-15.

Pech, J.C., Guis, M., Botondi, R., Ayub, R., Bouzayen, M., Lelievre, J.M., El Yahyaoui, F., Latche, A., 1999. Ethylene-dependent and ethylene-independent pathways in a climacteric fruit, the melon. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 105-110.

Percy, A.E., Melton, L.D., Jameson, P.E., 1997. Xyloglucan and hemicelluloses in the cell wall during apple fruit development and ripening. Plant Sci. 125, 31-39.

Percy, A.E., O'Brien, I.E.W., Jameson, P.E., Melton, L.D., MacRae, E.A., Redgwell, R.J., 1996. Xyloglucan endotransglycosylase activity during fruit development and ripening of apple and kiwifruit. Physiol. Plant. 96, 43-50.

Picchioni, G.A., Watada, A.E., Conway, W.S., Whitaker, B.D., Sams, C.E., 1995. Phospholipid, galactolipid, and steryl lipid composition of apple fruit cortical tissue following postharvest $CaCl_2$ infiltration. Phytochem. 39, 763-769.

Plocharski, W.J., Konopacka, D., 1999. The relation between mechanical and sensory parameters of apples and pears. Acta Hortic. 485, 309-317.

Pollard, J.E., 1974. Effects of SADH, ethephon and 2,4,5-T on colour and storage quality of "McIntosh" apples. J. Am. Soc. Hortic. Sci. 99, 341-343.

Poovaiah, B.W., Glenn, G.M., Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hortic. Rev. 10, 107-152.

Porritt, S.W., Lidster, P.D., 1978. The effect of pre-storage heating on ripening and senescence of apples during cold storage. J. Am. Soc. Hortic. Sci. 103, 584-587.

Porritt, S.W., Meheriuk, M., 1973. Influence of storage humidity and temperature on breakdown in Spartan apples. Can. J. Plant Sci. 53, 597-599.

Pratt, C., 1988. Apple flower and fruit: morphology and anatomy. Hortic. Rev. 10, 273-308.

Raese, J.T., Drake, S.R., 1993. Effects of preharvest calcium sprays on apple and pear quality. J. Plant Nut. 16, 1807-1819.

Redgwell, R.J., Fischer, M., Kendal, E., MacRae, E.A., 1997a. Galactose loss and fruit ripening: high-molecular-weight arabinogalactans in the pectic polysaccharides of fruit cell walls. Planta 203, 174-181.

Redgwell, R.J., MacRae, E.A., Hallett, I.C., Fischer, M., Perry, J., Harker, F.R., 1997b. In vivo and in vitro swelling of cell walls during fruit ripening. Planta 203, 162-173.

Reeve, R.M., 1953. Histological investigations of texture in apples. II Structure and intercellular spaces. Food Res. 18, 604-617.

Robertson, R.N., Turner, J.F., 1951. The physiology of growth in apple fruits. II Respiratory and other metabolic activities as functions of cell number and cell size in fruit development. Australian J. Sci. Res. B4, 92-107.

Rose, J.K.C., Bennett, A.B., 1999. Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening. Trends Plant Sci. 4, 176-183.

Rose, J.K.C., Hadfield, K.A., Labavitch, J.M., Bennett, A.B., 1998. Temporal sequence of cell wall disassembly in rapidly ripening melon fruit. Plant Physiol. 117, 345-361.

Ross, G.S., Wegrzyn, T., MacRae, E.A., Redgwell, R.J., 1994. Apple β-galactosidase. Plant Physiol. 106, 521-528.

Rupasinghe, H.P.V., Murr, D.P., Paliyath, G., Skog, L., 2000. Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. J. Hortic. Sci. Biotechnol. 75, 271-276.

Saftner, R.A., Conway, W.S., Sams, C.E., 1998. Effect of postharvest calcium chloride treatments on tissue water relations, cell wall calcium levels and postharvest life of 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 123, 893-897.

Saltveit, M.E., 1984. Effects of temperature on firmness and bruising of 'Starkrimson Delicious' and 'Golden Delicious' apples. HortScience 19, 550-551.

Sams, C.E., 1999. Preharvest factors affecting postharvest texture. Postharvest Biol. Technol. 15, 249-254.

Sams, C.E., Conway, W.S., 1984. Effect of calcium infiltration on ethylene production, respiration rate, soluble polyuronide content, and quality of 'Golden Delicious' apple fruit. J. Am. Soc. Hortic. Sci. 109, 53-57.

Sams, C.E., Conway, W.S., Abbott, J.A., Lewis, R.J., Ben-Shalom, N., 1993. Firmness and decay of apples following postharvest pressure infiltration of calcium and heat treatment. J. Am. Soc. Hortic. Sci. 118, 623-627.

Sharples, R.O., Munoz, G.C., 1974. The effects of delays in the period taken to cool and establish low oxygen conditions on the quality of stored Cox's Orange Pippin apples. J. Hortic. Sci. 49, 277-286.

Siddiqui, S., Bangerth, F., 1995. Effect of pre-harvest application of calcium on flesh firmness and cell wall composition of apples - influence of fruit size. J. Hortic. Sci. 70, 263-269.

Siddiqui, S., Bangerth, F., 1996. The effect of calcium infiltration on structural changes in cell walls of stored apples. J. Hortic. Sci. 71, 703-708.

Siddiqui, S., Brackmann, A., Streif, J., Bangerth, F., 1996. Controlled atmosphere storage of apples: cell wall composition and fruit softening. J. Hortic. Sci. 71, 613-620.

Sirit, Y., Bennett, A.B., 1998. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a re-examination. Plant Physiol. 116, 1145-1150.

Sisler, E.C., Serek, M., 1999. Control of ethylene responses at the receptor level. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C., Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 45-50.

Smith, C.J.S., Watson, C.F., Morris, P.C., Bird, C.R., Seymour, G.B., Gray, J.E., Arnold, C., Tucker, G.A., Schuch, W., Harding, D., Grierson, D., 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Mole. Biol. 14, 369-379.

Smith, W.H., 1940. The histological structure of the flesh of the apple in relation to growth and senescence. J. Pomol. Hortic. Sci. 18, 249-260.

Smith, W.H., 1950. Cell-multiplication and cell-enlargement in the development of the flesh of the apple fruit. Ann. Bot. 53, 23-38.

Smock, R.M., Blanpied, G.D., 1963. Some effects of temperature and rate of oxygen reduction on the quality of controlled atmosphere stored McIntosh apples. Proc. Am. Soc. Hortic. Sci. 83, 135-138.

Sozzi, G.O., Camperi, S.A., Cascone, O., Fraschina, A.A., 1998. Galactosidases in tomato fruit ontogeny: decreased galactosidase activities in antisense ACC synthase fruit during ripening and reversal with exogenous ethylene. Australian J Plant Physiol. 25, 237-244.

Stow, J., 1989a. The involvement of calcium ions in maintenance of apple fruit tissue structure. J. Exp. Bot. 40, 1053-1057.

Stow, J.R., 1989b. The response of apples cv. Cox's Orange Pippin to different concentrations of oxygen in the storage atmosphere. Ann. App. Biol. 114, 149-156.

Stow, J., 1990. The effects of removal of ethylene from low oxygen storage atmospheres on the quality of Coxs Orange Pippin apples. Sci. Hortic. 43, 281-290.

Stow, J., 1993. Effect of calcium ions on apple fruit softening during storage and ripening. Postharvest Biol. Technol. 3, 1-9.

Stow, J., Dover, C.J., Genge, P.M., 2000. Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18, 215-225.

Stow, J.R., Genge, P., 2000. The effects of storage conditions on the keeping quality of 'Gala' apples. J. Hortic. Sci. Biotechnol. 75, 393-399.

Tetley, U., 1930. A study of the anatomical development of the apple and some observations on the "pectic constituents" of the cell-walls. J. Pomol. Hortic. Sci. 8, 153-172.

Tetley, U., 1931. The morphology and cytology of the apple fruit, with special reference to the Bramleys seedling variety. J. Pomol. Hortic. Sci. 9, 278-297.

Thompson, J.E., Froese, C.D., Hong, Y., Hudak, K.A., Smith, M.D., 1997. Membrane deterioration during senescence. Can. J. Bot. 75, 867-879.

Tieman, D.M., Taylor, M.G., Ciardi, J.A., Klee, H.J., 2000. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. Proc. Nat. Acad. Sci. USA. 97, 5663-5668.

Tong, C., Krueger, D., Vickers, Z., Bedford, D., Luby, J., El-Shiekh, A., Shackel, K., Ahmadi, H., 1999. Comparison of softening-related changes during storage of 'Honeycrisp' apple, its parents, and 'Delicious'. J. Am. Soc. Hortic. Sci. 124, 407-415.

Tong, C., Labavitch, J.M., Yang, S.F., 1986. The induction of ethylene production from pear cell culture by cell wall fragments. Plant Physiol. 81, 929-930.

Tong, C.B.S., Gross, K.C., 1990. Stimulation of ethylene production by a cell wall component from mature green tomato fruit. Physiol. Plant. 80, 500-506.

Tu, K., De Baerdemaeker, J., Deltour, R., De Barsy, T., 1996. Monitoring post-harvest quality of Granny Smith apple under simulated shelf-life conditions: destructive, non-destructive and analytical measurements. Int. J. Food Sci. Technol. 31, 267-276.

Tu, K., Nicolai, B., De Baerdemaeker, J., 2000. Effects of relative humidity on apple quality under simulated shelf temperature storage. Sci. Hortic. 85, 217-229.

Tu, K., Waldron, K., Ingham, L., De Barsy, T., De Baerdemaeker, J., 1997. Effect of picking time and storage conditions on 'Coxs Orange Pippin' apple texture in relation to cell wall changes. J. Hortic. Sci. 72, 971-980.

Tukey, H.B., Young, J.O., 1942. Gross morphology and histology of developing fruit of the apple. Bot. Gaz. 104, 3-25.

Vendrell, M., Palomer, X., 1998. Hormonal control of fruit ripening in climacteric fruits. Acta Hortic. 463, 325-334.

Vincent, J.F.V., 1989. Relationship between density and stiffness of apple flesh. J. Sci. Food Agric. 47, 443-462.

Voisey, P.W., 1977. Examination of operational aspects of fruit pressure tests. J. Inst. Can. Sci. Technol. Aliment. 10, 284-294.

Wakabayashi, K., 2000. Changes in cell wall polysaccharides during fruit ripening. J. Plant Res. 113, 231-237.

Wallner, S.J., 1978. Apple fruit β-galactosidase and softening in storage. J. Am. Soc. Hortic. Sci. 103, 364-366.

Wang, C.Y., Conway, W.S., Abbott, J.A., Kramer, G.F., 1993. Postharvest infiltration of polyamines and calcium influences ethylene production and texture changes in 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 118, 801-806.

Wang, C.Y., Steffens, G.L., 1987. Postharvest responses of 'Spartan' apples to preharvest paclobutrazol treatment. HortScience 22, 276-278.

Wang, H.G., Gemma, H., Oogaki, C., Kurokawa, M., 1990. Influence of harvest maturity on chilling sensitivity, respiratory rate and ethylene production during storage in 'Sensyu' apple fruit. J. Jap. Soc. Hortic. Sci. 59, 163-169.

Wang, Z.Y., MacRae, E.A., Wright, M.A., Bolitho, K.M., Ross, G.S., Atkinson, R.G., 2000. Polygalacturonase gene expression in kiwifruit: relationship to fruit softening and ethylene production. Plant Mol. Biol. 42, 317-328.

Watada, A.E., Herner, R.C., Kader, A.A., Romani, R.J., Staby, G.L., 1984. Terminology for the description of developmental stages of horticultural crops. HortScience 19, 20-21.

Watkins, C.B., Hewett, E.W., Bateup, C., Gunson, A., Triggs, C.M., 1989. Relationships between maturity and storage disorders in 'Cox's Orange Pippin' apples as influenced by preharvest calcium or ethephon sprays. New Zeal. J. Crop Hortic. Sci. 17, 283-292.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Wills, R.B.H., Bambridge, P.A., Scott, K.J., 1980. Use of flesh firmness and other objective tests to determine consumer acceptability of delicious apples. Australian J. Exp. Agric. Animal Husbandry 20, 252-256.

Wu, Q., Szakacs-Dobozi, M., Hemmat, M., Hzrazdina, G., 1993. Endopolygalacturonase in apples (*Malus domestica*) and its expression during fruit ripening. Plant Physiol. 102, 219-225.

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35, 155-189.

Yoshioka, H., Aoba, K., Kashimura, Y., 1992. Molecular weight and degree of methoxylation in cell wall polyuronide during softening in pear and apple fruit. J. Am. Soc. Hortic. Sci. 117, 600-606.

Yoshioka, H., Kashimura, Y., Kaneko, K., 1995. β-D-galactosidase and α-L-arabinofuranosidase activities during the softening of apples. J. Jap. Soc. Hortic. Sci. 63, 871-878.

Zarembinski, T.I., Theologis, A., 1994. Ethylene biosynthesis and action: a case of conservation. Plant Mol. Biol. 26, 1579-1597.

Chapter 2 General materials and methods.

Due to the format of this thesis, materials and methods are described in later result-containing chapters (3 to 9) and therefore will not be described here. Locations for descriptions of materials and methods utilised for each variable are specified in Table 2-1. Fruit supply information is also specified within each result-containing chapter.

Table 2-1 Locations for descriptions of materials and methods used in this thesis.

Variable category	Variable	Location (section: *pg*)
Texture tests	Flesh firmness	3.3.5: *61*
	Cortical tensile strength	3.3.5: *61*
	Fruit density	5.3.2: *96*
Indicators of climacteric development	Internal ethylene concentration	4.3.2: *77*
	Respiration rate	5.3.2: *96*
Harvest indices	Background skin colour (Hue°)	5.3.2: *96*
	Total soluble solids	7.3.2: *132*
	Starch pattern index	7.3.2: *132*
	Titratable acidity	7.3.2: *132*
Carbohydrates	Starch concentration	8.3.2: *150*
	Sucrose, glucose and fructose	8.3.2: *150*
Mineral concentrations	Calcium, magnesium and potassium	8.3.2: *150*
	Nitrogen and phosphorus	8.3.2: *150*
Gas concentrations	Oxygen and carbon dioxide	9.3.1: *172*

Chapter 3 Physical change in apple texture with fruit temperature: effect of cultivar and time in storage.

3.1 Abstract

Flesh firmness is used to assess apple (*Malus domestica* Borkh.) quality both before and after low temperature storage. The effect of fruit temperature on determination of apple firmness at different times during postharvest handling is not known. Experiments were conducted to quantify physical change in apple texture with change in fruit temperature. 'Royal Gala', 'Granny Smith', and 'Pacific Rose™' apple cultivars were stored at 0°C, while 'Cox's Orange Pippin' was stored at 3°C. At different times during storage, flesh firmness and cortical tensile strength were measured on fruit at the storage temperature, after 24 hours at 20°C, or after 24 hours at 20°C followed by 24 hours at the storage temperature. 'Royal Gala', 'Granny Smith' and 'Cox's Orange Pippin' fruit were firmer at harvest, and softer after 50-100 days, when measured at 20°C than at 0-3°C. 'Pacific Rose™' had similar firmness and tensile strength when measured at 0°C and 20°C. 'Royal Gala' and 'Cox's Orange Pippin' were measured for firmness at different fruit temperatures at harvest and after storage. The relationship between firmness readings and fruit temperature between 0°C and 20°C was linear and positive at harvest and linear and negative for stored fruit. Firmness change with temperature was not affected by orchard or harvest maturity. These results suggest that physical changes in firmness with fruit temperature are common for the cultivars studied, and thus could be used to compare firmness values for fruit from different orchards that were measured at different temperatures.

Keywords: Malus domestica (Borkh.); Firmness; Tensile strength; Quality; Water loss; Maturity

3.2 Introduction

Apple texture is collectively influenced by many quality attributes, one of which is flesh firmness. Apple firmness is normally measured destructively using a penetrometer, where firmer fruit are generally considered to have better quality characteristics than softer fruit (Harker et al., 1997a). Commercially, firmness is used in combination with several other harvest indices to identify harvest dates for optimum maturity and storage

potential. Firmness is also used for allocating quality grades at harvest, and for assessing quality during storage. Thus, as firmness is such an important commercial indicator of apple quality, it is essential to understand how measurement conditions, such as fruit temperature, affect firmness.

There are conflicting results in the literature on the effect of fruit temperature on firmness of apples (Haller, 1941; Blanpied et al., 1978; Bourne, 1982; Saltveit, 1984). Saltveit (1984) found that 'Starkrimson Delicious' and 'Golden Delicious' individually had similar firmness when measured at either 0 or 20°C, and Haller (1941) found that 'Rome', 'Gallia', 'Wine Sap' and 'Stayman' individually had similar firmness at both 1 and 25°C. In contrast, Bourne (1982) showed that several apple cultivars were firmer when measured at 0°C than at 45°C, and Blanpied et al. (1978) found that 'Rome', 'Idared' and 'R.I. Greening' apples at 2°C were firmer than at 21°C. These inconsistent results could be explained if the magnitude of the physical effect of temperature on firmness changes with time at 0°C, as occurs with kiwifruit (Jeffery and Banks, 1994). The aim of this study was to determine whether temperature physically affects apple firmness with storage time, cultivar, orchard and harvest maturity as experimental factors. Cortical tensile strength was compared to firmness for physical effects of temperature during storage, as these techniques measure different textural properties of fruit (Harker and Hallett, 1992; Harker et al., 1997b).

3.3 Materials and methods

3.3.1 Storage time experiment

The objective of this experiment was to characterise the physical effect of fruit temperature on firmness and tensile strength readings at different times during storage. In 1998, export quality 'Royal Gala' (RG), 'Granny Smith' (GS), 'Pacific Rose™' (PR) and 'Cox's Orange Pippin' (COP) were transported from Hawkes Bay to Palmerston North, New Zealand within 48 hours of harvest. In 1999, RG and GS were obtained from Hawkes Bay within 48 hours of harvest, while PR and COP were obtained from storage (PR from Hawkes Bay after 21 days at 0°C, and COP from Nelson after 14 days at 3°C). While obtaining fruit after time in commercial storage in 1999 was less than optimal, storage conditions were known, and the effects of previous storage time on

firmness were accounted for using 1998 results. In both seasons, fruit were commercially grown, graded and packed. Fruit weight was 170±10g for RG and GS, 140±10g for COP, and 200±10g for PR. Fruit with excessive sunburn, yellow background skin colour, physical damage or skin greasiness were removed before storage at 0.5±0.5°C for RG, GS and PR, and at 3.0±0.5°C for COP. COP was stored in all experiments at 3°C to minimise low temperature breakdown.

Ten perforated polyethylene bags (35µm thickness; 50 x 5mm diameter perforations per m^2) each containing 18 fruit, and 20 bags each containing 30 fruit, were placed at 0°C in commercial Z-pack cartons (18kg) in 1998 and 1999 respectively. Three fruit were removed at regular intervals from each bag and randomly allocated to the following temperature regimes: storage temperature (f_1); 24 hours at 20°C (f_2), 24 hours at 20°C followed by 24 hours at 0°C (f_3), after which texture assessments were made. f_1 and f_3 were assessed at 3°C for COP. Texture was measured as flesh firmness in both seasons, with tensile strength measured in 1999 only.

3.3.2 Relationship between physical change in firmness and fruit temperature at harvest and after prolonged storage

The objective of this experiment was to determine if physical change in firmness was linear between 0°C and 20°C at harvest, and after prolonged storage at 0 or 3°C. In 1998, RG and COP fruit were randomly placed at 0.0, 2.5, 5.0, 12.0 or 20.0±0.5°C in perforated polyethylene bags (details above) within 48 hours of harvest. After 24 hours of storage, ten fruit had flesh firmness measured at the storage temperature.

In 2000, commercially grown and graded COP (Nelson) and RG (Hawkes Bay) were obtained within 72 hours of harvest, and stored at 0°C (3°C for COP) in perforated polyethylene bags (details above) in commercial Z-pack 18kg cartons. Once fruit were in the final slow softening phase (Chapter 4), 20 fruit were placed at 0.0°C (3.0°C for COP), 5.0, 10.0, 15.0 or 20.0±0.5°C. After 24 hours firmness was measured on 10 fruit at the storage temperature, while the remaining 10 fruit were transferred to 0°C (3°C for COP) for 24 hours before measuring firmness again.

3.3.3 Water loss experiment

Fruit water status was manipulated in an attempt to explain the physical firmness differences observed at harvest for fruit measured at different temperatures from the experiments detailed above. RG was harvested from the Fruit Crops Unit, Massey University, Palmerston North, and randomly allocated to 20°C non-pared, 0°C non-pared, and 0°C pared treatments (15 fruit per treatment) for 24 hours. Pared fruit had a 20x20 mm piece of skin removed from opposite sides of the fruit at the equator prior to being held at 0°C. Fruit from all treatments were weighed before and after 24 hours at either 0°C or 20°C, and firmness was measured after 24 hours at the storage temperature. Firmness of fruit in the pared 0°C treatment was measured after 24 hours at 0°C on tissue pared the previous day. For the intact (non-pared) treatments, fruit were pared immediately prior to firmness measurement.

3.3.4 Orchard and harvest date experiment

In 2000, effects of orchard and harvest date on the physical effect of temperature on firmness at harvest were investigated. To study effects of orchard, RG fruit from 6 Hawkes Bay orchards, and COP from 11 Nelson orchards were transported to Palmerston North within 72 hours of harvest. To study effects of harvest date, fruit were picked once before, three times during, and once after the commercial harvest period, at 7 to 14 day intervals from one Hawkes Bay orchard (RG and COP), from the Fruit Crops Unit, Massey University, Palmerston North (RG only), and from one Nelson Orchard (COP only). Firmness was measured on fifteen fruit from each orchard or harvest date after 24 hours at 0°C (3°C for COP) or 20°C.

3.3.5 Texture assessment

Flesh firmness was measured with a drill-press mounted 'Effegi' penetrometer fitted with an 11.1 mm diameter probe; the maximum force required to puncture pared tissue to a depth of 7.9 mm on opposite sides of the fruit equator was recorded.

Tensile strength was measured using the gripping method and tissue shape described by Stow (1989), and recorded as maximum force to separate a 10 by 5mm cross-sectional

area of tissue plug removed from the fruit cortex, at a crosshead speed of 1 $mm.s^{-1}$ with a Stable Micro Systems TA-XT2 Texture Analyser.

3.3.6 Data analysis

Analysis of variance and 5% least significant differences tests were performed using PROC GLM and the MEANS statement LSD option, linear regression using PROC REG, and t-tests using PROC TTEST in the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

3.4 Results

Firmness was not significantly different between f_1 and f_3 treatments for all cultivars regardless of time at 0°C (3°C for COP), except for COP after 1-3 days and 15 days at 3°C where f_3 had lower firmness than f_1 (Fig. 3-1). After 1-3 days at 0°C, f_2 had higher firmness than f_3 for RG and GS. However, from 100 to 200 days at 0°C, f_2 had lower firmness than f_3 for both RG and GS. For COP (that had 14 days at 3°C before the experiment started), f_2 had lower firmness than f_3 from 21 to 140 days at 3°C. There was no effect of temperature on PR firmness regardless of time at 0°C.

For all cultivars, f_1 had similar tensile strength to f_3 regardless of time at 0°C (Fig. 3-2). For GS, f_2 had higher tensile strength than f_3 after 22 days at 0°C, but had lower tensile strength than f_3 from 80 to 200 days at 0°C. For RG, f_2 had lower tensile strength than f_3 after 103, 143 and 206 days at 0°C. For PR, f_2 tensile strength was similar to f_3 regardless of time at 0°C, except after 206 days at 0°C where f_2 had lower tensile strength than f_3. For COP, f_2 had lower tensile strength than f_3 after 21, 43 and 59 days at 3°C. However, despite these statistical differences between tensile strength for COP measured at 3 or 20°C, these differences were relatively small when compared to RG and GS, and are probably commercially insignificant.

Firmness (Fig. 3-1) and tensile strength (Fig. 3-2) differences between f_2 and f_3 were used to calculate change with increasing temperature, in firmness (Δf_{temp}; $N.°C^{-1}$), and tensile strength (Δt_{temp}; $N.°C^{-1}$), after different times at 0 or 3°C (Fig. 3-3). After 1-3 days at 0°C (3°C for COP), Δf_{temp} was positive for RG, COP and GS. From 10 to 100

days at 0°C for RG and GS, and 10 to 50 days at 3°C for COP, Δf_{temp} was not significantly different from zero. From 100 to 200 days at 0°C for RG and GS, and 50 to 140 days at 3°C for COP, Δf_{temp} was negative. Throughout storage at 0°C, Δf_{temp} was not significantly different from zero for PR. However, like the other cultivars there was a tendency for PR Δf_{temp} to decrease with time at 0°C, but unlike the other cultivars Δf_{temp} did not become negative after 50-100 days at 0°C.

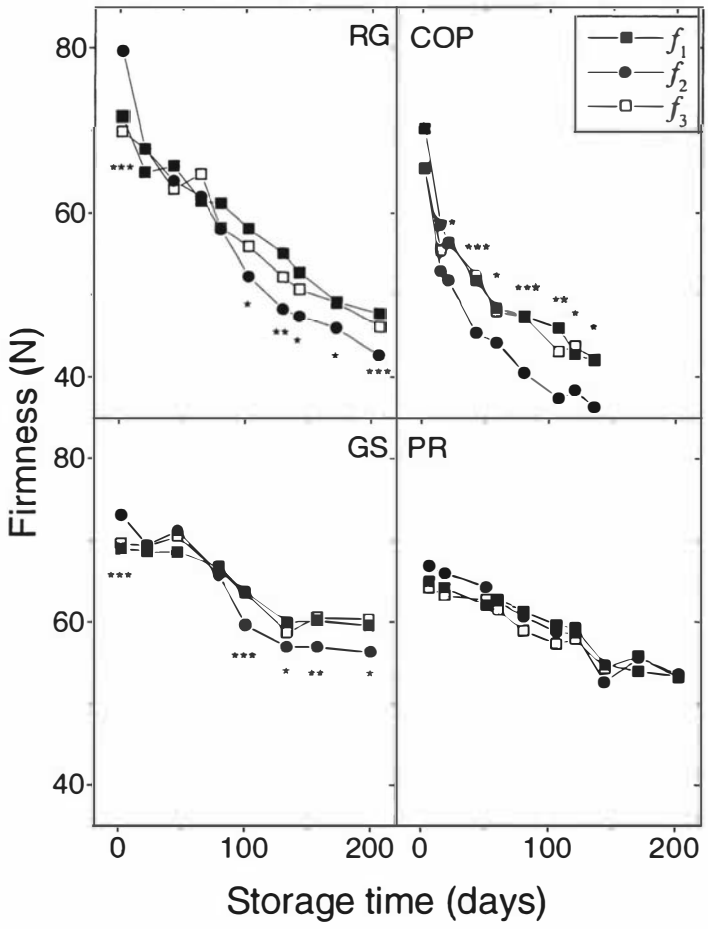

Fig. 3-1 Flesh firmness for 1999 'Royal Gala' (RG), 'Granny Smith' (GS), 'Pacific Rose' (PR) and 'Cox's Orange Pippin' (COP) measured at different fruit temperatures; 0°C (f_1), after removal from 0°C to 20°C for 24h (f_2), and after removal from 0°C to 20°C for 24h and returned to 0°C for 24h (f_3). f_1 and f_3 fruit were measured at 3°C for COP. Treatment means (n=20), and t-test significance levels (19 d.f. error) between f_2 and f_3 are shown. Significance levels were: $P>0.05$ (not significant); $P\leq0.05$ (*); $P\leq0.01$ (**); and $P\leq0.001$ (***).

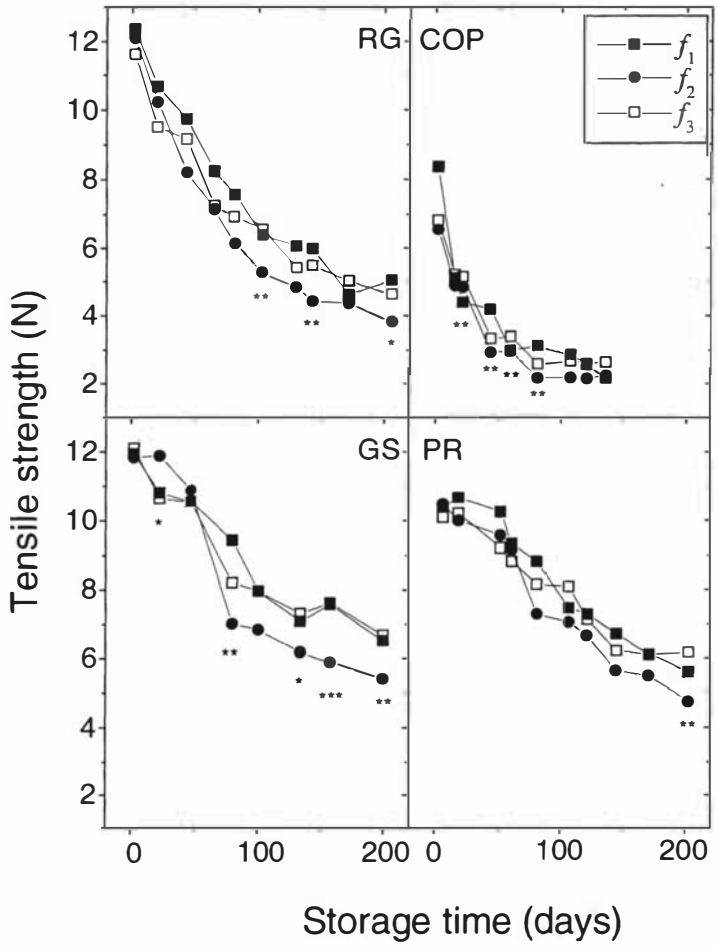

Fig. 3-2 Cortical tensile strength of 1999 'Royal Gala' (RG), 'Granny Smith' (GS), 'Pacific Rose' (PR) and 'Cox's Orange Pippin' (COP) measured at different fruit temperatures; 0°C (f_1), after removal from 0°C to 20°C for 24h (f_2), and after removal from 0°C to 20°C for 24h and returned to 0°C for 24h (f_3). f_1 and f_3 fruit were measured at 3°C for COP. Treatment means (n=20), and t-test significance levels (19 d.f. error) between f_2 and f_3 are shown. Significance levels were: $P>0.05$ (not significant); $P\leq0.05$ (*); $P\leq0.01$ (**); and $P\leq0.001$ (***).

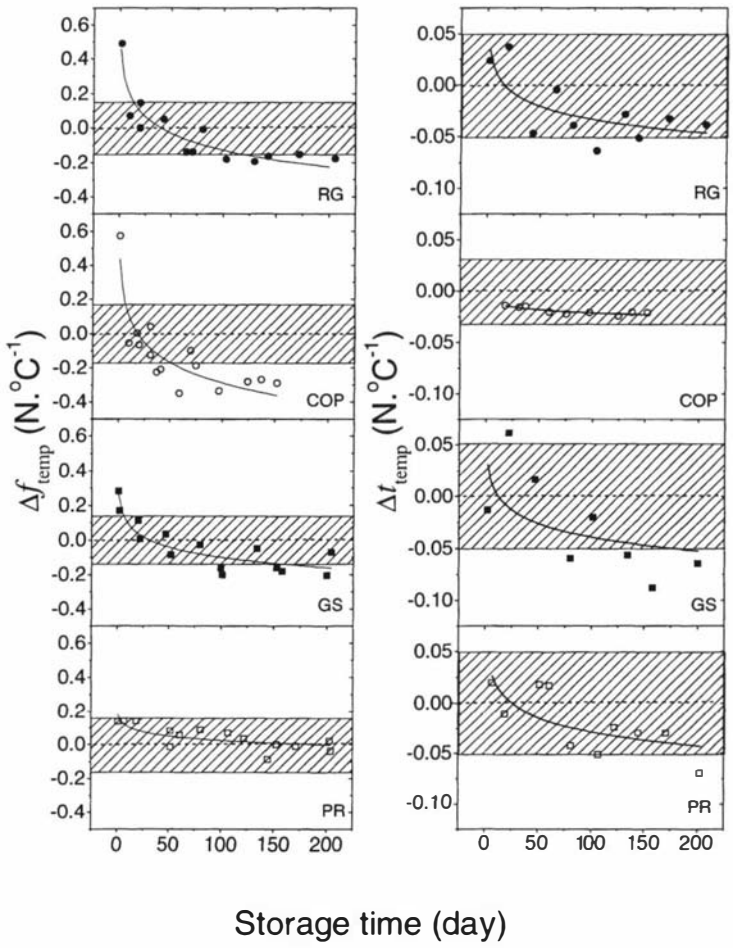

Fig. 3-3 Firmness change with increasing fruit temperature (Δf_{temp}) (1998 and 1999 season data), and tensile strength change with increasing fruit temperature (Δt_{temp}) (1999 season data), for 'Royal Gala' (RG), 'Granny Smith' (GS) and 'Pacific Rose' (PR) at 0°C, and 'Cox's Orange Pippin' (COP) at 3°C. Δf_{temp} and Δt_{temp} was calculated as Δfirmness or tensile strength ($f_2 - f_3$) / Δfruit temperature ($f_2 - f_3$). Data for COP and PR in 1999 were adjusted for prior time in commercial coolstores by adding 21 days to the PR and 14 days to the COP time scales used in Fig's 3-1 and 3-2. Shaded regions represent an LSD averaged across each time point in Fig's 3-1 and 3-2, and divided by the temperature difference (~20°C).

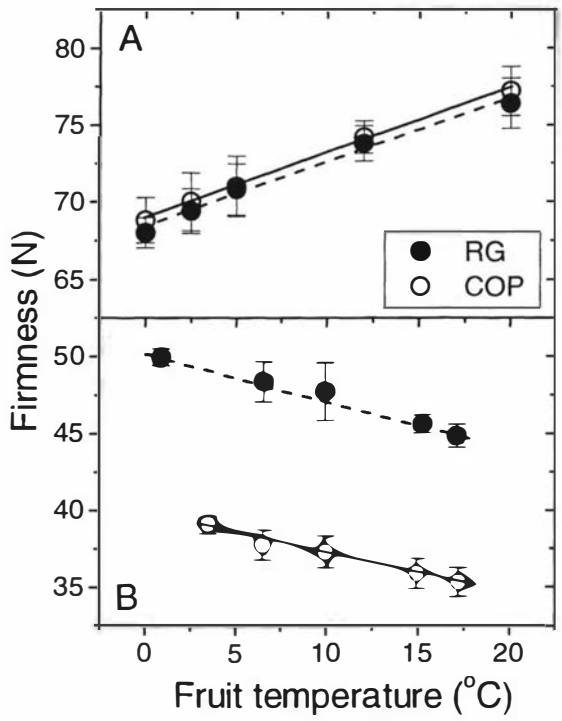

Fig. 3-4 Harvest (A) and minimum (B) flesh firmness for 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) fruit measured at different fruit temperatures. Means (n=10) and standard errors of the mean are shown. Linear regression slope estimates were 0.42 ± 0.03 and 0.42 ± 0.03 at harvest, and 0.31 ± 0.03 and 0.26 ± 0.03 after prolonged storage for RG and COP respectively.

The relationship between firmness and fruit temperature between 0 and 20°C was linear and positive for both RG and COP at harvest, and after prolonged storage at 0°C (3°C for COP) it was linear and negative (Fig. 3-4). The slope between fruit temperature and firmness was similar for both RG and COP at harvest and after prolonged storage. Fruit exposed to the different temperatures between 0 and 20°C after prolonged storage at 0°C or 3°C, had similar firmness when equilibrated back to 0 or 3°C (data not shown).

Magnitude of Δf_{temp} 1-3 days after harvest was not affected by orchard, even though harvest firmness was different between orchards for both RG and COP (Table 3-1). Likewise, harvest date did not affect magnitude of Δf_{temp} 1-3 days after harvest, even

though firmness decreased with advancing harvest date for RG and COP fruit from two orchards (Table 3-2).

Table 3-1 Firmness[1], and change in firmness with increasing fruit temperature (Δf_{temp}[2]) 1-3 days after harvest for 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) from different orchards. Means (n=15) and ANOVA *P* values are shown for firmness (RG = 112 d.f. error; COP = 157 d.f. error) and Δf_{temp} (RG = 72 d.f. error; COP = 116 d.f. error).

Cultivar	Orchard	Firmness (N)	Δf_{temp} ($N.^{\circ}C^{-1}$)
RG	1	78.3	0.26
RG	2	73.9	0.05
RG	3	72.5	0.20
RG	4	75.4	0.30
RG	5	71.9	0.27
RG	6	68.5	0.10
RG	7	75.3	0.23
RG	8	70.9	0.05
P value		0.011	ns
COP	2	77.4	0.04
COP	9	69.9	0.06
COP	10	86.4	0.31
COP	11	76.6	0.27
COP	12	66.4	0.21
COP	13	72.1	0.09
COP	14	79.8	0.19
COP	15	72.6	0.19
COP	16	81.3	0.06
COP	17	80.1	0.17
COP	18	78.4	0.16
COP	19	80.4	0.06
COP	20	81.1	0.19
P value		0.001	ns

[1]Firmness measured at 20°C.

[2]Calculated as Δ firmness / Δ temperature between fruit measured at 0°C and 20°C.

The role of fruit turgor in development of a positive Δf_{temp} at harvest was investigated with RG using fruit paring to initiate rapid water loss at 0°C. Firmness and mass loss for intact (non-pared) fruit was lower after 24 hours at 0°C than at 20°C (Fig. 3-5). However, firmness of pared fruit at 0°C was similar to that of intact fruit maintained at

20°C for one day. Weight loss was greater for pared fruit at 0°C than for intact fruit at both 0°C and 20°C.

Table 3-2 Firmness[1], and change in firmness with increasing fruit temperature (Δf_{temp}[2]) 1-3 days after harvest for 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) harvested on different dates from different orchards. Means (n=15) and ANOVA *P* values are shown for firmness (RG = 136 d.f. error; COP = 140 d.f. error) and Δf_{temp} (RG = 85 d.f. error; COP = 89 d.f. error).

Cultivar	Orchard	Harvest date	Firmness (N)	Δf_{temp} (N.°C^{-1})
RG	1	10-Feb-00	83.1	0.09
RG	1	18-Feb-00	82.0	0.16
RG	1	03-Mar-00	78.3	0.26
RG	1	13-Mar-00	69.5	0.05
RG	1	20-Mar-00	64.9	0.03
RG	2	01-Feb-00	88.7	0.17
RG	2	11-Feb-00	80.2	0.13
RG	2	22-Feb-00	73.9	0.05
RG	2	02-Mar-00	67.0	0.05
RG	2	16-Mar-00	62.2	0.00
P values	Harvest		0.006	ns
	Orchard		ns	ns
	Harvest x Orchard		0.008	ns
COP	2	01-Feb-00	90.7	0.32
COP	2	11-Feb-00	84.0	0.12
COP	2	22-Feb-00	77.4	0.04
COP	2	02-Mar-00	71.3	0.10
COP	2	16-Mar-00	62.8	0.05
COP	9	04-Feb-00	80.5	0.09
COP	9	16-Feb-00	74.6	0.21
COP	9	24-Feb-00	69.9	0.06
COP	9	06-Mar-00	67.0	0.22
COP	9	17-Mar-00	62.3	0.05
P values	Harvest		0.006	ns
	Orchard		0.023	ns
	Harvest x Orchard		0.001	ns

[1]Firmness measured at 20°C.

[2]Calculated as Δ firmness / Δ temperature between fruit measured at 0°C and 20°C.

Values of Δt_{temp} were not different from zero for any of the cultivars, with the exception of one PR data point, two RG data points, and four GS data points late in storage, where

Δt_{temp} was negative (Fig. 3-3). However, there was a tendency for Δt_{temp} to start slightly positive, and become increasingly negative with time at 0°C for RG, GS and PR.

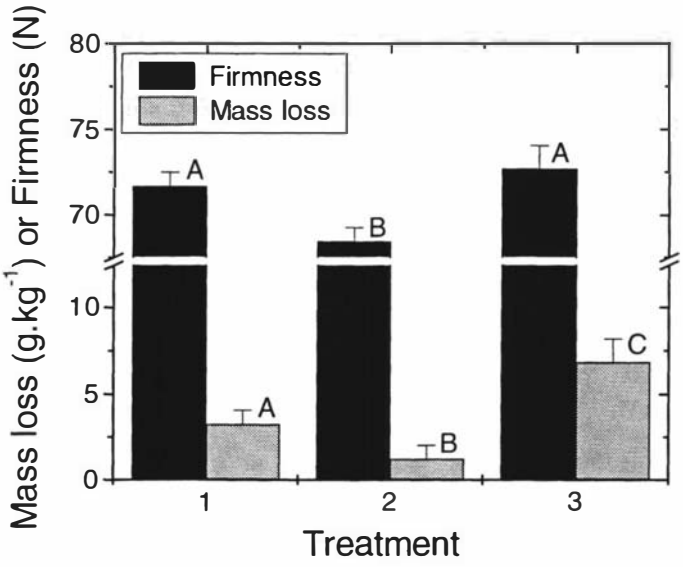

Fig. 3-5 Firmness and mass loss for 'Royal Gala' after one day of paring and temperature treatments; nonpared at 20°C (1), nonpared at 0°C (2), and pared 0°C (3). Means (n=15) and standard errors of the mean are shown. Treatments with different letters were determined as being significantly different using 5% least significant differences (42 d.f. error) of 2.9N for firmness and 0.4g/kg for mass loss. Analysis of variance significance levels for treatment were ** for firmness and *** for mass loss.

3.5 Discussion

The three temperature treatments (Fig. 3-1; f_1, f_2 and f_3) were used to differentiate between physiological (ripening) and physical changes in firmness associated with change in fruit temperature. Treatment f_1 represented the physical state at normal commercial storage temperature; treatment f_2 reflected a combination of physical change and enhanced ripening rate that occurred after transfer of fruit from 0 to 20°C, while treatment f_3 reflected the combination of physical change together with both enhanced and reduced ripening rates that occurred during the sequential fruit transfer from 0 to 20 to 0°C. For cultivars that ripen and soften rapidly at high temperatures, the difference in firmness between f_1 and f_2 may be due in part to a physiologically induced rather than

physically induced change in firmness. The relative degree of physiological and physical change in firmness between f_1 and f_2 can be estimated from f_3, as any physical change in firmness between f_1 and f_2 should be reversed when fruit are returned to 0°C in the f_3 treatment. Thus, the difference in firmness between f_1 and f_3 provided an estimate of change in physiological firmness between f_1 and f_2, while the firmness difference between f_2 and f_3 estimated change in physical firmness between f_1 and f_2. It is possible that some physiological change in firmness occurred between f_2 and f_3 resulting in an underestimation of physical change in firmness. However, physiological change is likely to be minimal as ripening rates for both RG and COP are considerably slower at 0°C (f_3) than at 20°C (f_2). As the difference between f_2 and f_3 appears to be best for estimating physical change, Δf_{temp} and Δt_{temp} were calculated using change in firmness (or tensile strength) between f_2 and f_3.

Magnitude of change in firmness and tensile strength with fruit temperature varied with time at 0°C (3°C for COP) for all cultivars (except PR), where Δf_{temp} was positive at harvest, but became negative after 50-100 days of storage. Similarly, there was a tendency for Δt_{temp} to be slightly positive at harvest and more negative after 50-100 days of storage. The changing physical response of Δf_{temp} with storage time may explain previous inconsistent findings that did not incorporate storage time as a factor. Blanpied et al. (1978) and Bourne (1982) found a negative Δf_{temp}, while others found no effect of temperature on firmness (Haller, 1941; Saltveit, 1984). Alternatively, previous inconsistent results may reflect large variation in ripeness between individual fruit; this would reduce sensitivity for detection of small treatment differences (in this instance, 3-5 N). In future texture-temperature studies, experimental sensitivity could be improved through repeated measurements on the same fruit across different temperatures using non-destructive devices, or by using internal ethylene concentration or other ripening related compounds as covariates during statistical analysis.

Positive Δf_{temp} after 1-3 days at 0°C for RG and GS, and 3°C for COP, could be due to cortical fruit tissue being more brittle under the compressive and fracturing forces of the penetrometer probe at 0°C than at 20°C, resulting in lower firmness readings at 0°C than at 20°C. The physical mechanism(s) or cellular components involved in rendering the

tissue more brittle at 0-3°C at harvest has not been elucidated, but it may be a consequence of high fruit water status and associated high cell turgor characteristic of recently harvested fruit. The hypothesis that fruit water status has a role in generating a positive Δf_{temp} at harvest is supported by the finding that firmness differences between fruit at 20 and 0°C were alleviated by fruit paring, and consequent rapid water loss from fruit at 0°C (Fig. 3-5). It is likely that pared fruit had reduced cell turgor from localised rapid water loss, and consequently had less brittle cells and higher firmness readings than intact fruit that probably had higher cell turgor at 0°C. Tissue brittleness at low temperatures may be reduced with time at 0-3°C through water loss, accounting for the gradual loss of a positive Δf_{temp} in the first 10-25 days at 0 or 3°C.

The mechanism for development of a negative Δf_{temp} after 50-100 days at 0°C (3°C for COP) has not been identified in apples, but it has been suggested that a negative Δf_{temp} for peaches could be caused by temperature-induced changes in the viscosity of water-soluble pectin (Werner and Frenkel, 1978). Assuming that the same mechanism in peaches is also responsible for a negative Δf_{temp} in apples, the development of a negative Δf_{temp} after 50-100 days at 0 or 3°C could be explained by an increased proportion of water-soluble pectin that occurs during apple ripening (Knee, 1973; Knee, 1978).

Δf_{temp} not only varied with time at 0°C (3°C for COP), but also between cultivars. COP and RG had the most positive Δf_{temp} 1-3 days after harvest, followed by GS, but Δf_{temp} for PR did not differ from ~0. After 50-100 days at 0°C (3°C for COP), COP had the most negative Δf_{temp}, followed by RG and GS, but again Δf_{temp} for PR did not differ from zero. Bourne (1982) also reported firmness-temperature coefficient variation between apple cultivars (Golden Delicious, -0.73%; Idared, -0.32%; Red Delicious, -0.20%; McIntosh, -0.39%; and Rome, -0.08%). The mechanism for cultivar Δf_{temp} differences is not known, although it is possible that cultivar differences in cell wall composition and cell packing may exist that facilitate Δf_{temp} differences. The structural characteristics of PR have not been compared to cultivars used in this study. However, PR has smaller cells, higher cell number and more intercellular spaces than its parents 'Splendour' and 'Gala' (Opara, 1999).

Orchard and harvest maturity did not affect magnitude of Δf_{temp} 1-3 days after harvest (Tables 3-1 and 3-2), indicating that a mean Δf_{temp} for a given cultivar could be used across the New Zealand apple industry to estimate temperature induced physical changes in firmness at harvest. Similarly, change in Δf_{temp} with time at low temperatures for RG, PR, GS and COP was consistent for two orchards harvested in different seasons (Fig. 3-3); this creates potential for estimating temperature induced physical changes in firmness through storage for a given cultivar from all orchards. As Δf_{temp} was linear between 0 and 20°C at harvest and during storage (Fig. 3-4), it is possible that one Δf_{temp} value for a given storage time could be used to compare firmness readings from fruit measured at different temperatures between 0 and 20°C.

In summary, the physical textural response of apples to temperature differed in magnitude between cultivars, texture test, and storage time at 0°C or 3°C. Change in firmness associated with change in fruit temperature was not affected by orchard or maturity at harvest, indicating that a cultivar and storage-time specific Δf_{temp} could be used to compare firmness readings for fruit from different orchards that were measured at different temperatures between 0 and 20°C. Similar studies are required to quantify the physical effect of temperature on firmness readings for other commercially important cultivars.

3.6 References

Blanpied, G.D., Bramlage, W.J., Dewey, D.H., LaBelle, R.L., Massey, L.M., Mattus, G.E., Stiles, W.C., Watada, A.E., 1978. A standardized method for collecting apple pressure test data. N. Y. Food Life Sci. Bull. 74, 8pp, Cornell University, Ithaca, New York.

Bourne, M.C., 1982. Effect of temperature on firmness of raw fruits and vegetables. J. Food Sci. 47, 440-444.

Haller, M.H., 1941. Fruit pressure testers and their practical applications. USDA Circular 627.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortScience 27, 1291-1294.

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., 1997a. Texture of fresh fruit. Hortic. Rev. 20, 121-224.

Harker, F.R., Stec, M.G.H., Hallett, I.C., Bennett, C.L., 1997b. Texture of parenchymatous plant tissue: a comparison between tensile and other instrumental and sensory measurements of tissue strength and juiciness. Postharvest Biol. Technol. 11, 63-72.

Jeffery, P.B., Banks, N.H., 1994. Firmness-temperature coefficient of kiwifruit. New Zeal. J. Crop Hort. Sci. 22, 97-101.

Knee, M., 1973. Polysaccharide changes in cell walls of ripening apples. Phytochemistry 12, 1543-1549.

Knee, M., 1978. Metabolism of polymethylgalacturonate in apple fruit cortical tissue during ripening. Phytochemistry 17, 1261-1264.

Opara, L.U., 1999. Bruise susceptibility and other postharvest quality attributes of 'Pacific Rose' apple and its parents. Paper presented: "Australasian Postharvest Conference", Waitangi, New Zealand, 3-8 October 1999.

Saltveit, M.E., 1984. Effects of temperature on firmness and bruising of 'Starkrimson Delicious' and 'Golden Delicious' apples. HortScience 19, 550-551.

Stow, J., 1989. The involvement of calcium ions in maintenance of apple fruit tissue structure. J. Exp. Bot. 40, 1053-1057.

Werner, R.A., Frenkel, C., 1978. Rapid changes in the firmness of peaches as influenced by temperature. HortScience 13, 470-471.

Chapter 4 Temperature induces differential softening responses in apple cultivars.

4.1 Abstract

Loss of firmness in 'Royal Gala', 'Cox's Orange Pippin', 'Granny Smith' and 'Pacific Rose™' apples (*Malus domestica* Borkh.) was quantified in fruit held continuously at temperatures from 0 to 35°C. Softening was triphasic, consisting of an initial slow softening phase, followed by a rapid softening phase, and a final slow softening phase. Loss of firmness at each temperature was described by an asymmetric, sigmoidal equation. Rate of firmness change (k) at different temperatures was described by a modified Arrenhius equation for 'Royal Gala' and 'Cox's Orange Pippin', where k increased with temperature from 0°C through ~22°C, and decreased through 35°C. This equation did not describe k at different temperatures for 'Granny Smith' and 'Pacific Rose™', as k was similar from 0 to 12°C, and could not be calculated for fruit at 20 to 35°C as rapid phase softening was not initiated. Transition between the initial slow softening phase and rapid softening phase was related to a rapid increase in internal ethylene concentration for all cultivars. 'Granny Smith' and 'Pacific Rose™' had slow softening at 20 to 35°C, and had delayed and slow increases in internal ethylene concentration. Equations used in this study could be used to estimate firmness loss at different parts of the commercial postharvest handling chain, especially where fruit are at non-optimal storage temperatures.

Keywords: *Malus domestica* (Borkh.); Quality; Firmness; Ethylene; Empirical modelling; Modified Arrenhius equation

4.2 Introduction

Flesh firmness is an important indicator of postharvest apple quality, with firmer fruit considered to have better quality characteristics than softer fruit (Harker et al., 1997). This is reflected in major international markets, where failure to meet firmness specifications can result in shipment rejections, reduced returns to growers and a damaged reputation as a supplier of top quality apples. Several studies have focused on understanding the effects of individual pre- and postharvest factors on apple firmness (Harker et al., 1997; DeEll et al., 1999), but little is known about how such factors

influence loss of firmness over time (softening). An initial step in understanding how these factors influence softening is to characterise softening with time after harvest, as done for pears (Bourne, 1968) and kiwifruit (MacRae et al., 1989). In contrast to other fruits, apple softening may be difficult to characterise, as apples only soften partially, and undergo a relatively small change in firmness during ripening (Bourne, 1979). Also in most apple studies, firmness is usually measured at harvest and after cold storage, with only a limited number of measurements made during storage, providing insufficient data to accurately characterise the softening curve.

Temperature strongly affects postharvest life of most horticultural produce. While the optimum temperature for slowing quality deterioration in apples is often 0°C to 3°C (depending on cultivar sensitivity to chilling injury), it is difficult to maintain apples consistently at those temperatures throughout the entire postharvest handling chain. Fruit are often exposed to non-optimal temperatures during packing (at harvest or after storage), ship loading, distribution to retailers, and in retail outlets. Quantifying the softening response of apples to a range of temperatures would allow producers and marketers to estimate loss of firmness during different phases of postharvest handling. While the effects of delayed cooling at harvest (Magness and Diehl, 1924; Blanpied, 1975), and short-term prestorage heat treatments on apple firmness have been extensively studied (Klein and Lurie, 1990; Klein et al., 1990; Ben Shalom et al., 1993), only Magness and Diehl (1924) and Landfald (1966) studied the effects of continuous temperature treatments on apple firmness. However, Landfald (1966) only measured firmness four times during storage, at four temperatures from 0°C to 12°C, with results averaged across three cultivars. Similarly, Magness and Diehl (1924) only measured firmness five times at 0°C, 2.2°C and 21°C in several cultivars. Studying a wider range of temperatures, with firmness measured more frequently during treatment, and comparing cultivar responses, may allow a more complete understanding of how apple softening is influenced by temperature.

Endogenous ethylene may play an important role in apple softening, as several inhibitors of ethylene action, applied at harvest, reduced loss of firmness during storage (Blankenship and Sisler, 1989; 1993; Fan et al., 1999; Watkins et al., 2000). One factor that influences onset of rapid ethylene production in apples is temperature, where

cultivars such as 'Granny Smith' (Jobling et al., 1991; Larrigaudiere and Vendrell, 1993; Larrigaudiere et al., 1997) and 'Golden Delicious' (Knee et al., 1983) initiated rapid ethylene biosynthesis earlier at 20°C when previously exposed to cold temperatures, while 'Cox's Orange Pippin' (Knee et al., 1983) and 'Royal Gala' (Larrigaudiere et al., 1997) rapidly initiated ethylene synthesis at 20°C without prior cold treatment. Thus, if the ethylene biosynthetic response to temperatures differs between cultivars, then it is likely that the softening response to temperature may also differ.

The aim of this study was to characterise loss of firmness at different temperatures for rapid ('Royal Gala' and 'Cox's Orange Pippin) and slow ('Granny Smith' and 'Pacific Rose™') softening apple cultivars, and determine if the softening responses were reflected in endogenous ethylene concentration changes for each cultivar.

4.3 Materials and methods

4.3.1 Fruit supply and treatments

Export quality 'Royal Gala' (RG), 'Cox's Orange Pippin' (COP), 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples were harvested at commercial export maturity, graded and then transported from Hawkes Bay to Massey University, Palmerston North, New Zealand within 48 hours of harvest. Fruit weights were 170 ± 10g for RG and GS, 140 ± 10g for COP, and 200 ± 20g for PR.

Ten perforated polyethylene bags (35µm thickness; 50 x 5mm diameter perforations per m^2), each containing 10 fruit were placed at 0.0, 2.5, 5.0, 12.0, 20.0, 24.0, 30.0 or 35.0±0.5°C in commercial cardboard cartons (18kg). Ten fruit (one fruit per bag) were randomly removed from storage for measurement of internal ethylene concentration (IEC) and flesh firmness on ten occasions during storage. Flesh firmness and IEC were measured on fruit at the temperature in which they were stored, and were measured more frequently at temperatures conducive to rapid softening. Oxygen and carbon dioxide concentrations were monitored weekly in perforated bags and the coolstore atmosphere to check that the concentrations of these gases were similar for fruit at each temperature. Disorders (predominantly senescent breakdown) and rots were

occasionally observed in fruit from all cultivars at each storage temperature; these fruit were not included in firmness or IEC datasets.

4.3.2 Flesh firmness and internal ethylene concentration measurements

Flesh firmness was measured with a drill-press mounted 'Effegi' penetrometer fitted with an 11.1 mm diameter probe; the maximum force required to puncture pared tissue to a depth of 7.9 mm on opposite sides of the fruit equator was recorded.

IEC was determined by injecting 1 ml gas samples, taken from the core cavity, into a gas chromatograph (Pye Unicam GCD) fitted with a flame ionisation detector (set at 140°C with H_2 and air flow rates of 30 ml.min^{-1} and 300 ml.min^{-1}, respectively), an activated alumina column (set at 100°C with N_2 as the carrier gas at 30 ml.min^{-1}), and a Hewlett Packard integrator (model 3390A) calibrated with external ethylene standards (certified as β-standard by B.O.C. Gases New Zealand Ltd).

4.3.3 Data analysis

A preliminary study (results not shown) was undertaken to compare several sigmoidal functions (logistic, Gompertz, Richards, Weibull, Michaelis-Menten and others) for ability to fit raw firmness data from a range of diverse apple softening curves, and to determine if the parameters for each model could be interpreted physiologically. Assessment of best fit between the functions was determined by examining residual patterns and residual variance. Sigmoidal models with only 4 or 5 parameters were assessed, as a minimum of 4 parameters are generally required for sigmoidal curves with a lower asymptote greater or less than 0, while models with greater than 5 parameters are considered to have increased likelihood of undesirable non-linear parameter behaviour (Ratkowsky, 1990). Also, if the selected model was to be used at harvest for predicting subsequent softening rates, it would be advantageous to minimise the number of parameters requiring prediction.

The following equation (Tablecurve 2D software, Jandel Scientific) was found to best describe firmness (f) as a function of time (t) at different temperatures (T):

$$f = f_{+\infty} + A \cdot \left(1 - \left(1 + \exp\frac{t + B \cdot \ln\left(2^{D^{-1}} - 1\right) - C}{B}\right)^{-D}\right) \qquad \text{(Eq. 4-1)}$$

where model parameters were a minimum firmness asymptote ($f_{+\infty}$, N), firmness range between maximum and minimum firmness asymptotes (A, N), and curvature parameters (B, C and D).

Apple firmness data was used to reduce the number of parameters in Eq. 4-1, to simplify parameter interpretation. Parameter D was constant and subsequently set to 0.2, parameter C was described as a function of B, parameter A was combined with the minimum firmness asymptote ($f_{+\infty}$, N) to generate an initial firmness asymptote ($f_{-\infty}$, N) parameter, and the inverse of B generated a rate of change parameter (k, day^{-1}). Firmness data at different temperatures was then fitted with the following modified three-parameter model using non-linear regression:

$$f = f_{-\infty} - (f_{-\infty} - f_{+\infty}) \cdot \left(1 - \left(1 + \exp\frac{t + k^{-1} \cdot \ln(31) - (5.2 \cdot k^{-1.0168})}{k^{-1}}\right)^{-0.2}\right) \qquad \text{(Eq. 4-2)}$$

where model parameters were an initial firmness asymptote ($f_{-\infty}$, N), a final firmness asymptote ($f_{+\infty}$, N) and rate of firmness change (k, day^{-1}).

Rate of firmness change (k, day^{-1}) at different temperatures (T, °C) was fitted with a simplified modified Arrenhius equation (Feng. et al., 1990) using non-linear regression:

$$k = \frac{k_a \cdot \exp\frac{-E_a \cdot R^{-1}}{T + 273.13}}{1 + \exp\left(\Delta S \cdot R^{-1} - \frac{\Delta H \cdot R^{-1}}{T + 273.13}\right)} \qquad \text{(Eq. 4-3)}$$

where model parameters were a rate constant (k_a), activation energy • gas constant^{-1} (E_a.R^{-1}, °K), increment of entropy • gas constant^{-1} (ΔS.R^{-1}), and increment of enthalpy • gas constant^{-1} (ΔH.R^{-1}, °K).

Non-linear regression was performed using the NLIN procedure of the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

4.4 Results

For RG at 0 to 5°C (Fig. 4-1), COP at 0 to 2.5°C (Fig. 4-2), and GS (Fig. 4-3) and PR (Fig. 4-4) at 0 to 12°C, there was a phase of little or no firmness loss for the first 5 to 25 days of storage (initial slow softening phase). This was followed by a phase of rapid firmness loss (rapid softening phase), and a final phase of little or no firmness loss (final slow softening phase). However, for RG at 12 to 35°C, and COP at 5 to 35°C, only the rapid and final slow softening phases were detected. Duration of initial slow softening phase, and rate of rapid phase softening, were temperature dependent for RG and COP, as duration of the initial slow softening phase decreased, and rate of rapid phase softening increased, as temperature increased from 0 to 20°C. From 20 to 35°C, the initial slow phase softening was not apparent, and rate of rapid phase softening decreased. PR and GS had similar initial slow and rapid softening phases at 0 to 12°C, but had no rapid phase softening at 20 to 35°C.

IEC of RG remained low (< 1.5 $\mu l.l^{-1}$) for the first 7 to 20 days at 0 to 5°C, increased rapidly to a peak between 32 to 80 days, and then decreased for the remainder of storage (Fig. 4-1). At 12 to 35°C, IEC in RG increased immediately after being placed in each temperature treatment, to a peak at 15 to 45 days, and decreased thereafter. In all temperature treatments, rapid ethylene production in COP had commenced (IEC =16 $\mu l.l^{-1}$) at the beginning of storage, and immediately increased to a peak at 15 to 50 days, before decreasing thereafter (Fig. 4-2). IEC of GS (Fig. 4-3) and PR (Fig. 4-4) was low (< 1.5 $\mu l.l^{-1}$) for the first 4 to 25 days at 0 to 12°C, increased to a peak at 125 to 175 days, and decreased for the remainder of storage. IEC of GS and PR fruit remained low (< 1.5 $\mu l.l^{-1}$) for the first 12 to 20 days at 20 to 35°C, and remained low or increased slightly during the remainder of storage.

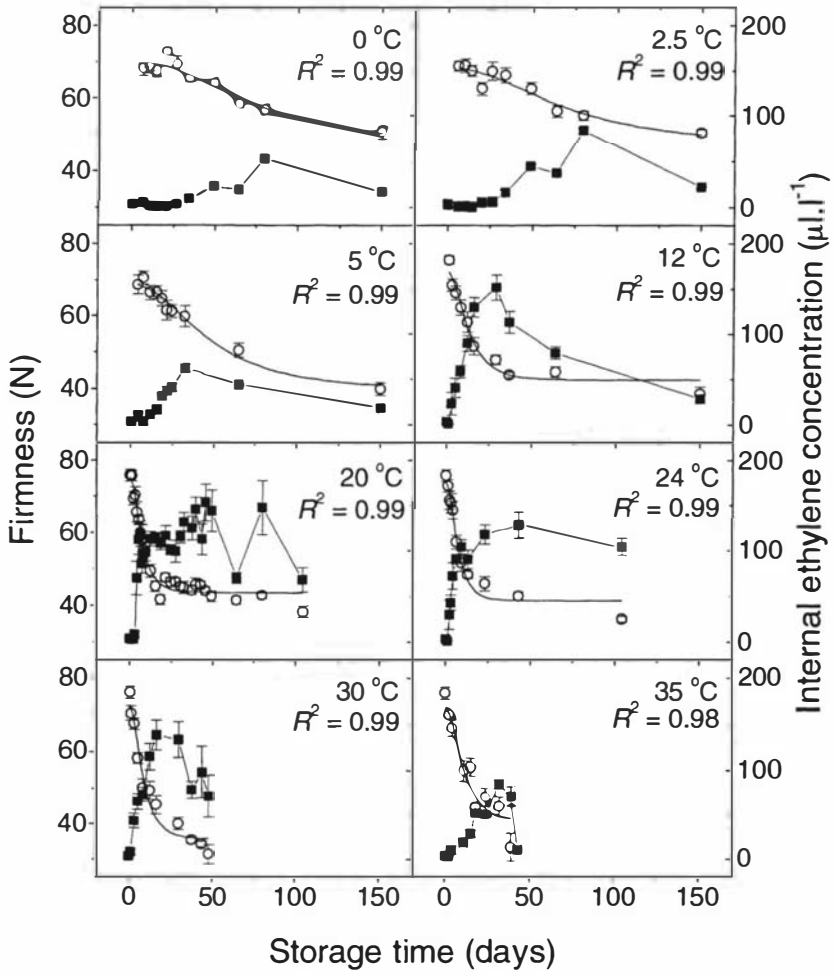

Fig. 4-1 Flesh firmness (○) and internal ethylene concentration (■) of 'Royal Gala' (RG) apples stored continuously at temperatures from 0 to 35°C. Means (n=10) and standard errors of the means are shown. Loss of firmness was fitted with Eq. 4-2 on individual fruit at each temperature, with resulting R^2 values ($P < 0.0001$; 97 d.f. error at each temperature) displayed.

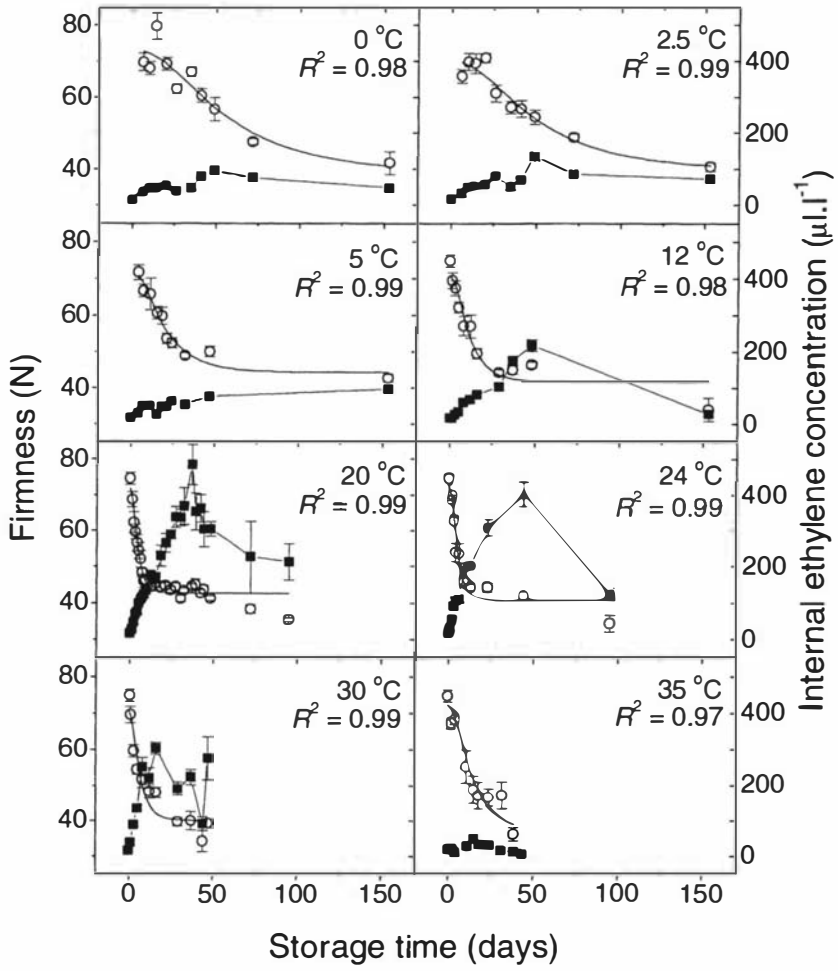

Fig. 4-2 Flesh firmness (○) and internal ethylene concentration (■) of 'Cox's Orange Pippin' (COP) apples stored continuously at temperatures from 0 to 35°C. Means (n=10) and standard errors of the means are shown. Loss of firmness was fitted with Eq. 4-2 on individual fruit at each temperature, with resulting R^2 values ($P < 0.0001$; 97 d.f. error at each temperature) displayed.

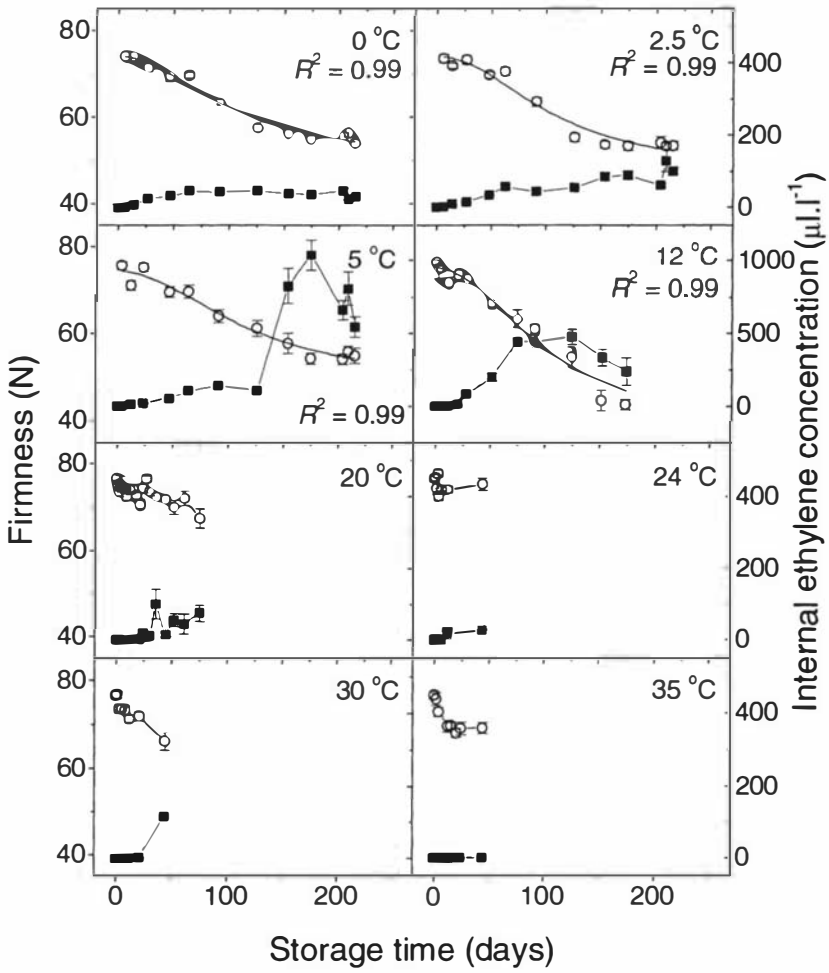

Fig. 4-3 Flesh firmness (○) and internal ethylene concentration (■) of 'Granny Smith' (GS) apples stored continuously at temperatures from 0 to 35°C. Means (n=10) and standard errors of the means are shown. Loss of firmness was fitted with Eq. 4-2 on individual fruit at 0 to 12°C, with resulting R^2 values ($P < 0.0001$; 97 d.f. error at each temperature) displayed. NB Scales for IEC data at 5 and 12°C differ from other temperatures.

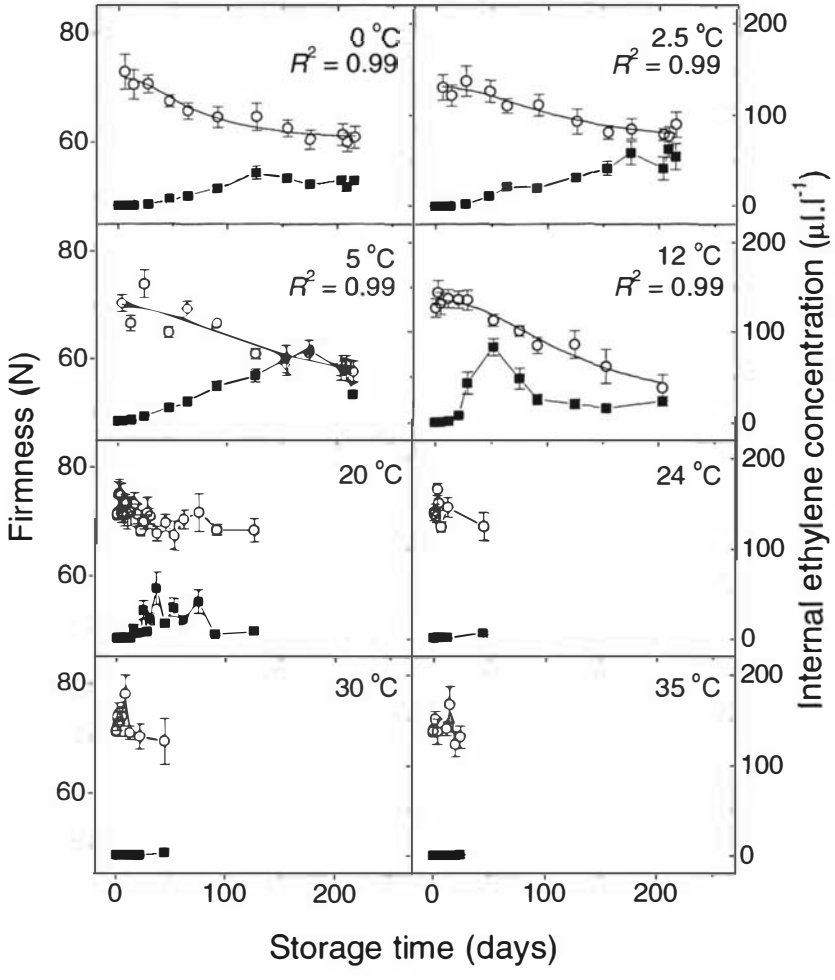

Fig. 4-4 Flesh firmness (○) and internal ethylene concentration (■) of 'Pacific Rose™' (PR) apples stored continuously at temperatures from 0 to 35°C. Means (n=10) and standard errors of the means are shown. Loss of firmness was fitted with Eq. 4-2 on individual fruit at 0 to 12°C, with resulting R^2 values ($P < 0.0001$; 97 d.f. error at each temperature) displayed.

The maximum IEC value attained by COP at each temperature, increased with temperature from 0°C to a maximum value of ~480 $\mu l.l^{-1}$ at 20°C, and then steadily decreased through 35°C (Fig. 4-5). In contrast, maximum IEC for GS increased sharply from 0°C to a peak of ~1050 $\mu l.l^{-1}$ at 5°C, and then progressively decreased as temperature increased to 35°C. There was little difference in the maximum IEC for RG between 12 and 30°C (~150 $\mu l.l^{-1}$), with lower values measured at 0 to 5°C and at 35°C. PR had the lowest peak IEC values of all four cultivars, with little difference between 5 and 20°C, and minimal values from 24 through 35°C. Peak IEC for GS at 5°C was approximately 10 and 2.5 fold higher than peak IEC at 20°C for RG and COP respectively, and approximately 10 times higher than peak IEC at 5 to 12°C for PR. Relative to 20°C, IEC was reduced in all cultivars at 35°C, where GS and PR had the lowest, and RG the highest, maximum IEC value at 35°C.

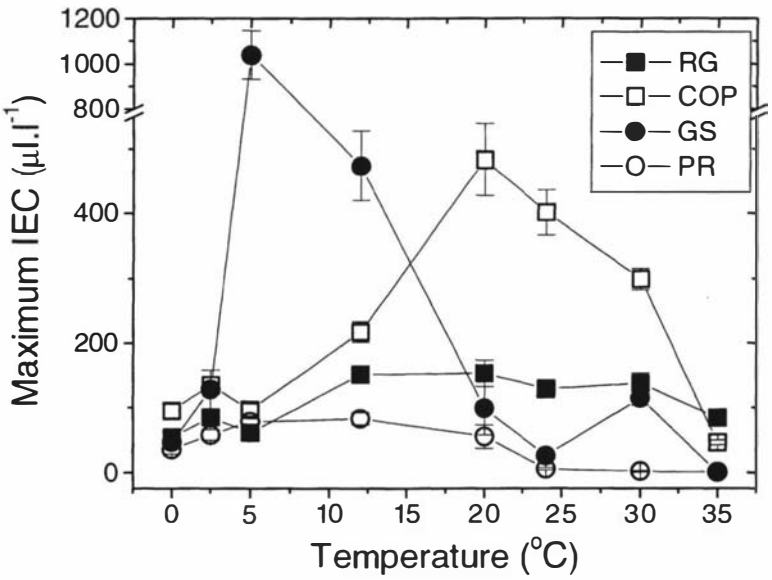

Fig. 4-5 Maximum internal ethylene concentration (IEC) at different temperatures for 'Royal Gala' (RG), 'Cox's Orange Pippin' (COP), 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples. Maximum IEC was the highest IEC measured through time at each temperature. Means (n=10) and standard errors of the mean are shown.

The initial slow softening phase for all cultivars coincided with a low IEC, and transition to rapid phase softening coincided with commencement of rapidly increasing

IEC (Fig's 4-1 to 4-4). The small change in firmness that occurred for GS and PR at 20 to 35°C also coincided with low but slight increases in IEC.

Rate of firmness change (k) for RG and COP increased slowly with temperature from 0 through 5°C, increased more rapidly from 5 to a maximum at 20-24°C, and then decreased from 24 through 35°C (Fig. 4-6). GS and PR had lower k values than RG and COP at all temperatures. GS and PR had similar k values at 0 to 12°C, but could not be calculated at 20 to 35°C, as rapid phase softening was not initiated. COP had the fastest k and lowest $f_{+\infty}$, while GS and PR had the slowest k, and PR the highest $f_{+\infty}$ at 0°C (Table 4-1).

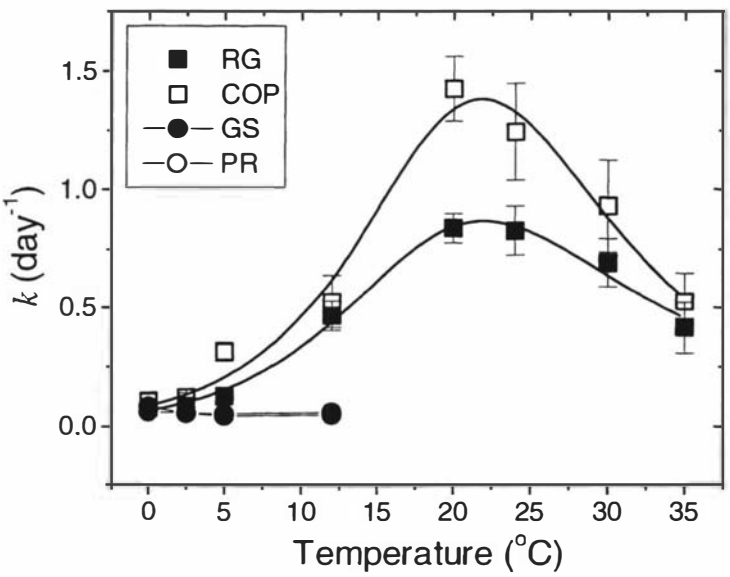

Fig. 4-6 Rate of firmness change (k, day^{-1}) at different temperatures for 'Royal Gala' (RG), 'Cox's Orange Pippin' (COP), 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples. Estimates and standard errors of k at each temperature were from firmness data individually fitted with Eq. 4-2 at each temperature (Fig's 4-1 to 4-4). RG and COP k values were fitted with a modified Arrenhius equation (Eq. 4-3) ($R^2 = 0.99$, $P = 0.0002$, 4 d.f. error for COP; and $R^2 = 0.99$, $P < 0.0001$, 4 d.f. error for RG).

Table 4-1 Rate of firmness change (k) and the minimum firmness asymptote ($f_{+\infty}$) at 0°C for 'Royal Gala', 'Cox's Orange Pippin', 'Granny Smith' and 'Pacific Rose™' apples. Parameter estimates and standard errors were derived from firmness data (Fig's 4-1 to 4-4) fitted with Eq. 4-2.

Cultivar	k (day^{-1})	$f_{+\infty}$ (N)
'Royal Gala'	0.078 ± 0.01	46.6 ± 3.9
'Cox's Orange Pippin'	0.107 ± 0.01	38.7 ± 3.8
'Granny Smith'	0.061 ± 0.01	52.2 ± 1.5
'Pacific Rose™'	0.054 ± 0.01	60.6 ± 1.7

4.5 Discussion

In general, postharvest apple softening at low temperatures was triphasic for all cultivars, consisting of an initial slow softening phase, a rapid softening phase, and a final slow softening phase (Fig's 4-1 to 4-4). An initial slow softening phase was not discernable at 12 to 35°C for COP and RG, as rapid phase softening began immediately when placed at these temperatures. In COP, rapid ethylene production had commenced before fruit were placed at storage temperatures (and may have commenced prior to harvest), resulting in an immediate rapid increase in IEC at all temperatures, and a short initial slow softening phase relative to other cultivars at 0 and 2.5°C. Kiwifruit harvested at an early maturity also had a similar triphasic softening curve (MacRae et al., 1989). However, kiwifruit harvested at a late maturity (MacRae et al., 1989), pears (Bourne, 1968) and nectarines (King et al., 1989) had biphasic softening curves with no discernable initial slow softening phase.

The nature of the apple softening curve will depend on the temperature at which fruit were stored and measured. When apples were stored and measured at the same temperature, as done in this study, the resulting softening curves were triphasic. However, when firmness was measured on fruit equilibrated to a temperature different from the storage temperature (i.e. stored at 0°C and measured at 20°C), the resultant softening curve had no initial slow softening phase and appeared biphasic (Chapter 3). This is because apples at harvest were physically firmer at 20°C than at 0°C, an effect that diminished within 25 to 50 days at low temperatures (Chapter 3). In contrast, apples were physically softer at 20°C than at 0°C after 100 days of storage at 0°C (Chapter 3), which could explain why RG had a higher minimum firmness at 0°C than at

higher temperatures. It is also possible that softening was physiologically incomplete at 0°C for RG, resulting in a higher minimum firmness value at 0°C than at higher temperatures where softening may have been more advanced.

The physical or physiological mechanisms that control transition between the three softening phases are not known. Duration of the initial slow softening phase could be influenced by maturity at harvest, as for kiwifruit (MacRae et al., 1989), physically through water loss, ripening induced increases in IEC, or as yet unspecified physiological events that occur during ripening. The initial slow softening phase of RG, PR and GS appeared to coincide with periods of low (< 1.5 $\mu l.l^{-1}$) IEC, and once IEC exceeded 1.5 $\mu l.l^{-1}$ rapid phase softening occurred. This suggests that ethylene may have a role in regulating the transition between the initial slow and rapid softening phases. Apple softening was reduced when inhibitors of ethylene action were applied at harvest (Blankenship and Sisler, 1989; 1993; Fan et al., 1999; Watkins et al., 2000), indicating that ethylene may have an important role in promoting apple softening. The threshold ethylene concentration for initiating rapid softening in controlled atmosphere storage was an external concentration of 1 $\mu l.l^{-1}$ (Liu, 1977) and IEC of 0.1 $\mu l.l^{-1}$ (Stow et al., 2000). Rapid softening is also initiated at the low external ethylene concentrations of 0.05 to 0.2 $\mu l.l^{-1}$ for pears (Wang et al., 1972), and 0.01 $\mu l.l^{-1}$ for kiwifruit (Jeffery and Banks, 1996). While the present results indicate that rapid phase softening is associated with IEC's higher than 1.5 $\mu l.l^{-1}$, the actual concentration required to initiate rapid softening of apples may be lower, and closer to the IEC of 0.1 $\mu l.l^{-1}$ suggested by Stow et al. (2000). It is not known if ethylene is required to sustain rapid phase softening once initiated, of if rate of rapid phase softening is IEC dependent. Modelling loss of firmness as a function of IEC may provide more information on the role of ethylene in apple softening.

Cultivar differences in occurrence of rapid phase softening at 20 to 35°C may reflect the influence of these temperatures on increasing IEC during storage. Both IEC and softening increased rapidly at 20 to 35°C for COP and RG, while both processes increased slowly in GS and PR at these temperatures. The delayed and slow increase in ethylene synthesis in GS at ~20°C was overcome by prior exposure to low temperatures

(Jobling et al., 1991; Larrigaudiere and Vendrell, 1993; Larrigaudiere et al., 1997). In contrast, low temperatures were not required to initiate rapid ethylene biosynthesis in RG (Larrigaudiere et al., 1997) and COP (Knee et al., 1983) at 17-20°C. The effect of temperature on ethylene physiology of PR is not known, but these results indicate that this cultivar may be similar to GS, where a period of low temperature is required before rapid ethylene synthesis occurs at 20 to 35°C. It is possible that the inability of PR to initiate rapid phase softening when held continuously at 20°C could be overcome with prior exposure to cold temperatures, as firmness at 20°C was reduced in GS previously exposed to 4°C for 10 days (Larrigaudiere and Vendrell, 1993).

The contrasting ethylene physiology between the four cultivars was also reflected in the maximum IEC at different temperatures, where peak IEC of GS and PR occurred at 5-12°C, compared with 20-24°C for RG and COP. A similar relationship for maximum IEC and k with temperature for COP and RG, suggests a possible role for IEC in regulating k at different temperatures for these cultivars. Substantially higher IEC in GS than other cultivars at 5-12°C, indicates that the enzymes required for rapid ethylene biosynthesis were present and functional in GS at these temperatures. Development of skin 'greasiness' at low temperatures may have reduced the skin permeance for ethylene (Dadzie et al., 1995), allowing IEC to accumulate more readily at 5-12°C than at other temperatures.

The k values for RG and COP at different temperatures were described by a modified Arrenhius equation. This equation is a combination of the Arrenhius equation, that describes change in rate of chemical reactions with temperature, and the Boltzman enzyme function that describes change in proportion of enzymes in active and inactive states at different temperatures (Johnson and Thornley, 1985; Feng et al., 1990). This is in contrast to the mechanistic model proposed by Tjiskens et al. (1999) for softening of 'Elstar' apples, that implicitly assumed the Arrenhius equation alone could describe softening at different temperatures. The increase in k between 0 and 20-24°C for RG and COP, as described by the Arrenhius component of the modified Arrenhius equation, is attributed to increased rates of chemical reactions. However, the decrease in k between 24 and 35°C, as described by the Boltzman component of the modified Arrenhius equation, was probably due to inactivation of enzymes directly or indirectly

involved in the cell wall disassembly process. The effect of heat treatments on the postharvest ripening behaviour of several crops has been reviewed by Lurie (1998), with reduced softening in apples at high temperatures being attributed to reduced ethylene biosynthesis (Klein and Lurie, 1990), and reduced cell wall degradation (Klein et al., 1990; Ben Shalom et al., 1993). The influence of temperature on RG and COP k values was similar to that found for postharvest skin yellowing of apples (Dixon and Hewett, 1998), changes in wheat shoot dry weight and leaf area (Feng et al., 1990), and growth rates of bacteria (Johnson and Thornley, 1985). The underlying cold requirement to initiate rapid ripening in GS, and probably PR, may explain why the modified Arrenhius equation could not describe k at different temperatures for these cultivars.

It is possible that water loss also influenced the softening rates of apple cultivars at different temperatures, as weight loss progressively increased as storage temperature increased from 0°C to 35°C (data not shown). The magnitude by which water loss differences may have influenced firmness at each temperature remains unclear, as accelerated water loss can increase (Porritt and Meheriuk, 1973; Hatfield and Knee, 1988), decrease (Blanpied, 1981; Lidster, 1990), and have no effect (Porritt and Meheriuk, 1973; Appendix A) on the firmness (as measured with a penetrometer) of apples during storage. Thus, it is possible that the magnitude of k at 22°C relative to k at 0°C, and the extent of inhibition of softening at 35°C relative to k at 22°C, may have both been influenced by water loss differences at each temperature. However, the overall shape of the curve relating k to temperatures between 0 and 35°C should be similar regardless of water loss effects.

The k and $f_{+\infty}$ values at 0°C differed substantially between cultivars. COP had the highest k value and softened by the greatest magnitude to generate the lowest $f_{+\infty}$, while PR had the slowest k value (along with GS) and softened the least to generate the highest $f_{+\infty}$. Fruit texture is influenced by cell wall composition, membrane integrity, cell turgor, cell size and shape, and neighbouring cell to cell interactions (Harker et al., 1997); cultivar differences in any of these components may cause different cultivar k and $f_{+\infty}$ values. In addition RG and COP are early season cultivars (picked in February-March, New Zealand), and GS and PR late season cultivars (picked in April-May, New

Zealand), which may influence softening potential. Early season cultivars generally have greater, and more rapid ethylene production, than late season cultivars (Hansen, 1945; Watkins et al., 1989).

In summary, the softening profile was triphasic for cultivars used in this study. The transition between the initial slow and rapid softening phases appeared to coincide with rapidly increasing IEC. The empirical equations describing loss of firmness at different temperatures for RG and COP have the potential to describe firmness changes during different phases of postharvest handling of apples. Further research is required to determine if k at a particular temperature is influenced by prior exposure to other temperatures for all cultivars, especially as GS and PR may require time at low temperatures before rapid softening is initiated at ambient temperatures.

4.6 References

Ben Shalom, N., Hanzon, J., Klein, J.D., Lurie, S., 1993. A postharvest heat treatment inhibits cell wall degradation in apples during storage. Phytochemistry 34, 955-958.

Blankenship, S.M., Sisler, E.C., 1989. 2,5-Norbornadiene retards apple softening. HortScience 2, 313-314.

Blankenship, S.M., Sisler, E.C., 1993. Response of apples to diazocyclopentadiene inhibition of ethylene binding. Postharvest Biol. Technol. 3, 95-101.

Blanpied, G.D., 1975. The effect of cooling rate on the market quality of 'McIntosh' apples. Proc. Am. Soc. Hortic. Sci. 70, 58-66.

Blanpied, G.D., 1981. A relationship between water loss and storage breakdown of 'McIntosh' apples. HortScience 16, 525-526.

Bourne, M.C., 1968. Texture profile of ripening pears. J. Food Sci. 33, 223-226.

Bourne, M.C., 1979. Texture of temperate fruits. J. Texture Stud. 10, 25-44.

Dadzie, B.K., Banks, N.H., Hewett, E.W., 1995. Reduced greasiness of 'Granny Smith' apples washed in tween 20 solution. New Zeal. J. Crop Hortic. Sci. 23, 219-222.

DeEll, J.R., Saad, F., Khanizadeh, S., 1999. Factors influencing apple fruit firmness. Compact Fruit Tree 32, 56-58.

Dixon, J., Hewett, E.W., 1998. Temperature affects postharvest color change of apples. J. Am. Soc. Hortic. Sci. 123, 305-310.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Feng, Y., Li, X., Boersma, L., 1990. The Arrhenius equation as a model for explaining plant responses to temperature and water stresses. Ann. Bot. 66, 237-244.

Hansen, E., 1945. Quantitative study of ethylene production in apple varieties. Plant Physiol. 20, 631-635.

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., 1997. Texture of fresh fruit. Hortic. Rev. 20, 121-224.

Hatfield, S.G.S., Knee, M., 1988. Effects of water loss on apples in storage. Int. J. Food Sci. Technol. 23, 575-583.

Jeffery, P.B., Banks, N.H., 1996. Effects of ethylene on kiwifruit softening in coolstorage. New Zeal. Kiwifruit J. 114, 9-10.

Jobling, J.J., McGlasson, W.B., Dilley, D.R., 1991. Induction of ethylene synthesizing competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1, 111-118.

Johnson, I.R., Thornley, J.H.M., 1985. Temperature dependence of plant and crop processes. Ann. Bot. 55, 1-24.

King, G.A., Henderson, K.G., Lill, R.E., 1989. Ultrastructural changes in the nectarine cell wall accompanying ripening and storage in a chilling-resistant and chilling-sensitive cultivar. New Zeal. J. Crop Hortic. Sci. 17, 337-344.

Klein, J.D., Lurie, S., 1990. Prestorage heat treatment as a means of improving poststorage quality of apples. J. Am. Soc. Hortic. Sci. 115, 265-269.

Klein, J.D., Lurie, S., Ben-Arie, R., 1990. Quality and cell wall components of 'Anna' and 'Granny Smith' apples treated with heat, calcium, and ethylene. J. Am. Soc. Hortic.Sci. 115, 954-958.

Knee, M., Looney, N.E., Hatfield, S.G.S., Smith, S.M., 1983. Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. J. Exp. Bot. 34, 1207-1212.

Landfald, R., 1966. Temperature effects on apples during storage. Bull. Int. Instit. Refrig. Ann. 1966-1, 453-460.

Larrigaudiere, C., Graell, J., Salas, J., Vendrell, M., 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Postharvest Biol. Technol. 10, 21-27.

Larrigaudiere, C., Vendrell, M., 1993. Cold-induced activation of 1-aminocyclopropane-1-carboxylic acid metabolism in rewarmed Granny Smith apples: Consequences on ripening. Sci. Hortic. 55, 263-272.

Lidster, P.D., 1990. Storage humidity influences fruit quality and permeability to ethane in 'McIntosh' apples stored in diverse controlled atmospheres. J. Am. Soc. Hortic. Sci. 115, 94-96.

Liu, F.W., 1977. Varietal and maturity differences of apples in response to ethylene in controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 102, 93-95.

Lurie, S., 1998. Postharvest heat treatments. Postharvest Biol. Technol. 14, 257-269.

MacRae, E.A., Lallu, N., Searle, A.N., Bowen, J.H., 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49, 413-430.

Magness, J.R., Diehl, H.C., 1924. Physiological studies on apples in storage. J. Agric. Res. 27, 1-38.

Porritt, S.W., Meheriuk, M., 1973. Influence of storage humidity and temperature on breakdown in Spartan apples. Can. J. Plant Sci. 53, 597-599.

Ratkowsky, D.A., 1990. Handbook of nonlinear regression models. Marcel Dekker Inc, New York.

Stow, J., Dover, C.J., Genge, P.M., 2000. Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18, 215-225.

Tijskens, L.M.M., Van Schaik, A.C.R., de Jager, A., Hertog, M.L.A.T.M., 1999. Modelling the firmness of 'Elstar' apples during storage and transport. Acta Hortic. 485, 363-371.

Wang, C.Y., Mellenthin, W.M., Hansen, E., 1972. Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. J. Am. Soc. Hortic. Sci. 97, 9-12.

Watkins, C.B., Bowen, J.H., Walker, V.J., 1989. Assessment of ethylene production by apple cultivars in relation to commercial harvest dates. New Zeal. J. Crop Hortic. Sci. 17, 327-331.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Chapter 5 Temperature and ethylene affect induction of rapid phase softening in slow softening apple cultivars.

5.1 Abstract

The occurrence of rapid softening in apples (*Malus domestica* Borkh.) at warm temperatures (20 to 35°C) varies between cultivars, as some cultivars require exposure to low temperatures before rapid ethylene biosynthesis occurs. The influence of time at low temperatures (0.5°C) on subsequent softening at warm temperatures (20°C) was investigated for the slow softening 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apple cultivars. Ethylene (100 $\mu l.l^{-1}$ for 24 hours) was also applied to apples at 20°C to determine if ethylene treatment could replace the effect of cold treatment. GS fruit that had no ethylene or cold treatment softened slowly at 20°C, while both ethylene and cold treated fruit induced rapid phase softening, and consequently softened more rapidly than non-treated fruit. Non-treated PR fruit also softened slowly at 20°C, but in contrast to GS, rapid phase softening did not occur after either ethylene or cold treatment. The mechanism by which both cold and ethylene treatments induced rapid phase softening in GS may be facilitated by ethylene, as these treatments induced internal ethylene concentrations (IEC) that were 2-3 fold greater than in non-treated fruit at 20°C. However, initiation of rapid phase softening at 20°C was delayed relative to the increase in IEC from low (<1.5 $\mu l.l^{-1}$) basal concentrations for cold-treated GS fruit. This delay for cold-treated GS fruit, and non-initiation of rapid phase softening in PR fruit at 20°C despite IEC's being in excess of 100 $\mu l.l^{-1}$, suggests that the fruits sensitivity to ethylene may be more important than the actual IEC *per se*. It is also suggested that PR may be a mutant genotype of apple with reduced capacity for ethylene biosynthesis and action. The role of ethylene in regulating rapid phase softening of both cultivars at shelf life temperatures may be clarified by research on changes in ethylene sensitivity that occur during maturation and ripening.

Keywords: Malus domestica (Borkh.); Firmness; Tensile strength; Ethylene; Temperature; Respiration; Quality.

5.2 Introduction

Rapid softening of apples during storage and transportation often results in poor quality fruit being delivered to markets and consumers. Harvested apples typically undergo three softening phases during postharvest handling; an initial slow softening phase, followed by a phase of more rapid softening, and then a final slow softening phase (Chapter 4). Studies are required that identify and quantify the influence of different pre- and postharvest factors on initiation, and rate of softening of each of these phases.

One factor that strongly influences rate of softening in harvested apples is temperature (Landfald, 1966), although responses to temperature differ between cultivars (Chapter 4). Rapid phase softening was initiated in 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) cultivars at temperatures from 0°C to 35°C, but was only initiated at 0°C to 12°C for 'Pacific Rose™' (PR) and 'Granny Smith' (GS), as these cultivars softened slowly at 20°C to 35°C (Chapter 4).

The different softening responses of these cultivars to temperature may have been mediated by ethylene, as initiation of rapid phase softening in all cultivars coincided with the time when internal ethylene concentration (IEC) rapidly exceeded 1.5 $\mu l.l^{-1}$, while the slow softening that occurred in GS and PR at 20°C to 35°C coincided with delayed and slow increases in IEC (Chapter 4). It is possible that rapid softening may have been initiated in GS and PR at 20 to 35°C if treatments had been imposed that rapidly increased ethylene production. One treatment known to rapidly initiate autocatalytic ethylene production in GS at 17-20°C is prior exposure to 0-4°C for 2-32 days (Jobling et al., 1991; Larrigaudiere and Vendrell, 1993; Lelievre et al., 1995; Larrigaudiere et al., 1997). Cold treatment, or exposure to exogenous ethylene or propylene (an ethylene analogue), also initiated earlier autocatalytic ethylene biosynthesis in several pear cultivars at 20°C, relative to untreated fruit at 20°C (Wang et al., 1972; Gerasopoulos and Richardson, 1996; 1997; Agar et al., 2000a, b). PR had a similar ethylene and firmness response to GS at different temperatures, suggesting that the ethylene physiology of these cultivars may be similar (Chapter 4). Thus, ethylene biosynthesis of PR may respond to cold treatments in a similar manner to GS.

Cold treated GS fruit also lost more firmness and had higher respiration rates at 20°C, than fruit stored at 20°C without prior cold treatment (Larrigaudiere and Vendrell, 1993). However, it is not known if initiation and rate of rapid phase softening in GS at 20°C was influenced by duration of the cold treatment. Rapid softening of cold-requiring 'Anjou' pears was initiated immediately upon transfer from –1°C to 20°C (Gerasopoulos and Richardson, 1997), while initiation was increasingly delayed for the cold-requiring 'Bartlett' pear as time at –1°C decreased (Agar et al., 2000a). Once rapid softening of these pear cultivars was initiated at 20°C, 'Bartlett' fruit that had more time at –1°C softened slower (Agar et al., 2000a), while 'Anjou' pears softened faster (Gerasopoulos and Richardson, 1997), than fruit that had less time at –1°C. It is possible that apple cultivars also exhibit different softening responses to cold treatments.

This study sought to determine if time at 0.5°C influenced subsequent initiation and rate of rapid phase softening in GS and PR at 20°C, and if a short-term ethylene treatment at harvest could initiate rapid ripening in these cultivars without cold treatment. Loss of firmness was compared with changes in tensile strength and fruit density as indicators of different textural properties, and with respiration rate and IEC as indicators of climacteric development.

5.3 Materials and methods

5.3.1 Fruit supply and treatments

'Pacific Rose™' (PR) apples (200 ± 20g) were harvested from the Fruit Crops Unit, Massey University, Palmerston North, New Zealand. Commercially grown, export quality 'Granny Smith' (GS) apples (170 ± 10g) were transported from Hawkes Bay to Massey University, Palmerston North within 48 hours of harvest.

Ten perforated polyethylene bags (35µm thickness; 50 x 5mm diameter perforations per m^2), each containing 10 fruit, were randomly allocated to: continuous storage at 0.5 ± 0.5°C or 20 ± 0.5°C; 10 (10d), 30 (30d) or 50 (50d) days at 0.5 ± 0.5°C before transfer to 20 ± 0.5°C; and treatment with 100 $\mu l.l^{-1}$ ethylene for 24 hours before being held at 20 ± 0.5°C. Ethylene treatment was performed in sealed 20 l opaque plastic containers (10

fruit per container) containing CO_2 adsorbent (Soda Lime), and a septum for initial ethylene injection. Gas samples (1 ml) were removed periodically during the 24 hour incubation to monitor ethylene, CO_2 and O_2 concentrations inside the containers.

Ten fruit (one fruit per bag) were randomly removed from storage on ten occasions during storage to measure respiration rate (r_{CO_2}), skin background colour (GS only), fruit density, IEC, flesh firmness and cortical tensile strength. Measurements were made before storage, immediately before and after each treatment (ethylene treatment, or transfer from 0.5 to 20°C for cold treatments), and thereafter at 5 to 10 day intervals at 20°C, and 20 to 30 day intervals at 0.5°C.

5.3.2 Storage measurements

All measurements were made on fruit at the treatment temperature. Fruit density was determined by dividing the individual weight of fruits by their volume, with fruit volume estimated by volume displacement in water. Skin background colour was determined by measuring hue angle of the skin twice in the equator region using a chroma-meter (Minolta, model CR-200) calibrated with a green colour standard (Commission Internationale de l'Éclairage units of Y=29.9, x=0.273, y=0.369 using illuminant C light source).

Respiration rate was determined by sealing individual apples in opaque plastic containers, and removing 1 ml headspace gas samples immediately, and one hour after sealing. Gas samples were injected into a gas analyser fitted with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddeson, UK), using O_2 free N_2 as carrier gas at 30 $ml.min^{-1}$, and a Hewlett Packard Integrator (model 3396A) calibrated with external CO_2 standards (certified as β-standard by B.O.C. Gases New Zealand Ltd). Respiration rate was calculated as change in the headspace CO_2 concentration, adjusting for time between gas sampling, container headspace volume, and fruit size.

IEC was determined by injecting 1 ml core cavity gas samples into a gas chromatograph (Pye Unicam GCD) fitted with a flame ionisation detector (set at 140°C with H_2 and air flow rates of 30 $ml.min^{-1}$ and 300 $ml.min^{-1}$ respectively), an activated alumina column

(set at 100°C with N_2 as carrier gas at 30 $ml.min^{-1}$), and a Hewlett Packard integrator (model 3390A) calibrated with external ethylene standards (certified as β-standard by B.O.C. Gases New Zealand Ltd).

Tensile strength was measured using the gripping method and tissue shape described in Stow (1989), and recorded as maximum force to separate a 10 by 5mm cross-sectional area of tissue plug removed from the fruit cortex, at a crosshead speed of 1 $mm.s^{-1}$ with a Stable Micro Systems TA-XT2 Texture Analyser.

Flesh firmness was measured with a drill-press mounted 'Effegi' penetrometer fitted with an 11.1 mm diameter probe; the maximum force required to puncture pared tissue to a depth of 7.9 mm on opposite sides of the fruit equator was recorded.

5.3.3 Data Analysis

Analysis of variance was performed, and least significant differences calculated using the general linear model procedure in SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

5.4 Results

Loss of firmness was triphasic for both GS and PR at 0.5°C, consisting of an initial slow softening phase for about 25 days, followed by a phase of more rapid softening lasting for approximately 125 days and then a final slow softening phase for the remainder of the experiment (Fig. 5-1). When both cultivars were stored at 20°C without cold or ethylene treatment, the fruit softened slowly, or increased slightly in firmness, and rapid phase softening did not occur. However, rapid phase softening was initiated in ethylene treated GS fruit after about 20 days at 20°C, while there was no effect of ethylene treatment on softening of PR fruit (Fig. 5-2). Maintaining GS fruit at 0.5°C for different times also initiated rapid phase softening once transferred to 20°C, but this did not occur in PR where fruit continued to soften slowly at 20°C after cold-treatment. However, rapid phase softening did not commence immediately after transfer from 0.5 to 20°C for GS, with the delay reduced from 20 to 3 days when previously stored for longer time at 0.5°C. Once rapid phase softening was initiated in GS at 20°C, rate of rapid phase

softening was slower as time at 0.5°C increased, resulting in convergence of firmness from all cold treatments after 40 days at 20°C. The rate of rapid phase softening in ethylene treated GS fruit at 20°C was similar to that of fruit that had 10 days at 0.5°C.

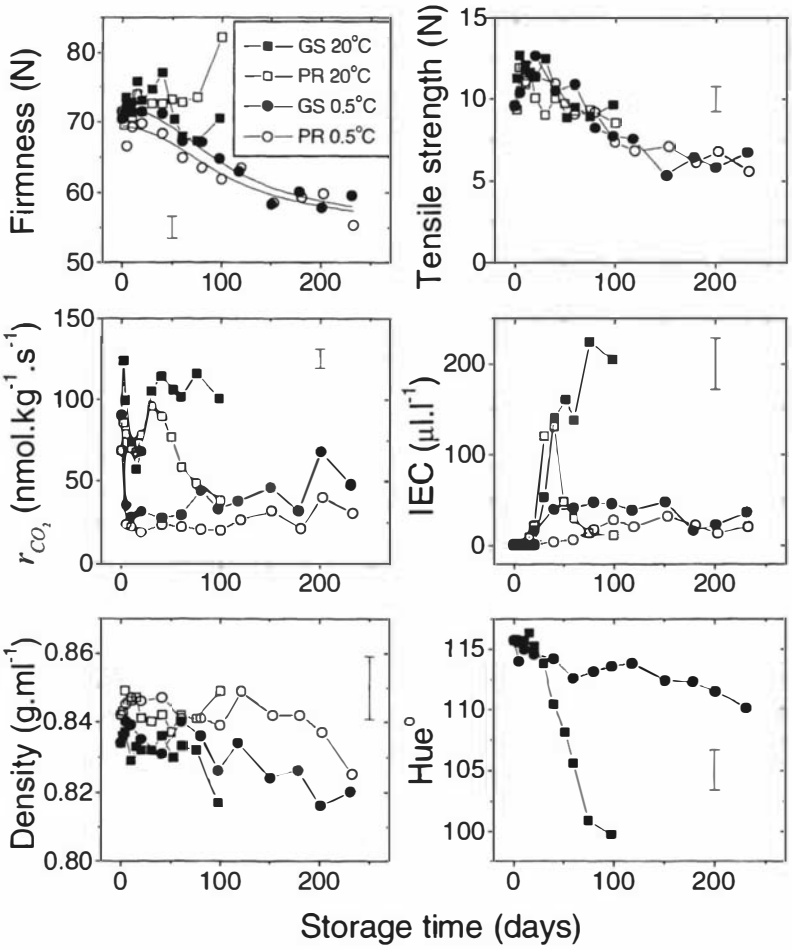

Fig. 5-1 Flesh firmness, cortical tensile strength, respiration rate (r_{CO_2}), internal ethylene concentration (IEC), density and skin background colour (Hue°) of 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples continuously stored at 0.5 and 20°C. Treatment means (n=10) and least significant differences (5%) are shown.

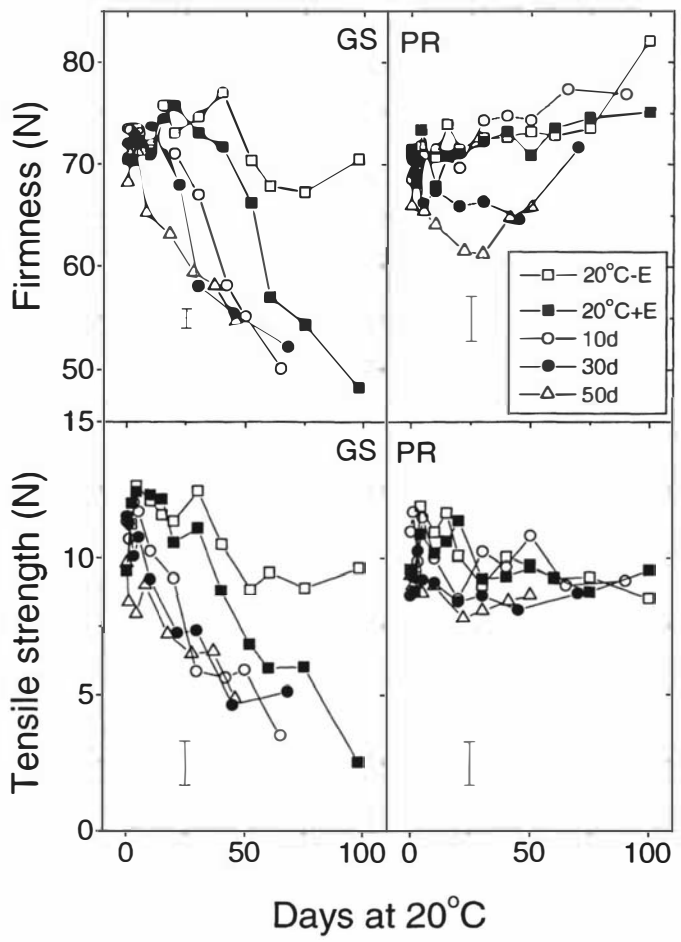

Fig. 5-2 Flesh firmness and cortical tensile strength of 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples ± ethylene (E) treatment at harvest and held at 20°C, or held at 0.5°C for 10 (10d), 30 (30d) and 50 (50d) days before being held at 20°C. Treatment means (n=10) and least significant differences (5%) are shown.

Change in tensile strength was also triphasic for both cultivars at 0.5°C (Fig. 5-1). Tensile strength for both GS and PR increased slightly for the first 10-20 days at 0.5°C, then decreased until 100-150 days at 0.5°C, after which there was little change. At 20°C, tensile strength also increased for the first 10-20 days for both cultivars, but then decreased slowly in fruit without ethylene or cold treatment. Rapid loss of tensile

strength was initiated by both ethylene and cold treatments in GS, but not in PR at 20°C (Fig. 5-2). In addition, initiation of rapid loss of tensile strength in GS at 20°C was delayed about 20 days after ethylene treatment, delayed about 5 days after the 10 day cold treatment, and was initiated immediately after 30 and 50 days of cold treatment. Once rapid loss of tensile strength was initiated at 20°C, the rate of texture loss was more rapid for fruit exposed to ethylene treatment or to10 days at 0.5°C, than for fruit that had been at 0.5°C for 30 and 50 days.

IEC remained low (<1.5 $\mu l.l^{-1}$) for both cultivars during the first 10-20 days at 0.5°C, increased slowly to 30-50 $\mu l.l^{-1}$ after 150 days at 0.5°C, and then decreased slightly for the remainder of storage (Fig. 5-1). IEC also remained low (<1.5 $\mu l.l^{-1}$) in non-treated fruit from both cultivars for the first 10-20 days at 20°C, after which it increased to a maximum concentration of about 200 $\mu l.l^{-1}$ in GS at 75 days, and a peak concentration of about 130 $\mu l.l^{-1}$ in PR at 40 days, before decreasing thereafter for PR. IEC increased immediately upon transfer from 0.5°C to 20°C for GS fruit from all cold treatments, and PR fruit that had 50 days at 0.5°C (Fig. 5-3). However, the increase in IEC at 20°C was delayed approximately 15 days after ethylene treatment for both cultivars, and delayed 10-15 days for PR fruit that had 10 and 30 days at 0.5°C. The maximum IEC attained in ethylene and cold treated GS fruit at 20°C was 2-3 fold higher than in non-treated fruit at 20°C, while the maximum IEC attained in PR at 20°C was similar for fruit from all treatments.

For both cultivars, r_{CO_2} remained low for 100 days at 0.5°C, then increased slowly to 40 $nmol.kg^{-1}.s^{-1}$ for PR, and 70 $nmol.kg^{-1}.s^{-1}$ for GS after about 200 days, and decreased thereafter (Fig. 5-1). For non-treated fruit at 20°C, r_{CO_2} decreased during the first 10-20 days by 50% for GS and by 20% for PR, then increased to the harvest value, before decreasing again after about 50 days for PR. GS fruit that had ethylene or cold treatment had a higher r_{CO_2} at 20°C than non-treated fruit (Fig. 5-3). In contrast r_{CO_2} of ethylene-treated PR fruit was only higher than non-treated fruit for the first 25 days at 20°C, after which r_{CO_2} was similar. PR fruit that had been maintained at 0.5°C had similar r_{CO_2} to non-treated fruit once transferred to 20°C. The increase in r_{CO_2} that followed the initial decrease after harvest, occurred 10 days earlier for ethylene-treated

GS fruit than for non-treated fruit at 20°C. For all GS treatments, r_{CO_2} and IEC increased simultaneously at 20°C (Fig. 5-3).

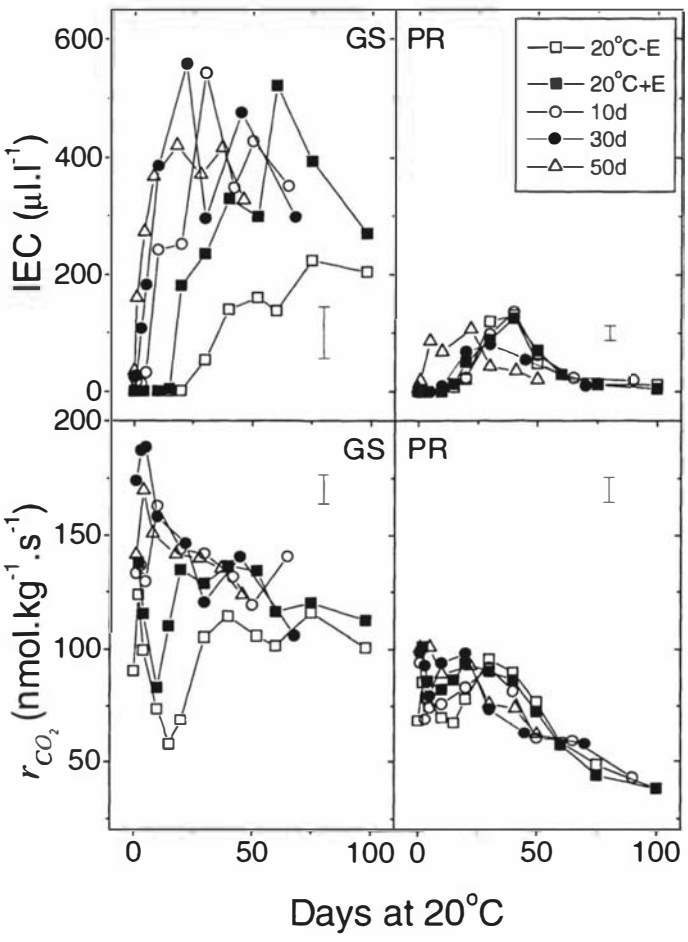

Fig. 5-3 Internal ethylene concentration (IEC) and respiration rate (r_{CO_2}) of 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples ± ethylene (E) treatment at harvest and held at 20°C, or held at 0.5°C for 10 (10d), 30 (30d) and 50 (50d) days before being held at 20°C. Treatment means (n=10) and least significant differences (5%) are shown.

While density was similar for GS fruit held at 0.5°C and 20°C for 100 days (Fig. 5-1), it decreased rapidly in ethylene treated fruit after 50 days at 20°C (Fig. 5-4). Density of PR at 20°C was similar regardless of ethylene treatment (Fig. 5-4), but decreased at 0.5°C after 175 days (Fig. 5-1). Time at 0.5°C did not influence density of either cultivar transferred to 20°C (Fig. 5-4).

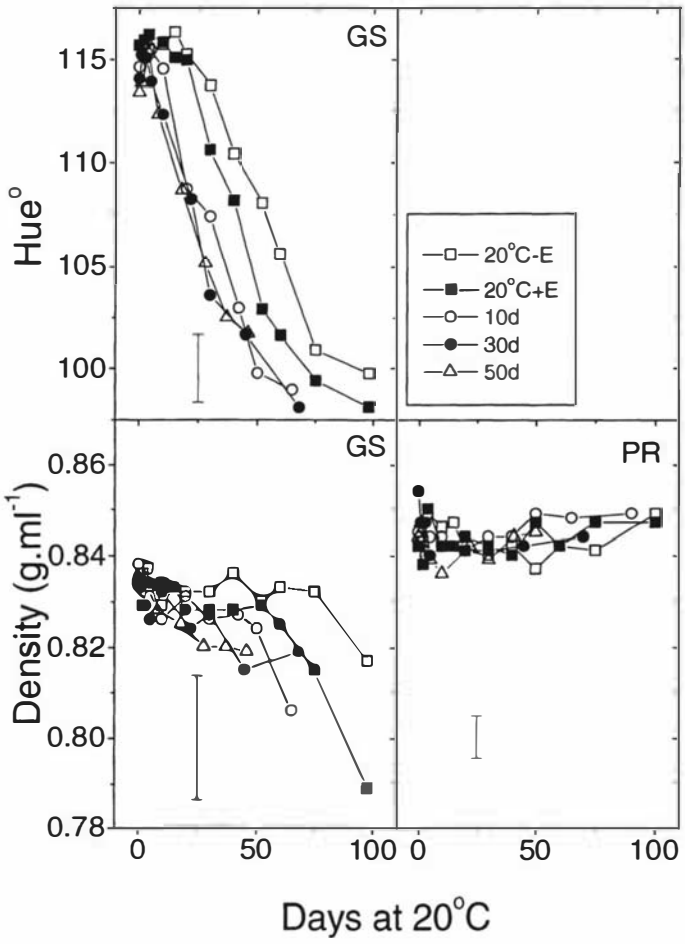

Fig. 5-4 Density and skin background colour (Hue°) of 'Granny Smith' (GS) and 'Pacific Rose™' (PR) apples ± ethylene (E) treatment at harvest and held at 20°C, or held at 0.5°C for 10 (10d), 30 (30d) and 50 (50d) days before being held at 20°C. Treatment means (n=10) and least significant differences (5%) are shown.

The skin of GS fruit yellowed considerably slower at 0.5°C than at 20°C (Fig. 5-1). Unlike softening, initiation of rapid skin yellowing occurred at 20°C without ethylene or cold treatment, although it occurred earlier at 20°C for fruit that had ethylene and cold treatment (Fig. 5-4). Once rapid skin yellowing was initiated at 20°C, the rate of yellowing was similar for fruit from all treatments, with initiation (Fig. 5-4) coinciding with increased IEC and r_{CO_2} (Fig. 5-3).

5.5 Discussion

The softening responses at different temperatures for the GS and PR cultivars used in this study, and for those cultivars reported previously (Chapter 4), suggest at least three softening responses to temperature occur among apple cultivars. Rapid phase softening occurred at shelf-life temperatures in 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) without prior cold or ethylene treatment (Chapter 4), it only occurred in GS when previously exposed to cold temperatures or exogenous ethylene, and did not occur in PR regardless of prior exposure to cold temperatures or ethylene.

The longer that GS fruit were exposed to 0.5°C the shorter the delay before initiation of subsequent rapid phase softening at 20°C, and the slower the rate of rapid softening once initiated. This also occurred with 'Bartlett' pears at 20°C after different times at –1°C (Agar et al., 2000a). However in 'Anjou' pears, rapid softening was initiated immediately upon transfer from –1°C to 20°C, and the softening rate at 20°C was faster for fruit that had longer time at –1°C (Gerasopoulos and Richardson, 1997). These pear cultivars are similar to GS in that they both required exposure to low temperatures or ethylene to initiate autocatalytic ethylene production and ripening at 20°C (Jobling et al., 1991; Gerasopoulos and Richardson, 1997; Agar et al., 2000a). However, it is not yet known what mechanism caused these pear cultivars to soften differently at 20°C following cold treatments of different duration.

As for pears, a short-term ethylene treatment was effective at initiating rapid phase softening and autocatalytic ethylene production at 20°C for GS fruit that were not previously exposed to low temperatures. However, initiation of rapid phase softening and autocatalytic ethylene production was delayed 15-20 days after ethylene treatment. A delayed ripening and ethylene production response to ethylene treatment has been observed in several fruits, including 'Cox's Orange Pippin' apples, bananas and pears (Peacock, 1972; Wang et al., 1972; Knee et al., 1987). These ripening delays for pears were reduced when using higher ethylene or propylene concentrations (Wang et al., 1972; Gerasopoulos and Richardson, 1996), higher application temperatures (Agar et al., 2000b), and use of fruit with more advanced harvest maturity (Wang et al., 1972; Agar et al., 1999). Similarly, the onset of autocatalytic ethylene production in apples

was hastened by increasing the concentration of ethylene during treatment, and by delaying the harvest date (Sfakiotakis and Dilley, 1973; Knee et al., 1987). Thus, the observed delay between ethylene treatment and initiation of rapid phase softening of GS in this experiment may have been influenced by the ethylene concentration utilised, and the maturity of the fruit at harvest.

In contrast to GS, ethylene treatment did not induce rapid phase softening in PR at 20°C. This may indicate that PR fruit were not sensitive to the ethylene concentration applied at harvest (Harkett et al., 1971; Sfakiotakis and Dilley, 1973), and/or that this cultivar is physiologically incapable of undergoing rapid softening at warm temperatures. Despite similar changes in IEC for ethylene treated and non-treated PR fruit at 20°C, r_{CO_2} was initially higher in ethylene treated fruit than non-treated fruit for 30 days at 20°C, indicating that ethylene treatment did induce a physiological response in this cultivar.

Different times at 0.5°C, like ethylene treatment, did not induce rapid phase softening in PR at 20°C. In addition, neither exogenous ethylene treatment nor cold treatment increased the maximum IEC attained in PR at 20°C when compared to non-treated fruit at 20°C, which contrasted with results obtained for GS fruit. However, like non-treated GS fruit, the peak IEC of 100µl.l^{-1} that occurred late in storage for PR fruit at 20°C should have been sufficient to initiate rapid softening. Because this did not occur, it is possible that PR fruit at 20°C may be deficient in the appropriate receptors and signal transduction pathway(s), and thus not be able to perceive the increased IEC thought to be needed to initiate rapid phase softening. Despite non-induction of rapid phase softening in PR at 20°C, it was induced at 0 to 12°C (Chapter 4). This suggests that continuous cold treatment may be required for this cultivar to be sensitive to, and act on, increased ethylene to initiate and sustain rapid phase softening.

The effect of ethylene and cold treatments on softening, skin yellowing and respiration rate in GS and PR, were probably mediated by ethylene. Rind yellowing, flesh softening and climacteric respiration were identified as being ethylene dependent processes in transgenic melons with suppressed ethylene biosynthesis (Guis et al., 1997; Pech et al., 1999) and in apples treated with inhibitors of ethylene action (Blankenship and Sisler, 1989; 1993; Fan et al., 1999; Fan and Mattheis, 1999; Watkins et al., 2000).

However, for both GS and PR at 20°C, initiation of rapid phase softening was disassociated from the rapid increase in IEC from low (<1.5 $\mu l.l^{-1}$) basal concentrations. This was clearly evident in PR, as rapid phase softening was not induced in this cultivar at 20°C despite having IEC's in excess of 100 $\mu l.l^{-1}$. For cold treated GS fruit at 20°C, the rapid increase in IEC's from low basal concentrations preceded initiation of rapid phase softening, with this delay being less apparent for fruit previously stored at 0.5 for increased time. In contrast initiation of other ripening pathways, such as skin yellowing and increased r_{CO_2}, coincided with the time when IEC's increased rapidly from a low basal concentration. Thus in both cultivars initiation of rapid phase softening was dissociated from increased ethylene production.

For pears, softening and chlorophyll degradation were simultaneously initiated before induction of autocatalytic ethylene production (Gerasopoulos and Richardson, 1997) and r_{CO_2} (Porritt, 1964). Gerasopoulos and Richardson (1997) explained this disassociation by suggesting that softening may have been initiated at lower ethylene concentrations than those attained during autocatalytic ethylene production. This hypothesis is supported by the finding that rapid softening was initiated at the low external ethylene concentrations of 0.05 to 0.2 $\mu l.l^{-1}$ for pears (Wang et al., 1972), and an external ethylene concentration of 0.01 $\mu l.l^{-1}$ for kiwifruit (Jeffery and Banks, 1996). Threshold ethylene concentrations for initiating softening of apples in controlled atmospheres have been suggested as an internal concentration of 0.1 $\mu l.l^{-1}$ (Stow et al., 2000), and an external concentration of 1 $\mu l.l^{-1}$ (Liu, 1977). Despite the results presented herein being for apples in air, this IEC threshold for apples was clearly exceeded in PR and GS at 20°C without immediate induction of rapid phase softening. Thus, IEC *per se* was not limiting for immediate induction of rapid phase softening in these cultivars at 20°C.

Disassociation between these different ripening pathways (particularly softening) could be facilitated by differential expression of ethylene receptors specific for each process. Multiple ethylene receptors have been identified in tomato, although definitive roles for each receptor in tomato development are currently not known (Klee et al., 1999). It is possible that each receptor regulates different developmental processes, or that each receptor is functionally redundant (Klee et al., 1999). Apple fruit may also have

multiple ethylene receptors, some of which may be developmentally regulated to control different ripening processes, such as autocatalytic ethylene production, skin yellowing, r_{CO_2} and softening. It is also possible that each ripening pathway may have a common ethylene receptor, and that there may be different signal transduction pathways with different response capacities for each ripening pathway.

Autocatalytic ethylene production requires upregulation of the key regulatory ethylene biosynthetic enzymes, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, and perhaps activation of receptors and signal transduction pathways that facilitate upregulation of these enzymes (Yang and Hoffman, 1984; Payton et al., 1996; Lelievre et al., 1997; Klee et al., 1999). The mechanism by which cold treatments induced earlier autocatalytic ethylene production in GS than in non-treated fruit at 20°C has been largely elucidated. Cold-treated GS fruit have been shown to accumulate ACC and ACC oxidase at 0-4°C (Jobling et al., 1991; Larrigaudiere and Vendrell, 1993; Lelievre et al., 1995; Larrigaudiere et al., 1997), and have increased ACC oxidase activity upon transfer from cold to warm temperatures (Larrigaudiere and Vendrell, 1993; Larrigaudiere et al., 1997).

In contrast to GS, the ethylene biosynthesis of PR at shelf-life temperatures was not stimulated by prior exposure to low temperatures. IEC's of PR fruit transferred to 20°C after 10-30 days at 0.5°C were similar to those for PR held continuously at 20°C with or without ethylene treatment. Furthermore, the maximum IEC's attained in PR at 20°C were similar for fruit from all treatments. Therefore, because PR does not have ethylene biosynthesis induced by cold treatment, and it has a maximum IEC at 20°C that is 70-80% less than that in GS (after cold treatment) and other cultivars such as 'Cox's Orange Pippin' and 'Royal Gala' at 20°C (Chapter 4), it is suggested that PR is a mutant genotype of apple with reduced capacity for ethylene biosynthesis and action.

The nature of the mutation in PR that imposes reduced ethylene production and softening relative to other apple cultivars is currently not known. This mutation was most dominant at shelf-life temperatures (20-35°C), as rapid phase softening was not initiated at these temperatures irrespective of prior cold or ethylene treatment. Other apple cultivars, such as 'Honeycrisp', 'NJ55' and 'PA14-238', have also been identified

as slow softening apple cultivars (Gussman et al., 1993; Tong et al., 1999). However, it is not known if these cultivars are like PR in not initiating rapid phase softening at shelf-life temperatures. Slow softening and low ethylene production mutants have also been identified in several other fruits, including nectarines, tomatoes and kiwifruit (Tigchelaar et al., 1978; Brecht et al., 1984; Hewett et al., 1999). Research is required to determine the molecular basis that causes PR to soften slowly, as done for several tomato ripening mutants (Lelievre et al., 1997).

Rapid softening is an undesirable trait in apples, as firmness is rapidly reduced to an unmarketable level during postharvest handling. Temperature strongly influenced both the initiation and rate of rapid phase softening, although the extent of this effect was cultivar dependent, and was dependent on the ethylene physiology of the cultivar. Further studies are required to determine if the softening behaviour of other commercially important apple cultivars at different temperatures also conform to the three classes identified in this study, and to determine the role of ethylene sensitivity in determining the softening response of different cultivars to temperature. Research is also required to determine the molecular basis for the slow softening characteristic of PR, especially at shelf-life temperatures.

5.6 References

Agar, I.T., Biasi, W.V., Mitcham, E.J., 1999. Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region. Postharvest Biol. Technol. 17, 67-78.

Agar, I.T., Biasi, W.V., Mitcham, E.J., 2000a. Cold storage duration influences ethylene biosynthesis and ripening of 'Bartlett' pears. HortScience 35, 687-690.

Agar, I.T., Biasi, W.V., Mitcham, E.J., 2000b. Temperature and exposure time during ethylene conditioning affect ripening of Bartlett pears. J. Agric. Food Chem. 48, 165-170.

Blankenship, S.M., Sisler, E.C., 1989. 2,5-Norbornadiene retards apple softening. HortScience 2, 313-314.

Blankenship, S.M., Sisler, E.C., 1993. Response of apples to diazocyclopentadiene inhibition of ethylene binding. Postharvest Biol. Technol. 3, 95-101.

Brecht, J.K., Kader, A.A., Ramming, D.W., 1984. Description and postharvest physiology of some slow-ripening nectarine genotypes. J. Am. Soc. Hortic. Sci. 109, 596-600.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Fan, X., Mattheis, J.P., 1999. Methyl Jasmonate promotes apple fruit degreening independently of ethylene action. HortScience 34, 310-312.

Gerasopoulos, D., Richardson, D.G., 1996. Effects of exogenous propylene and fruit calcium on ripening of non-chilled and chilled Anjou pears. Postharvest Biol. Technol. 8, 111-120.

Gerasopoulos, D., Richardson, D.G., 1997. Storage-temperature-dependent time separation of softening and chlorophyll loss from the autocatalytic ethylene pathway and other ripening events of 'Anjou' pears. J. Am. Soc. Hortic. Sci. 122, 680-685.

Guis, M., Botondi, R., Ben-Amor, M., Ayub, R., Bouzayen, M., Pech, J-C., Latche, A., 1997. Ripening-associated biochemical traits of cantaloupe charentais melons expressing an antisense ACC oxidase transgene. J. Am. Soc. Hortic. Sci. 122, 748-751.

Gussman, C.D., Goffreda, J.C., Gianfagna, T.J., 1993. Ethylene production and fruit-softening rates in several apple fruit ripening variants. HortScience 28, 135-137.

Harkett, P.J., Hulme, A.C., Rhodes, M.J.C., Wooltorton, L.S.C., 1971. The threshold value for physiological action of ethylene on apple fruits. J. Food Technol. 6, 39-45.

Hewett, E.W., Kim, H.O., Lallu, N., 1999. Postharvest physiology of kiwifruit: the challenges ahead. Acta Hortic. 498, 203-216.

Jeffery, P.B., Banks, N.H., 1996. Effects of ethylene on kiwifruit softening in coolstorage. New Zeal. Kiwifruit J. 114, 9-10.

Jobling, J.J., McGlasson, W.B., Dilley, D.R., 1991. Induction of ethylene synthesizing competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1, 111-118.

Klee, H., Tieman, D., Lashbrook, C., 1999. Ethylene perception in tomato: lots of genes, lots of functions. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J-C. , Grierson, D. (eds.), Biology and Biotechnology of the Plant Hormone Ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 351-356.

Knee, M., Hatfield, S.G.S., Bramlage, W.J., 1987. Response of developing apple fruits to ethylene treatment. J. Exp. Bot. 38, 972-979.

Landfald, R., 1966. Temperature effects on apples during storage. Bull. Int. Instit. Refrig. Ann. 1966-1, 453-460.

Larrigaudiere, C., Graell, J., Salas, J., Vendrell, M., 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Postharvest Biol. Technol. 10, 21-27.

Larrigaudiere, C., Vendrell, M., 1993. Cold-induced activation of 1-aminocyclopropane-1-carboxylic acid metabolism in rewarmed Granny Smith apples: Consequences on ripening. Sci. Hortic. 55, 263-272.

Lelievre, J-M., Latche, A., Jones, B., Bouzayen, M., Pech, J-C., 1997. Ethylene and fruit ripening. Physiol. Plant. 101, 727-739.

Lelievre, J-M., Tichit, L., Fillion, L., Larrigaudiere, C., Vendrell, M., Pech, J-C., 1995. Cold-induced accumulation of 1-aminocyclopropane-1-carboxylate oxidase protein in Granny Smith apples. Postharvest Biol. Technol. 5, 11-17.

Liu, F.W., 1977. Varietal and maturity differences of apples in response to ethylene in controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 102, 93-95.

Payton, S., Fray, R.G., Brown, S., Grierson, D., 1996. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. Plant Mol. Biol. 31, 1227-1231.

Peacock, B.C., 1972. Role of ethylene in the initiation of fruit ripening. Queensl. J. Agric. Animal Sci. 29, 137-145.

Pech, J-C., Guis, M., Botondi, R., Ayub, R., Bouzayen, M., Lelievre, J-M., El Yahyaoui, F., Latche, A., 1999. Ethylene-dependent and ethylene-independent pathways in a climacteric fruit, the melon. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J-C. , Grierson, D. (eds.), Biology and Biotechnology of the Plant Hormone Ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 105-110.

Porritt, S.W., 1964. The effect of temperature on postharvest physiology and storage life of pears. Can. J. Plant Sci. 44, 568-579.

Sfakiotakis, E., Dilley, D.R., 1973. Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. J. Am. Soc. Hortic. Sci. 98, 504-508.

Stow, J., 1989. The involvement of calcium ions in maintenance of apple fruit tissue structure. J. Exp. Bot. 40, 1053-1057.

Stow, J., Dover, C.J., Genge, P.M., 2000. Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18, 215-225.

Tigchelaar, E.C., McGlasson, W.B., Buescher, R.W., 1978. Genetic regulation of tomato fruit ripening. HortScience 13, 508-513.

Tong, C., Krueger, D., Vickers, Z., Bedford, D., Luby, J., El-Shiekh, A., Shackel, K., Ahmadi, H., 1999. Comparison of softening-related changes during storage of 'Honeycrisp' apple, its parents, and 'Delicious'. J. Am. Soc. Hortic. Sci. 124, 407-415.

Wang, C.Y., Mellenthin, W.M., Hansen, E., 1972. Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. J. Am. Soc. Hortic. Sci. 97, 9-12.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Yang, S.F., Hoffman, N.E., 1984. Ethylene biosynthesis and its regulation in higher plants. Ann. Rev. Plant Physiol. 35, 155-189.

Chapter 6 Postharvest coolchain effects on softening of apples.

6.1 Abstract

'Royal Gala' and 'Cox's Orange Pippin' apples (*Malus domestica* Borkh.) soften rapidly after harvest, resulting in poor fruit quality after extended periods of storage and transportation. This study sought to characterise the effects of delayed cooling, intermittent warming and shelf life temperatures on softening of these cultivars during postharvest handling. Freshly harvested fruit that were held for increased time at 20°C before cooling to 0.5-3°C were softer at the beginning of low-temperature storage, and thereafter at 0.5-3°C, than fruit cooled without delay after harvest. Fruit temporarily transferred from 0.5-3°C to ambient temperatures (12 and 20°C) were subsequently softer once returned to 0.5-3°C, when either temperature or duration of the intermittent warming period was increased. Softening rates for either cultivar at 20°C were similar regardless of prior temperature (0.5-12°C) or time at 0.5-3°C. Using softening rates calculated from constant temperature treatments, it was possible to describe softening in treatments with stepwise temperature changes, indicating that rate of softening at a given temperature was not affected by prior exposure to temperatures between 0.5°C and 20°C. Results from this research could be used to develop models that describe the effects of temperature on softening of both cultivars during different phases of postharvest handling.

Keywords: Malus domestica (Borkh.); Firmness; Ethylene; Coolchain; Quality; Empirical modelling.

6.2 Introduction

The optimum temperature for minimising quality deterioration of harvested apples (*Malus domestica* Borkh.) is usually 0 to 3°C, depending on cultivar sensitivity to chilling injury. However, it is difficult to maintain optimum temperatures throughout the entire postharvest handling chain. Fruit are often exposed to non-optimal temperatures during packing (at harvest or after storage), ship loading, distribution to retailers, and in retail outlets. Detailed knowledge on the comparative effects of these non-optimal temperatures on the softening rates of harvested apples is lacking.

'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) are commercially important early season apple cultivars in New Zealand that soften rapidly after harvest. While the rate of softening has been estimated for these cultivars when stored continuously at 0°C to 35°C (Chapter 4), it is not known if the rate of softening at a given temperature is affected by prior exposure to other temperatures. Time at 0.5°C influenced softening of the 'Granny Smith' (GS) cultivar at 20°C, as fruit with increased time at 0.5°C had a shorter initial slow softening phase, and slower rapid softening phase once transferred to 20°C (Chapter 5). However, GS required prior chilling to rapidly initiate autocatalytic ethylene production (Jobling et al., 1991) and rapid softening at 20°C (Larrigaudiere and Vendrell, 1993; Chapter 5), which was not required for RG and COP (Knee et al., 1983; Larrigaudiere et al., 1997; Chapter 4). Thus, the softening response of RG and COP to temperature perturbations may be quite different to that previously observed in GS.

The influence of temperature perturbations on softening may be mediated by changes in ethylene production. An increased delay between harvest and cooling increased both fruit softening and ethylene production in 'McIntosh' apples in controlled atmosphere storage at 3°C (Liu, 1986). Evidence for the importance of ethylene in promoting apple softening was strengthened following studies using 1-methylcyclopropene, as softening was reduced in several cultivars when this inhibitor of ethylene action was applied at harvest (Fan et al., 1999; Watkins et al., 2000b). More research is required to determine if other instances of non-optimal temperatures during postharvest handling also affect softening through changes in endogenous ethylene concentrations.

Although several studies have determined the effect of cooling delays on softening in storage, limited knowledge is available on the comparative effects of other instances of non-optimal temperatures that occur during postharvest handling. Furthermore, the influence of time at a given temperature on the subsequent rate of softening at another temperature is not known. Thus, this study sought to determine the effect of sequential temperature treatments on subsequent softening rates of RG and COP by exposing fruit to cooling delays at harvest, temporarily removing fruit from low-temperatures to shelf-life temperatures, and transferring fruit from different temperatures to shelf-life temperatures. An additional aim was to determine if the effect of these instances of

non-optimal temperatures on softening reflected changes in endogenous ethylene concentrations for both cultivars.

6.3 Materials and methods

6.3.1 Fruit supply and treatments

Export quality RG and COP apples were harvested in 1999 and 2000 at commercial export maturity, graded and transported from Hawkes Bay (RG only) and Nelson (COP only) to Massey University, Palmerston North, New Zealand within 48 hours of harvest. Fruit sizes used in the experiments were 170 ± 10g for RG and 140 ± 10g for COP.

Fruit from both cultivars were randomly distributed across four experiments to evaluate the effect of: delayed cooling at harvest in 1999 and 2000; temporary transfer of fruit from low temperatures (0.5-3°C) to ambient temperatures (10-20°C) in 1999 and 2000; different times at low temperatures before transfer to 20°C in 1999; and exposure to different temperatures before transfer to 20°C in 1999. Details of experimental treatments are in Table 6-1. Fruit were also stored continuously at 0.5°C (3°C for COP), 5°C, 10°C, 12°C or 20°C as control treatments for all experiments.

For each treatment, 100 fruit were evenly distributed among ten perforated polyethylene bags (35µm thickness; 50 x 5mm diameter perforations per m^2), that in turn were stored in commercial cardboard cartons (18kg). Ten fruit (one fruit per bag) were randomly removed from storage for measurement of internal ethylene concentration (IEC) and flesh firmness at the start of the experiment, before and after changes in treatment temperature, and thereafter at 2-10 day intervals at 10-20°C, and at 10-30 days intervals at 0.5-5°C. Fruit with rots and disorders were omitted from the firmness and IEC datasets.

Table 6-1 Treatment structure for delayed cooling, intermittent warming, and shelf-life experiments conducted on 'Cox's Orange Pippin' (COP) and 'Royal Gala' (RG) apples in 1999 and 2000.

Season	Stepwise temperature treatments	Treatment code
	Delayed cooling experiments	
1999	1d at 20°C ⇒ 0.5[a] or 3°C[b].	DC_{1d}
	3d at 20°C ⇒ 0.5 or 3°C.	DC_{3d}
	5d at 20°C ⇒ 0.5 or 3°C.	DC_{5d}
2000	1d at 20°C ⇒ 0.5 or 3°C.	DC_{1d}
	2d at 20°C ⇒ 0.5 or 3°C.	DC_{2d}
	4d at 20°C ⇒ 0.5 or 3°C.	DC_{4d}
	Intermittent warming experiment	
1999	50d at 0.5°C[a] or 20d at 3°C[b] ⇒ 1d at 12°C ⇒ 0.5[a] or 3°C[b]	IW_{1d}
	50d at 0.5°C or 20d at 3°C ⇒ 3d at 12°C ⇒ 0.5 or 3°C	IW_{3d}
	50d at 0.5°C or 20d at 3°C ⇒ 7d at 12°C ⇒ 0.5 or 3°C	IW_{7d}
2000	10d at 0.5 or 3°C ⇒ 2d at 10°C ⇒ 0.5 or 3°C	$IW_{2d\text{-}10^oC}$
	10d at 0.5 or 3°C ⇒ 2d at 20°C ⇒ 0.5 or 3°C°C	$IW_{2d\text{-}20^oC}$
	Shelf-life experiment	
1999	10d at 3°C[b] ⇒ 20°C	t_{10d}
	20d at 3°C ⇒ 20°C	t_{20d}
	30d at 3°C ⇒ 20°C	t_{30d}
1999	25d at 0.5°C[a] ⇒ 20°C	t_{25d}
	50d at 0.5°C ⇒ 20°C	t_{50d}
	75d at 0.5°C ⇒ 20°C	t_{75d}
	100d at 0.5°C ⇒ 20°C	t_{100d}
	125d at 0.5°C ⇒ 20°C	t_{125d}
1999	25d at 0.5°C ⇒ 20°C	$T_{0.5}$[a]
	10d at 3°C ⇒ 20°C	T_3[b]
	25d at 5°C ⇒ 20°C	T_5[a]
	10d[b] or 25d[a] at 12°C ⇒ 20°C	T_{12}

Abbreviations: then transferred to (⇒); days (d); time (t); temperature (T); delayed cooling (DC); intermittent warming (IW).
[a] 'Royal Gala' only.
[b] 'Cox's Orange Pippin' only.

6.3.2 *Flesh firmness and internal ethylene concentration measurement*

Flesh firmness was measured with a drill-press mounted 'Effegi' penetrometer fitted with an 11.1 mm diameter probe; the maximum force required to puncture pared tissue

to a depth of 7.9 mm on opposite sides of the fruit equator was recorded. Firmness was measured at the treatment temperature.

IEC was determined on 1 ml gas samples from the core cavity, injected into a gas chromatograph (Pye Unicam GCD) fitted with a flame ionisation detector (set at 140°C with H_2 and air flow rates of 30 ml.min^{-1} and 300 ml.min^{-1}, respectively), an activated alumina column (set at 100°C with N_2 as the carrier gas at 30 ml.min^{-1}), and a Hewlett Packard integrator (model 3390A) calibrated with external ethylene standards (certified as β-standard by B.O.C. Gases New Zealand Ltd).

6.3.3 Data analysis

Firmness (f, N) data after different times (t, day) at 0.5°C to 20°C were fitted with an empirical sigmoidal function previously used for apple softening (Chapter 4), using non-linear regression:

$$f = f_{-\infty} - (f_{-\infty} - f_{+\infty}) \cdot \left(1 - \left(1 + \exp\frac{t + k^{-1} \cdot \ln(31) - (5.2 \cdot k^{-1.0168})}{k^{-1}}\right)^{-0.2}\right) \qquad \text{(Eq. 6-1)}$$

where model parameters were an initial firmness ($f_{-\infty}$, N) and minimum firmness asymptote ($f_{+\infty}$, N), and rate of firmness change (k, day^{-1}). Non-linear regression was performed using the NLIN procedure in the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA). Softening in treatments with more than one temperature was described using parameter estimates from constant temperature treatments obtained in this study.

6.4 Results

In both seasons softening of RG fruit held continuously at 0.5°C was triphasic, with an initial slow softening phase for 10-25 days, then a phase of more rapid softening for about 150 days, and a final slow softening phase thereafter (Fig's 6-1 and 6-2). COP softening was also triphasic when continuously held at 3°C in 2000 (Fig. 6-2), but was biphasic in 1999 with no discernible initial slow softening phase (Fig. 6-1). The initial slow softening phase of 2000 COP fruit lasted for approximately 10 days at 3°C, and the phase of more rapid softening continued for 80-90 days in both seasons.

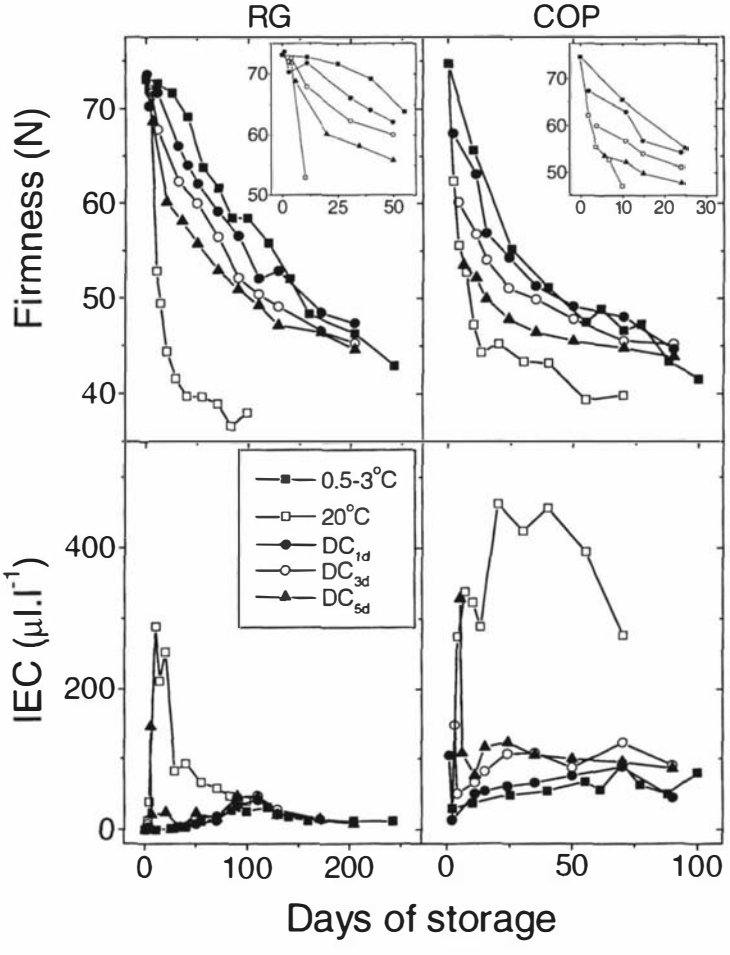

Fig. 6-1 Flesh firmness and internal ethylene concentration (IEC) of 1999 season 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples from different delayed cooling (DC) treatments. Figure insets depict firmness data on a reduced firmness and time scale. Details of delayed cooling treatments are given in Table 6-1. Treatment means (n=10) are shown.

Delayed cooling after harvest reduced the duration of the initial slow softening phase for both cultivars. The initial slow softening phase in RG fruit was reduced from 10-25 days in fruit with no cooling delay, to 0 days after a cooling delay of 2-3 days at 20°C in both seasons (Fig's 6-1 and 6-2). Similarly for 2000 season COP fruit, the initial slow

softening phase was reduced from approximately 10 days in fruit with no cooling delay, to 0 days after a cooling delay of 2 days at 20°C (Fig. 6-2). For 1999 season COP fruit that had no discernible initial slow softening phase, softening occurred at a rate of 3-4 $N.day^{-1}$ while at 20°C (Fig. 6-1). Once both cultivars were at 0.5°C or 3°C, fruit with less time at 20°C were firmer through the rapid softening phase than fruit that had increased time at 20°C (Fig's 6-1 and 6-2). However, firmness differences between fruit from the different delayed cooling treatments were progressively reduced with time at low temperatures, so that firmness converged in the final slow softening phase.

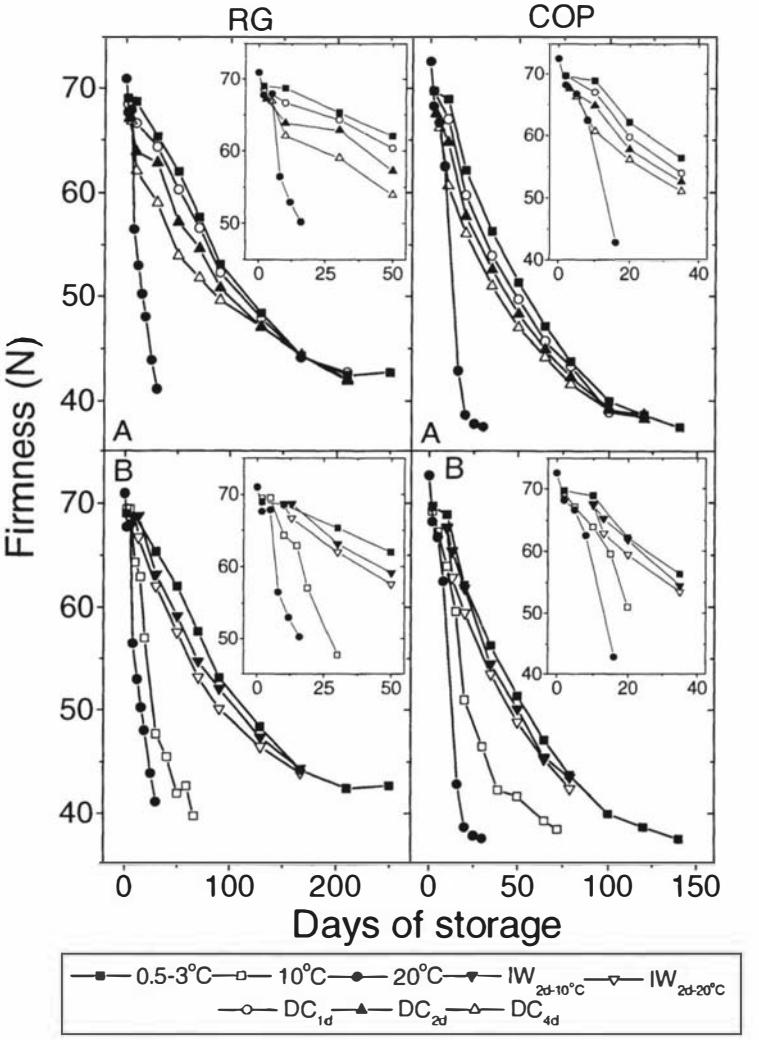

Fig. 6-2 Flesh firmness of 2000 season 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples from different delayed cooling (A) or intermittent warming (B) treatments. Figure insets depict firmness data on a reduced firmness and time scale. Delayed cooling (DC) and intermittent warming (IW) treatments are described in Table 6-1. Treatment means (n=10) are shown.

The IEC of fruit increased rapidly at 20°C to maximum concentrations of about 300 $\mu l.l^{-1}$ for RG and 450 $\mu l.l^{-1}$ for COP (Fig. 6-1). While an increased cooling delay resulted in higher IECs on transfer from 20°C to 0.5-3°C, IECs were immediately reduced after 1-3 days at 0.5-3°C. The maximum IECs attained in COP at 3°C were approximately two fold higher for fruit that had 3 and 5 day cooling delays when compared to fruit that had cooling delays of 1 and 0 days. In RG fruit, a longer delay before cooling after harvest induced an earlier increase in IEC, and a greater peak IEC attained at 0.5°C, than fruit held at 20°C for less time before cooling.

The longer fruit were at 12°C in the intermittent warming experiment, the softer they were on return to, and during subsequent storage at 0.5-3°C (Fig. 6-3). Likewise, increasing the intermittent warming temperature from 10°C to 20°C resulted in softer fruit when returned to 0.5-3°C (Fig. 6-2). However, the effect of intermittent warming on firmness was progressively reduced with storage time, as firmness converged in the final slow softening phase.

Intermittent warming to 12°C temporarily increased IEC in fruit from both cultivars (Fig. 6-3). IEC's of RG fruit increased from about 10 $\mu l.l^{-1}$ for fruit with no intermittent warming, to approximately 20, 40 and 150 $\mu l.l^{-1}$ after 1, 3 and 7 days at 12°C, respectively. Likewise, IEC of COP increased from about 50 $\mu l.l^{-1}$ in fruit with no intermittent warming, to 75 $\mu l.l^{-1}$ after 1 and 3 days at 12°C, and 110 $\mu l.l^{-1}$ after 7 days at 12°C. On return to 0.5°C or 3°C, the IEC in fruit from both cultivars in the 1 and 3 day intermittent warming treatments were similar to that in fruit held continuously at 0.5°C or 3°C. However, the IEC's in fruit from the 7 day intermittent warming treatment were higher for the following 25 and 20 days at 0.5-3°C for COP and RG respectively, than in fruit from other intermittent warming treatments.

Rapid softening was initiated immediately upon transfer to 20°C in both cultivars, regardless of prior time at 0.5°C or 3°C (Fig. 6-4), or prior temperature (Fig. 6-5). Prior time at 0.5°C or 3°C did not influence the rate at which rapid phase softening subsequently occurred at 20°C for either COP or RG (Fig. 6-4). Likewise, a constant time at different temperatures had no influence on the rate of rapid phase softening once

at 20°C (Fig. 6-5). COP fruit from all treatments tended to converge to similar firmness values in the final slow softening phase, while RG fruit were not stored for sufficient time at 20°C for the final slow softening phase to occur in all treatments (Fig's 6-4 and 6-5).

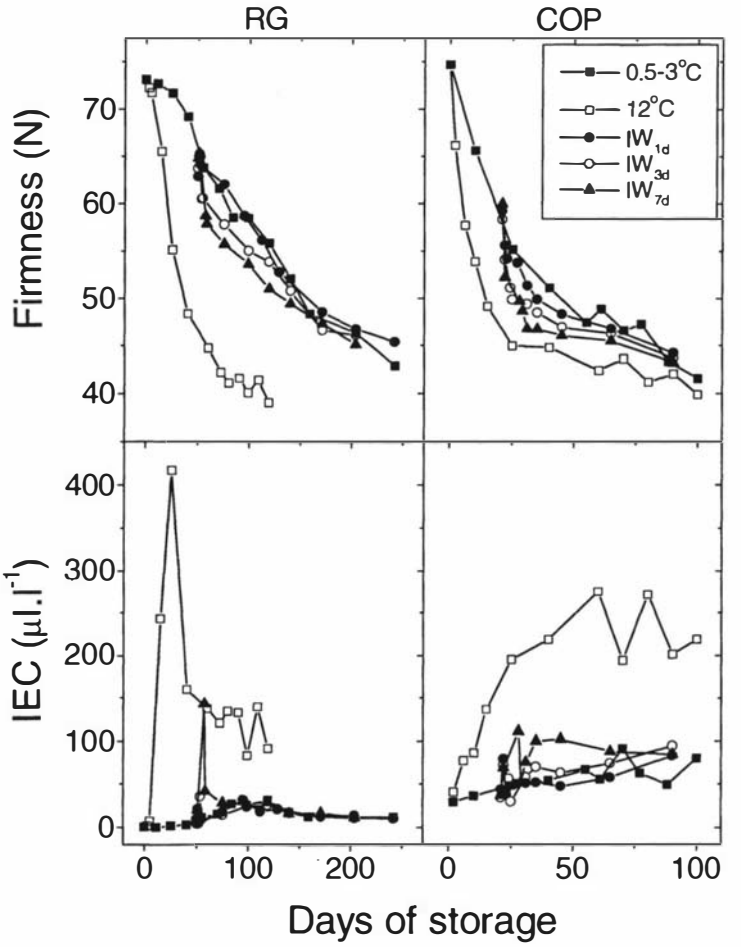

Fig. 6-3 Flesh firmness and internal ethylene concentration (IEC) of 1999 season 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples from different intermittent warming (IW) treatments. Intermittent warming treatments are described in Table 6-1. Treatment means (n=10) are shown.

IEC of both cultivars increased rapidly after transfer to 20°C for all preceding temperature treatments (Fig's 6-4 and 6-5). The maximum IEC attained at 20°C for

these treatments was similar for COP, while both prior time at 0.5°C (Fig. 6-4), and constant time at different temperatures (Fig. 6-5), influenced the peak IEC attained in RG at 20°C. The maximum IEC attained in RG at 20°C progressively increased as prior time at 0.5°C increased from 0 to 100 days, but then decreased after 125 days at 0.5°C (Fig. 6-4). The peak IEC attained in RG at 20°C was approximately 4 fold higher after 25 days at 5°C or 12°C, than after 25 days at 0.5°C (Fig. 6-5).

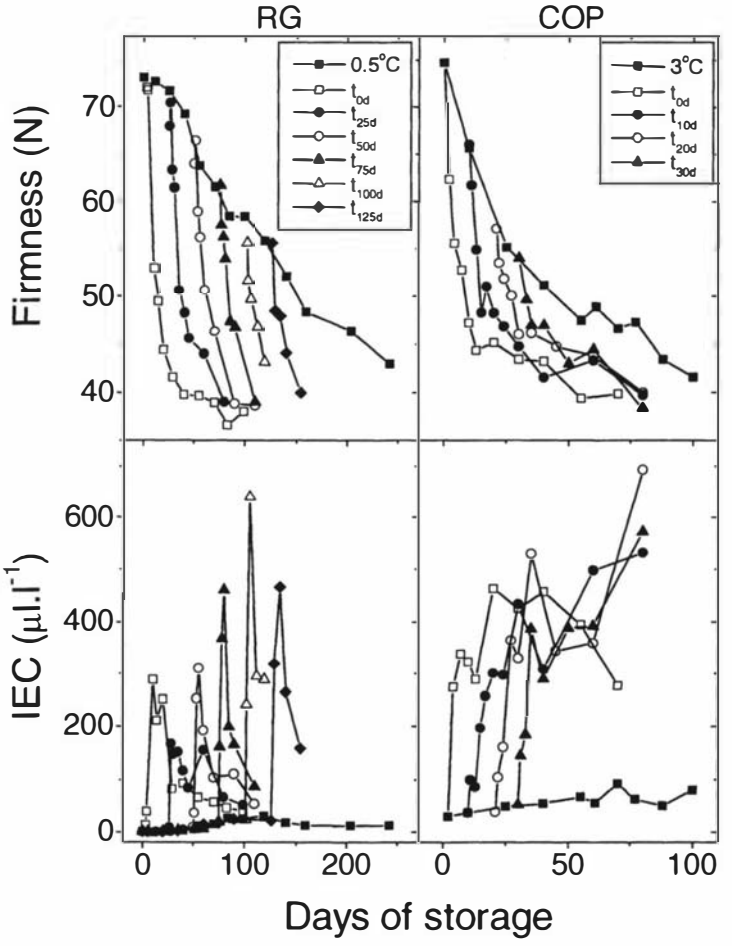

Fig. 6-4 Flesh firmness and internal ethylene concentration (IEC) of 1999 season 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples held at 0.5°C or 3°C for different times before transfer to 20°C. Treatment details are given in Table 6-1. Treatment means (n=10) are shown.

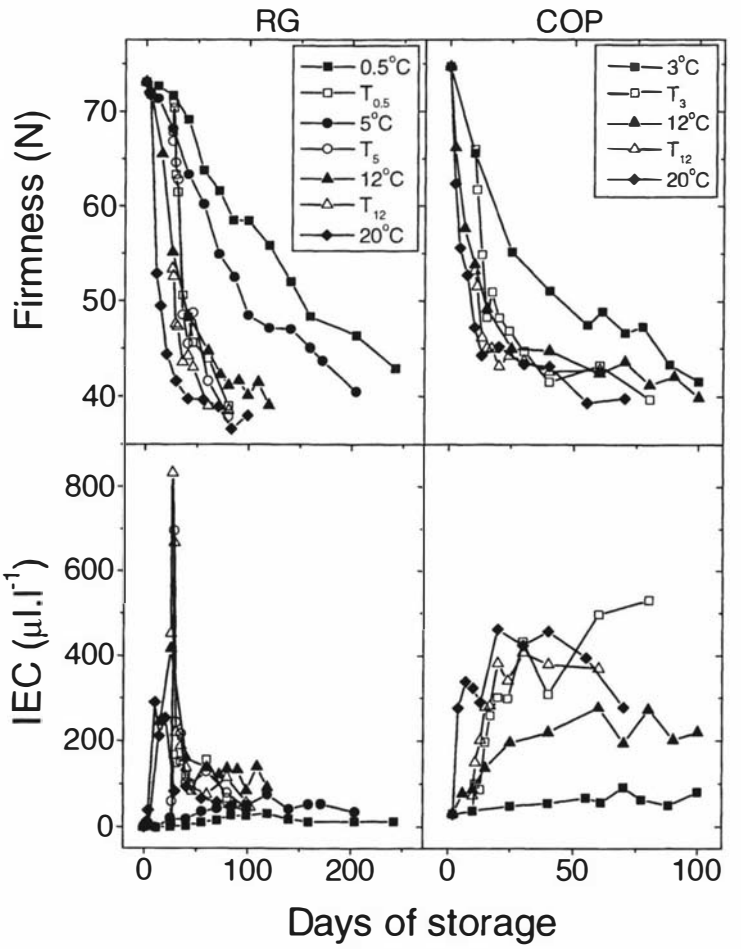

Fig. 6-5 Flesh firmness and internal ethylene concentration (IEC) of 1999 season 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples held at different temperatures before transfer to 20°C. Treatment details are given in Table 6-1. Treatment means (n=10) are shown.

The estimates of *k* (values not shown) for fruit stored at constant temperatures were used to describe softening in treatments where fruit were exposed to more than one temperature (Fig. 6-6). For each of the coolchain scenarios explored, a single *k* value for each temperature closely described most of the softening data. However, predicted lines tended to underestimate firmness of fruit approaching the final slow softening phase when transferred to 20°C.

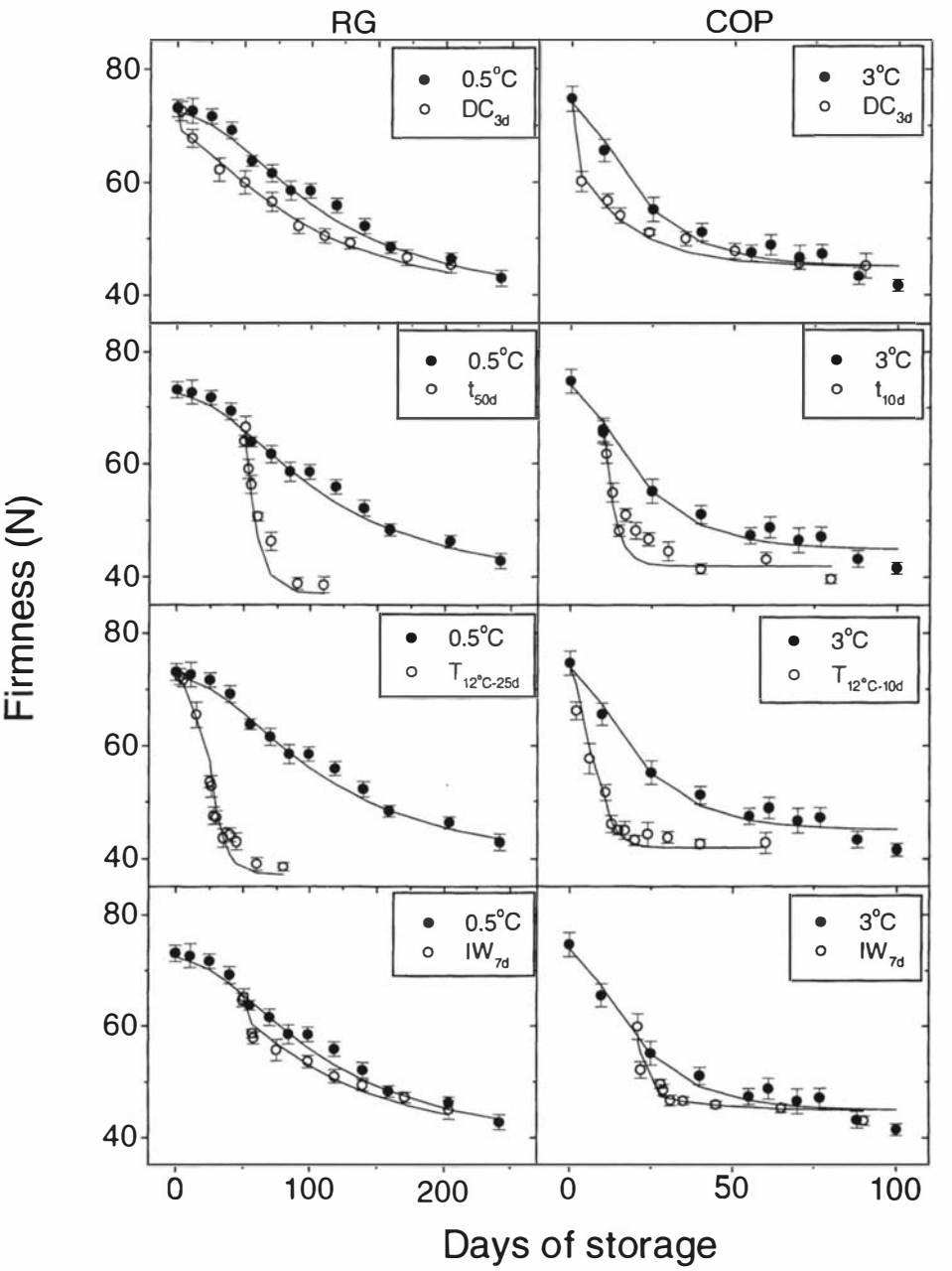

Fig. 6-6 Predicted and actual firmness of 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples exposed to different coolchain scenarios. Details of coolchain scenarios are given in Table 6-1. Constant temperature treatments were fitted with Eq. 6-1 using non-linear regression. Predicted lines for coolchain scenarios were then determined using the rate of firmness change values (k) estimated for each constant temperature treatment.

6.5 Discussion

In general, softening was triphasic in both cultivars at low-temperatures, consisting of an initial slow softening phase, followed by a phase of more rapid softening, and a final slow softening phase. However, an initial slow softening phase was not discernible for 1999 season COP fruit, probably because fruit were more mature at harvest in 1999 than in 2000 with this initial phase having been completed on the tree. Kiwifruit harvested at a mature stage had a shorter initial slow softening phase than earlier harvested fruit (MacRae et al., 1989). The IEC in 1999 season COP fruit at harvest was higher than in 2000 (data not shown), and IEC increased immediately when fruit was placed at 3°C, indicating that rapid ethylene production may have commenced before this experiment started. RG fruit at 0.5°C showed an initial slow softening phase and a delayed increase in IEC, similar to that previously demonstrated in RG, GS and 'Pacific Rose™' (PR) cultivars at 0 to 5°C (Chapter 4).

The longer that freshly harvested RG and COP fruit were held at 20°C before cooling, the shorter the initial slow softening phase, and the softer the fruit were at 0.5°C or 3°C. Rapid cooling improved poststorage firmness of 'York Imperial', 'Delicious', 'Ben Davis', 'Rhode Island Greening', 'Winesap' (Magness and Diehl, 1924), 'Sturmer Pippin' (Padfield, 1953), 'McIntosh' (Liu, 1986), 'Red Delicious' (King and Henderson, 1988), 'Stayman' (D'Souza and Ingle, 1989), 'Rome Beauty' (Magness and Diehl, 1924; D'Souza and Ingle, 1989) and 'Spartan' (Lidster and Porritt, 1978) apple cultivars, but not 'Golden Delicious' (King and Henderson, 1988), 'Jonared' (D'Souza and Ingle, 1989) or COP (Sharples and Munoz, 1974). The benefit of delayed cooling on firmness retention in COP and RG was slowly reduced with increased time at 0.5°C or 3°C, an effect also seen in 'McIntosh' apples (Blanpied, 1975), and may explain why Sharples and Munoz (1974) found no effect of delayed cooling on the firmness in COP after 156 days in controlled atmosphere storage. Thus, the influence of delayed cooling on apple softening depends on cultivar (Magness and Diehl, 1924; King and Henderson, 1988; D'Souza and Ingle, 1989), and time in storage before firmness is measured (Blanpied, 1975).

The mechanism by which delayed cooling reduced duration of the initial slow softening phase, and reduced firmness at 0.5°C for RG and 3°C for COP, was probably mediated by ethylene. Delayed cooling induced earlier ethylene production, and a greater rate of ethylene production thereafter at 0.5-3°C. Similarly, rapidly cooled 'McIntosh' apples had lower ethylene production and were firmer during low-ethylene (<1 $\mu l.l^{-1}$) controlled atmosphere storage than fruit cooled more slowly (Liu, 1986). Evidence for the importance of ethylene in promoting apple softening was strengthened following studies using inhibitors of ethylene action, as softening was reduced in several apple cultivars treated with 1-methylcyclopropene at harvest (Fan et al., 1999; Watkins et al., 2000b).

Intermittent warming influenced firmness and IEC for both cultivars. Fruit from intermittent warming periods of increased duration or increased temperature were softer and had higher IEC's on return to 0.5-3°C, and thereafter at 0.5-3°C, than fruit with no intermittent warming. These firmness and IEC results are similar to those obtained for 'Cortland' and 'Delicious' apples after intermittent warming to 20°C for different durations (Watkins et al., 2000a). However, the same intermittent warming treatments had a minimal effect on the IEC and firmness of cultivars such as GS and PR (Watkins et al., 2000a). As for delayed cooling, the effect of intermittent warming on subsequent softening at 0.5-3°C was reduced with increased storage time, with convergence of firmness values in the final slow softening phase in all treatments.

Neither time at 0.5 or 3°C, nor constant time at different temperatures, affected subsequent initiation, or rate, of softening in COP and RG at 20°C. A similar effect occurred in nectarines, where rate of softening at 15°C was similar regardless of prior exposure to different temperatures from -0.5°C to 7°C (Von Mollendorff et al. 1992a). Maintaining nectarines for different times at –0.5°C or 3°C also did not influence the rate of softening in fruit subsequently placed at 10, 15 or 20°C (Von Mollendorff et al. 1992b). This contrasted to the softening response in GS, several pear cultivars, and kiwifruit. Increasing the time that GS apples (Chapter 5) and 'Bartlett' pears (Agar et al., 2000) were at –1°C or 0°C before transfer to 20°C shortened the subsequent initial slow softening phase, decreased the rate of rapid phase softening, and increased the softening rate of 'Anjou' pears (Gerasopoulos and Richardson, 1997) and kiwifruit

(Zoffoli et al., 1999). Different softening responses in these fruits at 20°C, after varying times at 0°C, were probably due to differential effects of low temperature on induction of autocatalytic ethylene production. Prior time at low-temperatures was required to rapidly initiate autocatalytic ethylene production in 'Anjou' (Gerasopoulos and Richardson, 1997) and 'Bartlett' (Agar et al., 2000) pears, and GS apples (Jobling et al., 1991) at shelf-life temperatures. This contrasted with COP (Knee et al., 1983) and RG (Larrigaudiere et al., 1997), where initiation of rapid ethylene production occurred immediately at 20°C without prior exposure to low-temperatures. Thus, RG and COP may soften at similar rates at 20°C, regardless of prior time at low temperature, as these cultivars do not require chilling to initiate rapid ethylene production and ripening at 20°C.

The *k* values estimated from continuous temperature treatments described both the influence of delayed cooling and intermittent warming on subsequent softening at 0.5-3°C, and the influence of time at different temperatures on subsequent softening at 20°C in COP and RG. This indicates that the *k* values for these cultivars at a given temperature were not influenced by prior exposure to different temperatures between 0.5°C and 20°C. Thus, a combination of the empirical softening model used herein, and the modified Arrenhius equation used previously to describe *k* at temperatures from 0 to 35°C (Chapter 4), have the potential to describe the influence of temperature perturbations during postharvest handling on softening of these cultivars. However, it is unlikely that this simple model could be used for GS, or similar apple cultivars, that have a requirement for chilling prior to initiation of rapid phase softening at 20°C (Chapter 5).

In summary, softening rates of COP and RG at a given temperature were similar regardless of prior exposure to different temperatures. However, different coolchain treatments did influence IECs and firmness retention in storage. Reduced time at 20°C before cooling after harvest resulted in firmer fruit and lower IECs at 0.5-3°C than in fruit that had increased cooling delays after harvest. Fruit that had longer periods of intermittent warming were softer, and had higher IECs than fruit with shorter periods of intermittent warming. At shelf-life temperatures, both softening and IEC increased immediately after transfer from low-temperatures. The *k* values estimated from constant

temperature treatments were able to describe softening in treatments with more than one temperature, which indicates that k at a given temperature was not influenced by exposure to different prior temperatures. Thus, k has the potential to describe the effects of temperature on softening of the RG and COP cultivars during postharvest handling. These results also confirm the importance of minimising the exposure of rapid softening apple cultivars to ambient temperatures during postharvest handling.

6.6 References

Agar, I.T., Biasi, W.V., Mitcham, E.J., 2000. Cold storage duration influences ethylene biosynthesis and ripening of 'Bartlett' pears. HortScience 35, 687-690.

Blanpied, G.D., 1975. The effect of cooling rate on the market quality of 'McIntosh' apples. Proc. Am. Soc. Hortic. Sci. 70, 58-66.

D'Souza, M.C., Ingle, M., 1989. Effect of delayed cooling on the poststorage flesh firmness of apples. J. Food Sci. 54, 493-494.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Gerasopoulos, D., Richardson, D.G., 1997. Storage-temperature-dependent time separation of softening and chlorophyll loss from the autocatalytic ethylene pathway and other ripening events of 'Anjou' pears. J. Am. Soc. Hortic. Sci. 122, 680-685.

Jobling, J.J., McGlasson, W.B., Dilley, D.R., 1991. Induction of ethylene synthesizing competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1, 111-118.

King, G.A., Henderson, K.G., 1988. Changes in quality of 'Red Delicious' and 'Golden Delicious' apples following delayed cooling versus delayed establishment of controlled atmosphere storage. New Zeal. J. Exp. Agric. 16, 341-348.

Knee, M., Looney, N.E., Hatfield, S.G.S., Smith, S.M., 1983. Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. J. Exp. Bot. 34, 1207-1212.

Larrigaudiere, C., Graell, J., Salas, J., Vendrell, M., 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Postharvest Biol. Technol. 10, 21-27.

Larrigaudiere, C., Vendrell, M., 1993. Cold-induced activation of 1-aminocyclopropane-1-carboxylic acid metabolism in rewarmed Granny Smith apples: Consequences on ripening. Sci. Hortic. 55, 263-272.

Lidster, P.D., Porritt, S.W., 1978. Influence of maturity and delay of storage on fruit firmness and disorders in 'Spartan' apple. HortScience 13, 253-254.

Liu, F.W., 1986. Effects of delayed cooling and delayed low-ethylene CA storage on the keeping quality of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 111, 719-723.

MacRae, E.A., Lallu, N., Searle, A.N., Bowen, J.H., 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49, 413-430.

Magness, J. R., Diehl, H. C., 1924. Physiological studies on apples in storage. J. Agric. Res. 27, 1-38.

Padfield, C.A.S., 1953. Effects of delay between harvesting and cool-storing on Sturmer Pippin apples. New Zeal. J. Sci. Technol. A35, 152-158.

Sharples, R.O., Munoz, G.C., 1974. The effects of delays in the period taken to cool and establish low oxygen conditions on the quality of stored Cox's Orange Pippin apples. J. Hortic. Sci. 49, 277-286.

Von Mollendorff, L.J., Jacobs, G., De Villiers, O.T., 1992a. The effects of storage temperature and fruit size on firmness, extractable juice, woolliness and browning in two nectarine cultivars. J. Hortic. Sci. 67, 647-654.

Von Mollendorff, L.J., Jacobs, G., De Villiers, O.T., 1992b. Effect of temperature manipulation during storage and ripening on firmness, extractable juice and woolliness in nectarines. J. Hortic. Sci. 67, 655-662.

Watkins, C.B., Bramlage, W.J., Brookfield, P.L., Reid, S.J., Weis, S.A., Alwan, T.F., 2000a. Cultivar and growing region influence efficacy of warming treatments for amelioration of superficial scald development on apples after storage. Postharvest Biol. Technol. 19, 33-45.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000b. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Zoffoli, J.P., Gil, G.F., Crisosto, C.H., 1999. Low temperature storage enhances subsequent softening of kiwifruit at high temperatures. Acta Hortic. 498, 225-230.

Chapter 7 Harvest date and fruit size affect postharvest apple softening.

7.1 Abstract

The influence of harvest maturity and fruit size on the three phases of apple softening in the 'Royal Gala' and 'Cox's Orange Pippin' apple (*Malus domestica* Borkh.) cultivars is currently not known. These factors were studied by harvesting fruit from two orchards once before, three times during, and once following the commercial harvest period. In addition, small (<70 mm diameter), medium (70-75 mm) and large (>75 mm) fruit were picked from one orchard on the three harvests that occurred during the commercial harvest period. During storage at 0.5°C or 3°C, late-harvested fruit had a shorter initial slow softening phase and required less time before the internal ethylene concentration exceeded 1.5 $\mu l.l^{-1}$ than earlier harvested fruit. However, the rate of rapid phase softening was similar at 0.5-3°C for fruit from each harvest date. Unlike harvest date, fruit size did not consistently affect the maturity attributes of fruit from either cultivar at harvest. During storage at 0.5°C or 3°C, the softening profiles of different sized 'Royal Gala' and 'Cox's Orange Pippin' fruit from harvests two and three were similar. However, small fruit from harvest four had a longer initial slow softening phase for both cultivars, and a slower rapid softening phase for 'Cox's Orange Pippin', than medium and large sized fruit from the same harvest. Results in this study indicate that softening of these apple cultivars was not linear in storage, and that both harvest date, and to a lesser extent fruit size, influenced the degree of non-linearity by influencing the length of the initial slow softening phase. It is also suggested that the initial slow softening phase may be a continuation of the slow softening that occurs in fruit while attached to the tree.

Keywords: Malus domestica (Borkh.); Firmness; Ethylene; Ripening; Maturation; Quality.

7.2 Introduction

'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples are commercially important early season cultivars in New Zealand. The propensity for these cultivars to soften rapidly after harvest makes it difficult for producers to meet minimum firmness

standards set by markets. Failure to comply with market standards can result in shipment rejections, reduced grower returns, and a damaged reputation as a supplier of quality fruit. Management of this softening problem could be improved through increased understanding of the effects of different pre- and postharvest factors on softening.

Harvested apples typically have an initial slow softening phase, followed by a phase of more rapid softening, and a final slow softening phase (Chapter 4). The influence of temperature on each of these softening phases has been characterised for several apple cultivars (Chapters 4 and 5), although the influence of harvest date and fruit size on the three phases of softening is currently not known. Harvest date is an important factor to be considered for softening effects, given that the harvest date can vary considerably between growers, districts and years for a given cultivar.

While the influence of harvest date on firmness before and after prolonged storage has been studied extensively, the softening curves for apples harvested at different stages of maturity has not been characterised. Fruit harvested at a later maturity were generally softer than earlier picked fruit at harvest, while the influence of advancing harvest date on poststorage firmness was less consistent (Lidster and Porritt, 1978; Liu, 1978; Olsen and Martin, 1980; Marmo et al., 1985; Ingle and Morris, 1989; Knee and Smith, 1989; Ingle et al., 2000; Stow and Genge, 2000). Knee and Smith (1989) found that the firmness of COP fruit was similar after prolonged CA storage at 3°C, despite there being differences in firmness at harvest. Earlier harvested 'Rome' apples softened faster at 0 and 20°C (Ingle and Morris 1989) and 'Spartan' apples were softer after four months at –1°C (Lidster and Porritt 1978) than fruit that were picked later. In contrast, earlier harvested fruit from several other cultivars were firmer after prolonged storage than later harvested fruit (Liu, 1978; Olsen and Martin, 1980; Blanpied, 1986; Wang et al., 1990; Ingle et al., 2000; Stow and Genge, 2000). A study using tensile, compression, and acoustic impulse response tests to assess apple texture also found that earlier harvested fruit were firmer than later harvested fruit both before and after storage (Tu et al., 1997). Furthermore, the longer that fruit were left on the tree the greater the incidence of mealiness, an undesirable textural disorder of apples (Harker and Hallett, 1992).

In general small fruit are considered to be firmer than large fruit (Harker et al., 1997). Larger fruit tend to have bigger cells, reduced cell wall material per unit volume of fruit, and as a consequence, reduced tissue strength and lower firmness than smaller fruit (Harker et al., 1997). However, this physical effect of fruit size on firmness is difficult to separate from the effects of harvest date in apples, as fruit size increases in parallel with declining firmness during maturation. Large RG apples were softer and had more advanced maturity attributes than smaller fruit (Koorey and Brookfield, per. com.). Similarly, large apples from other apple cultivars were softer than smaller apples at harvest and after storage (Blanpied et al., 1978; Marmo et al., 1985; Siddiqui and Bangerth, 1995). However, maturity data for each fruit size was not presented in these studies, and therefore maturity differences cannot be eliminated as an underlying cause of these results. Research is required to determine if the influence of fruit size on apple firmness was independent from the effects of harvest maturity.

Apple softening was reduced when inhibitors of ethylene action were applied at harvest, suggesting that ethylene has an important role in promoting apple softening (Blankenship and Sisler, 1989; 1993; Fan et al., 1999; Watkins et al., 2000). The onset of rapid phase softening coincided with the time when internal ethylene concentrations (IEC's) increased rapidly from low (<1.5 $\mu l.l^{-1}$) concentrations in both RG and COP at a range of temperatures (Chapter 4). Thus, IEC may regulate the onset of rapid phase softening in these cultivars. This study sought to determine the effect of harvest date and fruit size on the three phases of apple softening, and to ascertain if these effects reflect IEC changes in the rapid softening RG and COP apple cultivars.

7.3 Materials and methods

7.3.1 Fruit supply and treatments

Medium sized fruit were randomly picked from mature trees once before, three times during, and once after commercial harvest, from two orchards per cultivar (Table 7-1). In addition, small and large fruit were randomly picked three times during the commercial harvest period from one orchard per cultivar (Table 7-1).

Maturity was assessed on 15 fruit from each harvest date and fruit size within 24 hours of harvest through measuring flesh firmness, IEC, titratable acidity (TA), starch pattern index (SPI), total soluble solids (TSS), and background skin colour (Hue°; COP only). For each harvest, ten randomly selected fruit were placed into each of ten perforated polyethylene bags (35μm thickness; 50x 5mm diameter perforations per m^2) that in turn were packed into commercial cardboard cartons and placed into storage at 0.5±0.5°C (3.0±0.5°C for COP). Fruit weight, flesh firmness and IEC were measured on 10 fruit (one fruit randomly removed from each bag) after 2-3 days in storage, and thereafter at 20-30 day intervals. Fruit firmness and IEC were measured at the storage temperature.

Table 7-1 Summary of harvest dates for different sized 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples from different orchards in 2000. Fruit sizes were small (S, <70 mm diameter, <120 g for COP and <140 g for RG), medium (M, 70-75 mm diameter, 120-150 g for COP and 140-170 g for RG), and large (L, >75 mm diameter, >150 g for COP and >170 g for RG).

Cultivar	Orchard location and label	Harvest date and label	Fruit size
RG	Palmerston North (orchard 1)[1]	10-Feb (H1)	M
		18-Feb (H2)	S, M and L
		3-Mar (H3)	S, M and L
		13-Mar (H4)	S, M and L
		20-Mar (H5)	M
RG	Hawkes Bay (orchard 2)[2]	1-Feb (H1)	M
		11-Feb (H2)	M
		22-Feb (H3)	M
		2-Mar (H4)	M
		16-Mar (H5)	M
COP	Hawkes Bay (orchard 2)[2]	1-Feb (H1)	M
		11-Feb (H2)	S, M and L
		22-Feb (H3)	S, M and L
		2-Mar (H4)	S, M and L
		16-Mar (H5)	M
COP	Nelson (orchard 3)[2]	4-Feb (H1)	M
		16-Feb (H2)	M
		24-Feb (H3)	M
		6-Mar (H4)	M
		17-Mar (H5)	M

[1]Fruit Crops Unit, Massey University.
[2]Commercial orchard.

7.3.2 Measurements

Fruit diameter was determined using a Cranston fruit gauge (Cranston Machinery Company, Oregon, USA). Flesh firmness, IEC and skin background colour measurements were measured as described in Chapter 5. TSS was determined by placing a drop of juice, released from the outer cortex during firmness measurement, onto a hand-held temperature compensating refractometer (Atago N20, Tokyo, Japan). SPI was assessed by placing the cut surface of half an apple in an iodine solution (2.5 $g.l^{-1}$ iodine and 10 $g.l^{-1}$ potassium iodide, in distilled water) for 30 seconds, and assigning each fruit a score ranging from 0 (cut surface completely stained) to 6 (cut surface with no staining) according to a commercial (ENZAFRUIT New Zealand International) starch pattern index chart for apples. Juice for TA was extracted by placing longitudinal apple slices into an electronic juicer and the juice was stored at –80°C until analysis. TA was determined by titrating 1 ml of juice in 50 ml distilled water against 0.1M NaOH to an endpoint pH of 7.1 with an auto-titrator (Mettler DL21 Titrator, Greifensee, Switzerland). The NaOH concentration was confirmed by a titration with 0.1M HCL (Convol standard, BDH Chemicals New Zealand Ltd) to an endpoint pH of 7.0. Titration results were calculated as malic acid equivalents per unit volume of juice using a malic acid standard curve.

7.3.3 Data analysis

Firmness (f, N) after different times (t, day) at 0, 3 and 20°C were fitted with an empirical asymmetric sigmoidal function (Chapter 4) using non-linear regression:

$$f = f_{-\infty} - (f_{-\infty} - f_{+\infty}) \cdot \left(1 - \left(1 + \exp \frac{t + k^{-1} \cdot \ln(31) - (5.2 \cdot k^{-1.0168})}{k^{-1}}\right)^{-0.2}\right) \qquad \text{(Eq. 7-1)}$$

where model parameters were an initial firmness asymptote ($f_{-\infty}$, N), a minimum firmness asymptote ($f_{+\infty}$, N), and rate of firmness change (k, day^{-1}). Non-linear regression was performed using the NLIN procedure, and 5% least significant differences calculated from analysis of variance using the GLM procedure in the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

7.4 Results

All maturity attributes (flesh firmness, TSS, SPI, TA, IEC and hue°) measured at harvest were affected by harvest date for both RG and COP (Fig. 7-1). Firmness decreased in both cultivars while fruit was attached to the tree, with rate of softening varying between orchards. Firmness of RG and COP apples from orchard 2 (located in Hawkes Bay) decreased by 27N over the 43 day harvest period, while firmness of fruit from orchards 1 (located in Palmerston North) and 3 (located in Nelson) decreased by 18N over a similar period. TSS was slightly lower in RG fruit than in COP fruit at harvest one, and increased by 2% in both cultivars during the 43 day harvest period so that COP fruit had a slightly higher TSS than RG fruit at the last harvest. The SPI of fruit from both cultivars were similar at harvest one (0-1), but then increased rapidly in RG fruit to 5.2-5.5 at harvest five, compared with 4.2-4.8 in COP fruit at harvest five. The TA in COP fruit was approximately 2 fold higher than in RG fruit at harvest one, and then decreased rapidly in COP, and slowly in RG, through the 43 day harvest period. IEC of RG fruit progressively increased during the 43 day harvest period to 4 $\mu l.l^{-1}$ at harvest five, while IEC of COP was similar for the first three harvests before increasing to 1 $\mu l.l^{-1}$ at harvest five. Background skin colour (hue°) decreased in COP fruit while attached to the tree, with the rate of yellowing being similar for both orchards.

The predominant effects of harvest date on softening of RG at 0.5°C (Fig. 7-2) and 20°C (Fig. 7-4), and COP at 3°C (Fig. 7-3) and 20°C (Fig. 7-4), were that later harvested fruit were softer at the beginning of storage, and had a shorter initial slow softening phase, than earlier harvested fruit. Harvest date did not appear to influence rate of rapid phase softening in either cultivar at 0.5°C (3°C for COP) or 20°C. The effect of harvest date on the final slow softening phase was difficult to discern, as the experiment did not continue long enough to accurately characterise this phase of softening. However, COP data at 3°C indicated that given sufficient time, each of the softening curves from different harvest dates might converge to a similar minimum firmness value (Fig. 7-3).

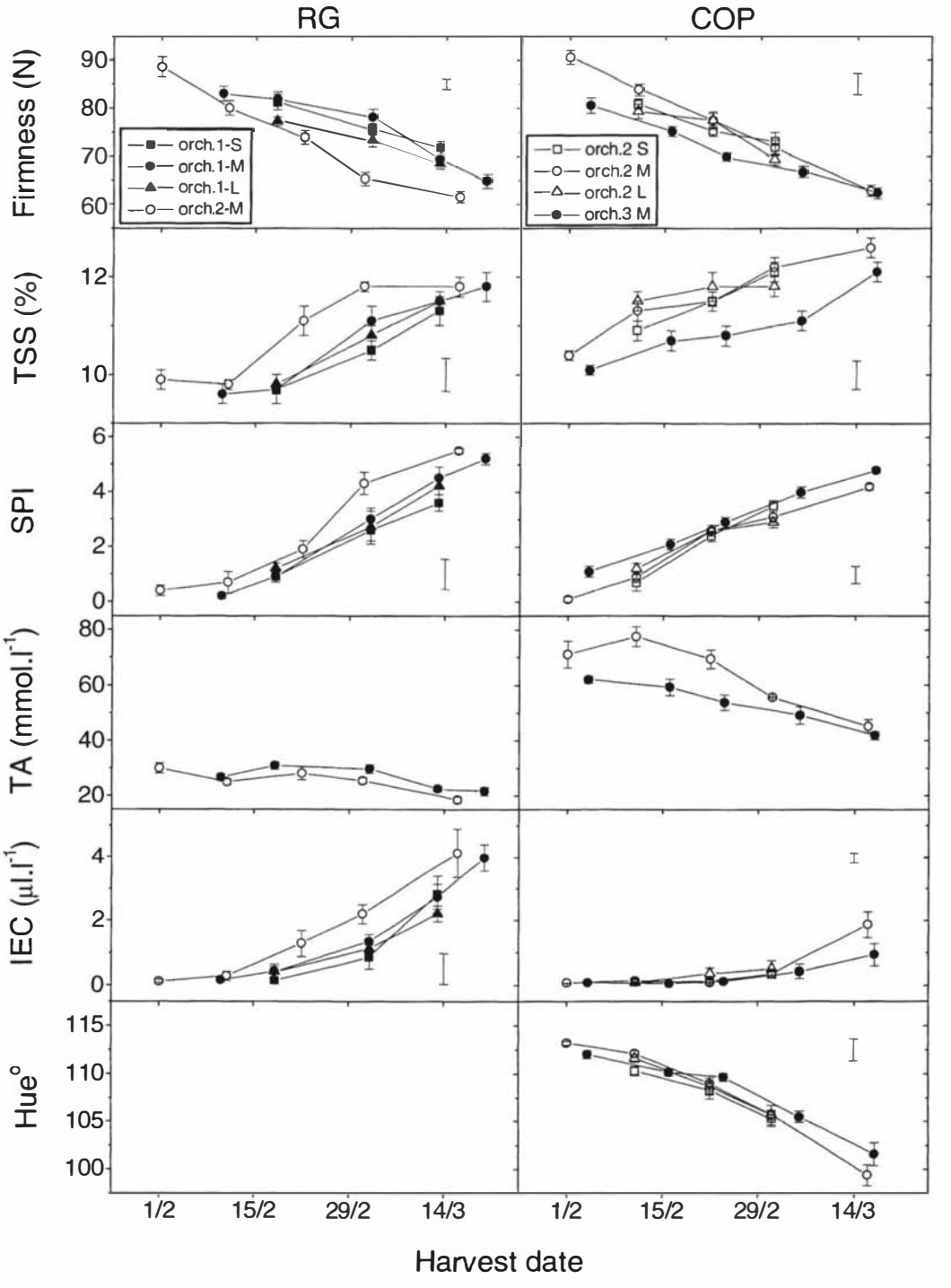

Fig. 7-1 Flesh firmness, total soluble solids (TSS), starch pattern index (SPI), titratable acidity (TA), internal ethylene concentration (IEC) and skin background colour (hue°) of small (S), medium (M) and large (L) 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples at harvest, when harvested at different times from two orchards (orch.1 and orch.2 for RG, and orch.2 and orch.3 for COP). Details of orchards, fruit sizes and harvest dates are given in Table 7-1. Means, standard error of the means (n = 15), and 5% least significant differences (96 d.f. error) are shown.

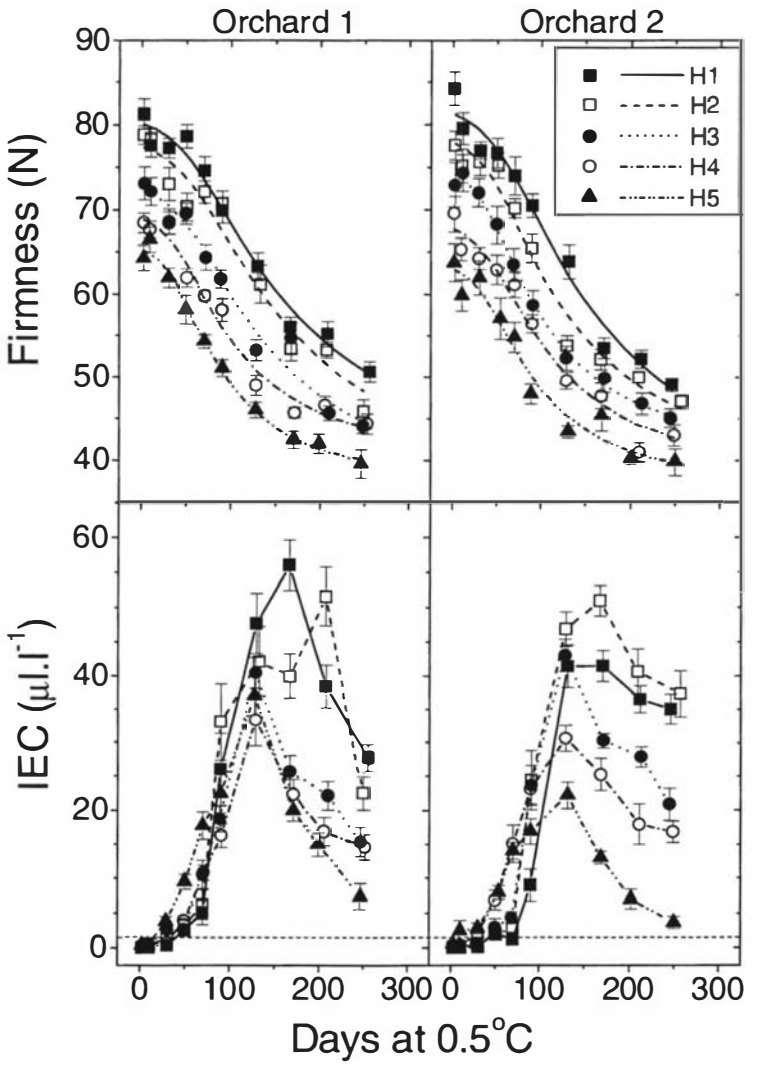

Fig. 7-2 Flesh firmness and internal ethylene concentration (IEC) of 'Royal Gala' (RG) apples at 0.5°C, that were harvested on five dates (H1 to H5) from two orchards. Harvest dates for both orchards are given in Table 7-1. Means (n=10) and standard errors of the mean are shown. Firmness data was fitted with Eq. 7-1 using non-linear regression. Dashed line is an IEC of 1.5 $\mu l.l^{-1}$.

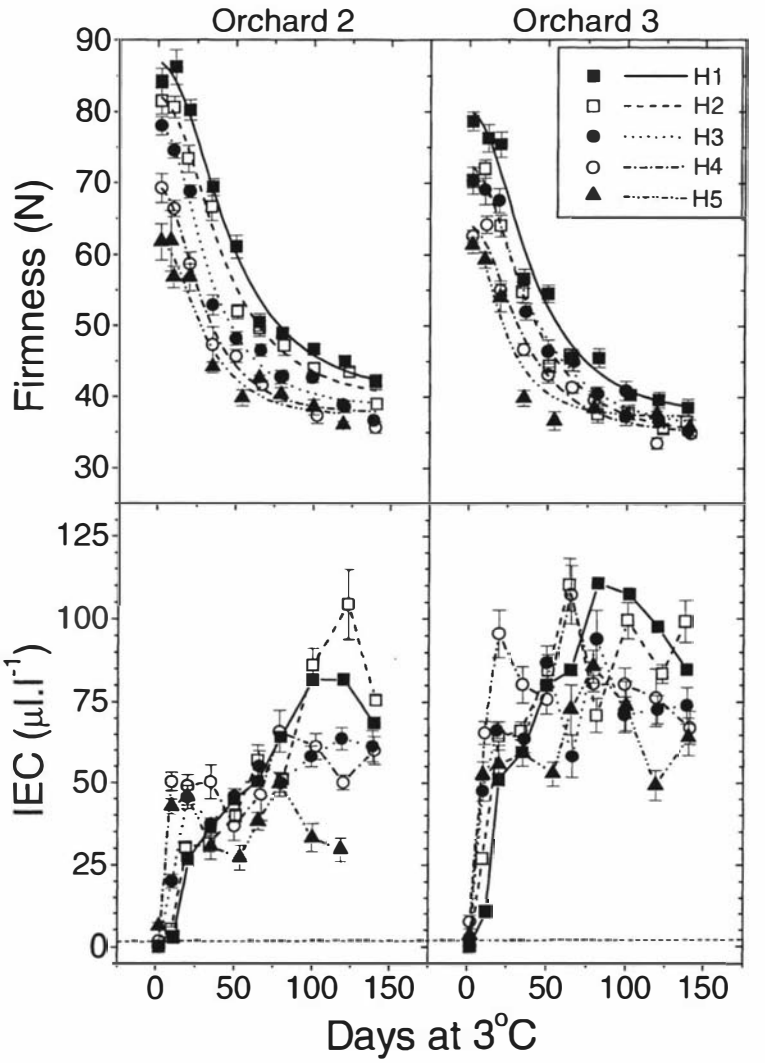

Fig. 7-3 Flesh firmness and internal ethylene concentration (IEC) of 'Cox's Orange Pippin' (COP) apples at 3°C, that were harvested on five dates (H1 to H5) from two orchards. Harvest dates for both orchards are given in Table 7-1. Means (n=10) and standard errors of the mean are shown. Firmness data was fitted with Eq. 7-1 using non-linear regression. Dashed line is an IEC of 1.5 $\mu l.l^{-1}$.

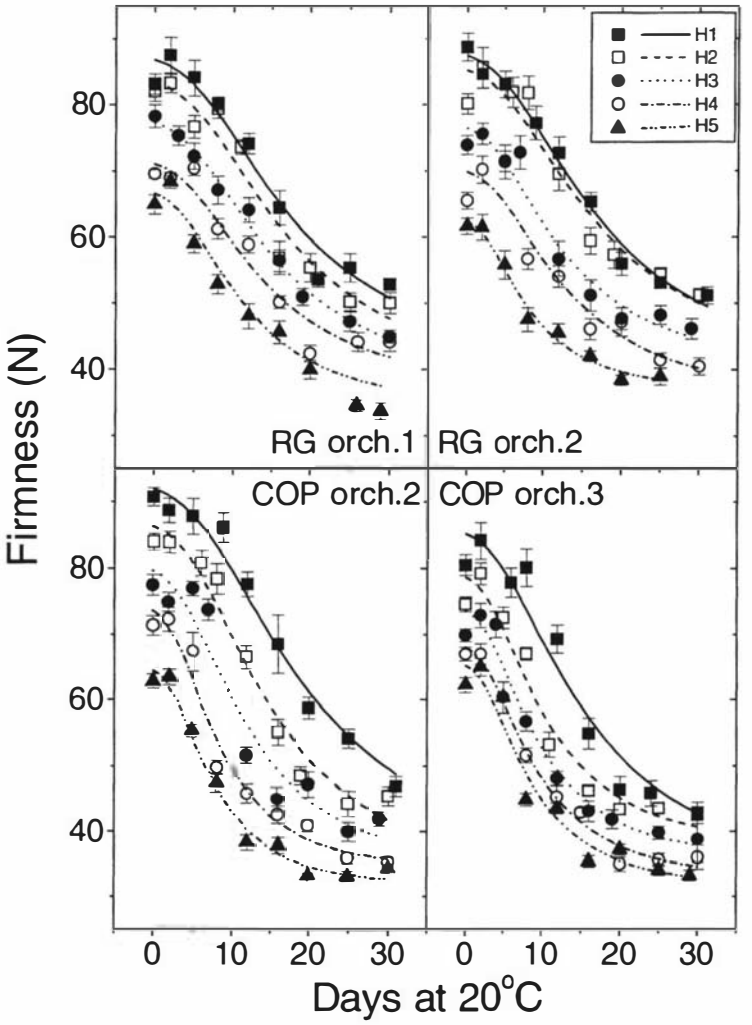

Fig. 7-4 Flesh firmness of 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples at 20°C, that were harvested on five dates (H1 to H5) from two orchards (orch.1 and orch.2 for RG, and orch.2 and orch.3 for COP). Harvest dates for both orchards and cultivars are given in Table 7-1. Means (n=10) and standard errors of the mean are shown. Firmness data was fitted with Eq. 7-1 using non-linear regression.

IEC of fruit from both RG (Fig. 7-2) and COP (Fig. 7-3) was low (<1.5 $\mu l.l^{-1}$) at the beginning of storage, and then increased rapidly to a peak (RG) or plateau concentration (COP). Harvest date influenced both the time at which IEC increased from a low concentration, and the maximum IEC attained thereafter, for both cultivars at 0.5°C or 3°C. IEC of RG fruit exceeded 1.5 $\mu l.l^{-1}$ after 30-40 days at 0.5°C for fruit from

harvests one to four for orchard 1, and harvests one to three for orchard 2, but occurred approximately 20 days earlier for fruit of later harvests from both orchards (Fig. 7-2). RG fruit from harvests four and five had harvest IEC's of 2.5 and 4 $\mu l.l^{-1}$ (Fig. 7-1), which then decreased below 1.5 $\mu l.l^{-1}$ once in storage at 0.5°C (Fig. 7-2). The IEC in COP fruit from harvests one to three increased rapidly after 2-10 days at 3°C, but increased immediately in fruit from harvests four and five when placed at 3°C (Fig. 7-3). Maximum IEC's attained at 0.5°C for RG fruit from orchard 1 decreased from about 55 $\mu l.l^{-1}$ in fruit from harvests one and two, to approximately 35 $\mu l.l^{-1}$ in fruit from harvests four and five (Fig. 7-2). Likewise, the maximum IEC of RG fruit from orchard 2 decreased from about 50 $\mu l.l^{-1}$ in fruit from harvest two, to approximately 20 $\mu l.l^{-1}$ in fruit from harvest five. For COP at 3°C, the maximum IEC attained also decreased from about 100 $\mu l.l^{-1}$ in fruit from harvest two to approximately 50 $\mu l.l^{-1}$ in fruit from harvest five (Fig. 7-3). However, the maximum IEC of COP fruit from orchard 3 was similar in fruit from harvests one to five, with some evidence that harvest five fruit had a slightly lower maximum IEC than fruit from the earlier harvests.

In contrast to harvest date, size had a minimal effect on maturity attributes of RG and COP fruit at harvest (Fig. 7-1). The three RG size classes had similar TSS, SPI and IEC for fruit from all harvest dates, while large fruit were slightly softer than both medium and small fruit from the second harvest date. The three COP size classes had similar TSS, SPI, IEC, firmness and hue angle for all three harvests. Although not statistically significant, there was a trend for large COP fruit from harvest four, and for large RG fruit from harvest three, to be slightly softer than both medium and small fruit from the same harvests.

The softening curves (Fig. 7-5), and *k* values (Fig. 7-6), for the three fruit sizes at harvests two and three were similar within both cultivars. However, small fruit from harvest four had a lower *k* value than both medium and large sized fruit from the same harvest for both cultivars (Fig. 7-6). Small COP fruit from harvest four had a longer initial slow softening phase, and slower rapid softening phase, while the small RG fruit from the same harvest had a longer initial slow softening phase, than medium and large sized fruit (Fig. 7-5).

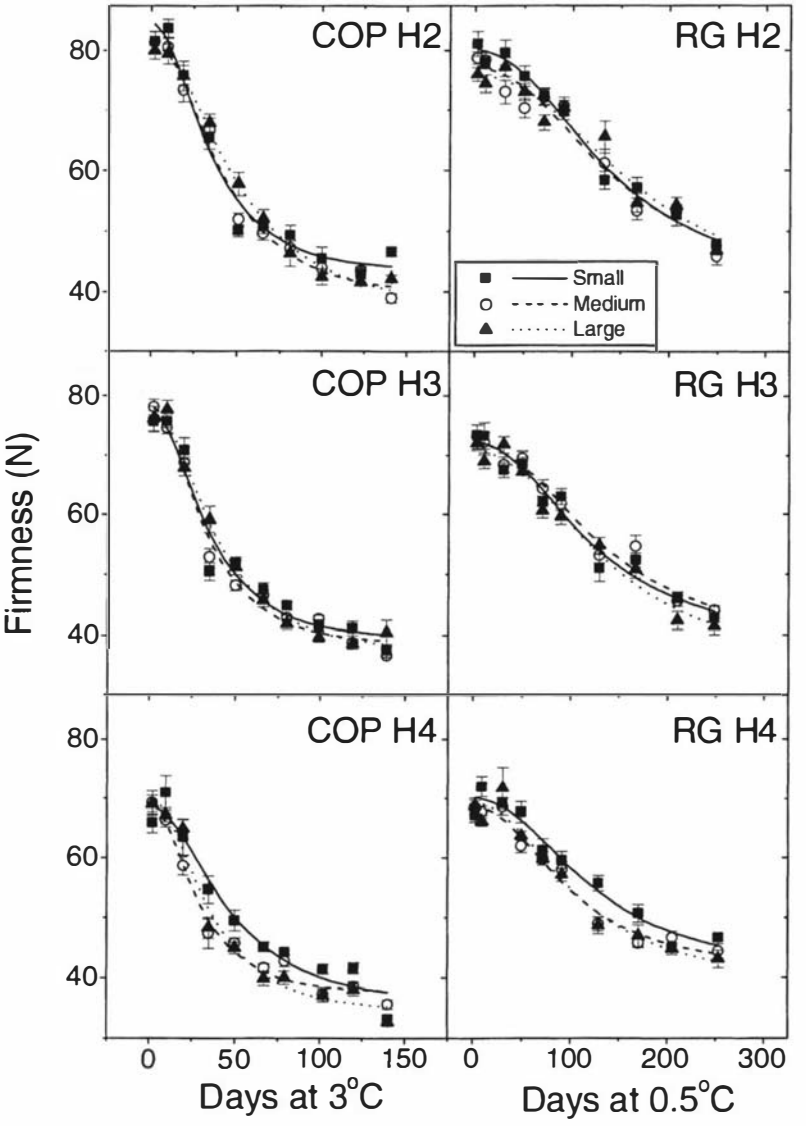

Fig. 7-5 Flesh firmness of small (<70 mm diameter), medium (70 to 75 mm), and large (>75 mm) 'Royal Gala' (RG) apples at 0.5°C and 'Cox's Orange Pippin' (COP) apples at 3°C, that were harvested on three dates (H2 to H4) from orchards 1 (RG) and 2 (COP). Harvest date details for both cultivars are given in Table 7-1. Means (n=10) and standard errors of the mean are shown. Firmness data was fitted with Eq. 7-1 using non-linear regression.

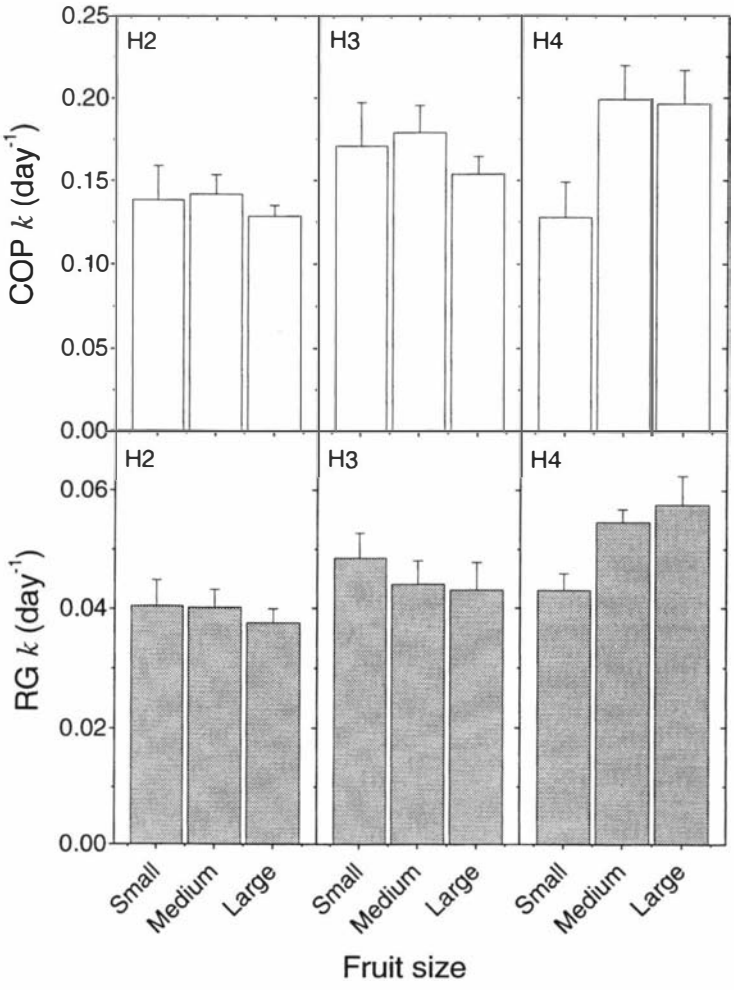

Fig. 7-6 Rate of firmness change (k, day^{-1}) of small (<70 mm diameter), medium (70 to 75 mm), and large (>75 mm) 'Royal Gala' (RG) apples at 0.5°C and 'Cox's Orange Pippin' (COP) apples at 3°C, that were harvested on three dates (H2 to H4) from orchards 1 (RG) and 2 (COP). Harvest date details for both cultivars are given in Table 7-1. Estimates and standard errors of k were determined from non-linear regression of firmness data (Fig. 7-5) with Eq. 7-1.

7.5 Discussion

Consistent with studies using COP (Knee and Smith, 1989; Tu et al., 1997) and other apple cultivars (Lidster and Porritt, 1978; Liu, 1978; Olsen and Martin, 1980; Marmo et al., 1985; Ingle and Morris, 1989; Ingle et al., 2000; Stow and Genge, 2000), firmness decreased with advancing harvest date as fruit matured while attached to the tree.

Despite attached fruit from both cultivars softening by 18-27N during the 43 day harvest period, the rate of firmness loss was slower than previously observed in detached fruit. The rates of rapid phase softening in detached apples at 12 to 35°C were between 1.5 and 3.5 $N.day^{-1}$ for COP and 1.5 and 2.7 $N.day^{-1}$ for RG (Chapter 4) as compared with 0.45 to 0.65 $N.day^{-1}$ for attached fruit in this experiment. Thus it would appear that on-tree softening could be considered part of the initial slow softening phase that occurs before onset of the rapid softening phase in harvested apples.

Fruit left on the tree for longer time had yellower skin (decreased hue°), less starch (increased SPI), acid and firmness, and higher TSS at harvest than earlier harvested fruit (Fig. 7-1). These changes are typical in apples during maturation and ripening (Kingston, 1991). The progressive change in each of these maturity indices with advancing harvest date indicates that subsequent softening curves were from fruit harvested at different physiological stages of maturity. Thus, the influence of harvest date on softening in this study could be interpreted as harvest maturity effects.

Later-harvested fruit had a shorter initial slow softening phase, but a similar rate of rapid phase softening, when compared with fruit harvested less mature. Data in Stow and Genge (2000) indicated that the effect of harvest date on the softening profile of 'Gala' was similar to that measured here for COP and RG. Kiwifruit also had a longer initial slow softening phase when harvested less mature (MacRae et al., 1989). Advanced harvest maturity also decreased pre- and poststorage firmness in peaches (Shewfelt et al., 1987) and in several other apple cultivars (Liu, 1978; Olsen and Martin, 1980; Blanpied, 1986; Wang et al., 1990; Ingle et al., 2000), and increased the softening rate in 'Bartlett' pears (Agar et al., 1999). Knee and Smith (1989) found that firmness of COP apples harvested on different dates was similar after 7 months CA storage, possibly because fruit had entered the final slow softening phase where firmness converged to a similar minimum value. It is not known why poststorage firmness, and softening rates at 0 and 20°C, increased with advancing harvest date in Lidster and Porritt (1978) and Ingle and Morris (1989).

Earlier harvested fruit had a longer initial slow softening phase, and required more time to exceed an IEC of 1.5 $\mu l.l^{-1}$, than fruit picked at a later maturity for both cultivars.

Onset of rapid phase softening in fruit from each harvest date coincided with the time when IEC exceeded 1.5 $\mu l.l^{-1}$. This also occurred for the same cultivars when stored at temperatures from 0 to 35°C (Chapter 4), indicating that IEC may regulate duration of the initial slow softening phase in these cultivars. The importance of ethylene in promoting softening in apples was evident in studies using inhibitors of ethylene action, as softening was reduced in apples treated with 1-methylcyclopropene at harvest (Fan et al., 1999; Watkins et al., 2000).

Threshold ethylene concentrations for initiating softening of apples in controlled atmospheres were estimated to be 0.1 $\mu l.l^{-1}$ internally (Stow et al., 2000) and 1.0 $\mu l.l^{-1}$ externally (Liu, 1977). In other fruits, softening was initiated at external ethylene concentrations of 0.01 $\mu l.l^{-1}$ in kiwifruit (Jeffery and Banks, 1996), and 0.05 to 0.2 $\mu l.l^{-1}$ in pears (Wang et al., 1972). A threshold ethylene concentration for initiating rapid phase softening could not be determined accurately in this study as the transition between the initial slow and rapid softening phases was continuous and not discrete. Nonetheless, onset of rapid phase softening appeared to occur when IEC began to increase rapidly from a base level to exceed 1.5 $\mu l.l^{-1}$ in fruit from each harvest date. While the onset of rapid phase softening was associated with an IEC of 1.5 $\mu l.l^{-1}$, the actual concentration required for initiation of rapid softening may be somewhat lower and closer to the IEC threshold of 0.1 $\mu l.l^{-1}$ suggested by Stow et al. (2000). The 0.1 $\mu l.l^{-1}$ IEC threshold suggested by Stow et al. (2000) may be the threshold required to initiate softening in the initial slow softening phase, while 1.5 $\mu l.l^{-1}$ may be the threshold required to initiate rapid phase softening.

While IEC may be responsible for initiation of rapid phase softening in apples, it is not known what initiates or controls the rate of softening during the initial slow softening phase. This phase could be considered a combination of slow softening during maturation on the tree and slow softening during early stages of low temperature storage. Lau et al. (1986) and Blankenship and Unrath (1988) found that the IEC was low in apples during maturation and concluded that IEC did not initiate the softening that occurred in this period of development. However, IEC may still be high enough to sustain a basal rate of slow softening during maturation as proposed for kiwifruit

(Hewett et al., 1999). Also, sensitivity to ethylene increases in apples during maturation (Harkett et al., 1971; Sfakiotakis and Dilley, 1973), which may influence the effectiveness of a particular IEC to induce softening during maturation and ripening. While ethylene may have an important role in regulating on-tree softening, it is possible that other growth regulators also have an important regulatory role. On-tree firmness may also decline through the physical consequences of cell expansion during the final stages of fruit growth.

Large fruit were softer than small fruit at harvest two for RG, and although not statistically different, were also softer than smaller fruit at harvest three for RG and harvest four for COP. However, fruit size did not affect the TSS, SPI, IEC and background skin colour in fruit from both cultivars, suggesting that harvest maturity was similar for fruit of each size. Thus, the effect of fruit size on RG firmness and subsequent softening at 0.5-3°C was probably not due to maturity differences, but may have resulted from physical variation in tissue strength caused by differences in cell size and cell number between fruit of different sizes. Large apples were softer than smaller fruit in several other cultivars at harvest (Blanpied et al., 1978; Marmo et al., 1985; Siddiqui and Bangerth, 1995; Koorey and Brookfield, per. com.).

The softening curve for different sized fruit from harvests two and three were similar at 0.5 or 3°C for both cultivars. However, at harvest four, small fruit softened slower at 0.5 or 3°C than both medium and large fruit. This was mediated by a longer initial slow softening phase in both cultivars, while COP also had a slower rapid softening phase in smaller fruit than in larger fruit. In nectarines, large fruit were slightly firmer than smaller fruit after four weeks at –0.5°C, but then softened more rapidly once transferred to 15°C (Von Mollendorff et al., 1992). The interaction between harvest date and fruit size on loss of firmness has not been reported previously. The reason for this interaction is not known, but it is possible that delayed harvest date may accentuate the physical differences in cell size and number between fruit of different sizes, which in turn may influence softening. It is also possible that the longer initial slow softening phase that occurred in small fruit, compared with that in medium and large fruit, was a result of delayed induction of autocatalytic ethylene production. However, the IEC in different

sized fruit at harvest four were similar, indicating that the different sized fruit were probably at a similar stage of development at harvest.

In summary, harvest maturity, and to a lesser extent fruit size, both influenced flesh firmness at harvest and subsequent softening in storage for the COP and RG apple cultivars. Earlier harvested fruit were firmer at the beginning of storage, and had a longer initial slow softening phase in storage, than fruit harvested more mature. Fruit size had a minimal effect on firmness at harvest and during storage of both cultivars. However, small fruit from the last commercial harvest had a longer initial slow softening phase than both medium and large sized fruit of both cultivars. The mechanism by which advancing harvest maturity shortened the initial slow softening phase appeared to be mediated by earlier induction of autocatalytic ethylene production. These results confirm the importance of harvesting rapid softening cultivars at an early maturity to maximise firmness in the market place, although not so early as to compromise other aspects of postharvest quality.

7.6 References

Agar, I.T., Biasi, W.V., Mitcham, E.J., 1999. Exogenous ethylene accelerates ripening responses in Bartlett pears regardless of maturity or growing region. Postharvest Biol. Technol. 17, 67-78.

Blankenship, S.M., Sisler, E.C., 1989. 2,5-Norbornadiene retards apple softening. HortScience 2, 313-314.

Blankenship, S.M., Sisler, E.C., 1993. Response of apples to diazocyclopentadiene inhibition of ethylene binding. Postharvest Biol. Technol. 3, 95-101.

Blankenship, S.M., Unrath, C.R., 1988. Internal ethylene levels and maturity of 'Delicious' and 'Golden Delicious' apples destined for prompt consumption. J. Am. Soc. Hortic. Sci. 113, 88-91.

Blanpied, G.D., 1986. A study of the relationship between fruit internal ethylene concentration at harvest and post-storage fruit quality of cv Empire apples. J. Hortic. Sci. 61, 465-470.

Blanpied, G.D., Bramlage, W.J., Dewey, D.H., LaBelle, R.L., Massey, L.M., Mattus, G.E., Stiles, W.C., Watada, A.E., 1978. A standardized method for collecting apple pressure test data. N. Y. Food Life Sci. Bull. 74, 8pp, Cornell University, Ithaca, New York.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Harker, F.R., Hallett, I.C., 1992. Physiological changes associated with development of mealiness of apple fruit during cool storage. HortScience 27, 1291-1294.

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., 1997. Texture of fresh fruit. Hortic. Rev. 20, 121-224.

Harkett, P.J., Hulme, A.C., Rhodes, M.J.C., Wooltorton, L.S.C., 1971. The threshold value for physiological action of ethylene on apple fruits. J. Food Technol. 6, 39-45.

Hewett, E.W., Kim, H.O., Lallu, N., 1999. Postharvest physiology of kiwifruit: the challenges ahead. Acta Hortic. 498, 203-216.

Ingle, M., D'Souza, M.C., Townsend, E.C., 2000. Fruit characteristics of 'York' apples during development and after storage. HortScience 35, 95-98.

Ingle, M., Morris, J.C., 1989. Predicting firmness changes of 'Rome' apples in refrigerated storage. J. Am. Soc. Hortic. Sci. 114, 90-94.

Jeffery, P.B., Banks, N.H., 1996. Effects of ethylene on kiwifruit softening in coolstorage. New Zeal. Kiwifruit J. 114, 9-10.

Kingston, C.M., 1991. Maturity indices for apple and pear. Hortic. Rev. 13, 407-432.

Knee, M., Smith, S.M., 1989. Variation in quality of apple fruits stored after harvest on different dates. J. Hortic. Sci. 64, 413-419.

Lau, O.L., Liu, Y., Yang, S.F., 1986. Effects of fruit detachment on ethylene biosynthesis and loss of flesh firmness, skin color, and starch in ripening 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 111, 731-734.

Lidster, P.D., Porritt, S.W., 1978. Influence of maturity and delay of storage on fruit firmness and disorders in 'Spartan' apple. HortScience 13, 253-254.

Liu, F.W., 1977. Varietal and maturity differences of apples in response to ethylene in controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 102, 93-95.

Liu, F.W., 1978. Effects of harvest date and ethylene concentration in controlled atmosphere storage on the quality of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 103, 388-392.

MacRae, E.A., Lallu, N., Searle, A.N., Bowen, J.H., 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49, 413-430.

Marmo, C.A., Bramlage, W.J., Weis, S.A., 1985. Effects of fruit maturity, size, and mineral concentrations on predicting the storage life of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 110, 499-502.

Olsen, K.L., Martin, G.C., 1980. Influence of apple bloom date on maturity and storage quality of 'Starking Delicious' apples. J. Am. Soc. Hortic. Sci. 105, 183-186.

Sfakiotakis, E., Dilley, D.R., 1973. Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. J. Am. Soc. Hortic. Sci. 98, 504-508.

Shewfelt, R.L., Myers, S.C., Resurreccion, A.V.A., 1987. Effect of physiological maturity at harvest on peach quality during low temperature storage. J. Food Qual. 10, 9-20.

Siddiqui, S., Bangerth, F., 1995. Effect of pre-harvest application of calcium on flesh firmness and cell wall composition of apples - influence of fruit size. J. Hortic. Sci. 70, 263-269.

Stow, J., Dover, C.J., Genge, P.M., 2000. Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18, 215-225.

Stow, J.R., Genge, P., 2000. The effects of storage conditions on the keeping quality of 'Gala' apples. J. Hortic. Sci. Biotechnol. 75, 393-399.

Tu, K., Waldron, K., Ingham, L., De Barsy, T., De Baerdemaeker, J., 1997. Effect of picking time and storage conditions on 'Coxs Orange Pippin' apple texture in relation to cell wall changes. J. Hortic. Sci. 72, 971-980.

Von Mollendorff, L.J., Jacobs, G., De Villiers, O.T., 1992. The effects of storage temperature and fruit size on firmness, extractable juice, woolliness and browning in two nectarine cultivars. J. Hortic. Sci. 67, 647-654.

Wang, C.Y., Mellenthin, W.M., Hansen, E., 1972. Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. J. Am. Soc. Hortic. Sci. 97, 9-12.

Wang, H., Gemma, H., Oogaki, C., Kurokawa, M., 1990. Influence of harvest maturity on chilling sensitivity, respiratory rate and ethylene production during storage in 'Sensyu' apple fruit. J. Jap. Soc. Hortic. Sci. 59, 163-169.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Chapter 8 Softening rate variation for apples from different orchards.

8.1 Abstract

Variation in softening rates of 'Royal Gala' and 'Cox's Orange Pippin' apples (*Malus domestica* Borkh.) during postharvest handling makes it difficult for producers to consistently meet market requirements for firmness. Management of this softening problem could be improved if the softening rates of fruit from different orchards could be predicted before storage. This study sought to identify the extent of variation in softening rates for fruit from 23 different orchards over two seasons, and to determine if this variation can be predicted by stage of maturity and fruit mineral concentrations before storage. Most of the variation (75-80% for 'Royal Gala' and 90-92% for 'Cox's Orange Pippin') in fruit softening rates between orchards was accounted for by differences in firmness at harvest, where fruit with low firmness had a shorter market life than fruit with higher firmness. Addition of dry matter, total soluble solids and glucose concentration to the firmness model improved prediction of 'Royal Gala' softening at 0.5°C by 10-15%. Mineral concentrations in fruit from different orchards were poorly associated with subsequent softening rates in both cultivars. However, calcium concentration may be involved in cultivar differences in softening, as 'Royal Gala' fruit generally had higher calcium concentrations at harvest, and slower softening than 'Cox's Orange Pippin' during low temperature storage. These results demonstrate that variation in postharvest softening rates between orchards in two seasons was largely due to maturity differences at harvest. However, further research is required to determine if maturity differences can be used to predict softening rates in seasons that produce fruit with abnormally fast softening rates.

Keywords: Malus domestica (Borkh.); Firmness; Maturity; Minerals; Calcium; Prediction; Modelling; Quality.

8.2 Introduction

The 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apple cultivars soften rapidly after harvest. Variation in their softening rates during postharvest handling makes it difficult for apple producers to estimate the postharvest life of fruit from different

orchards and seasons, so that firmness specifications (often >65N) in markets are met. Management of this problem would be improved if softening rates in fruit from different orchards and seasons could be predicted before storage.

Softening in harvested RG and COP is triphasic, consisting of an initial slow softening phase, a phase of more rapid softening, and a final slow softening phase (Chapter 4). The influence of temperature (Chapters 4 and 5), harvest date and fruit size (Chapter 7) on softening in each of these phases has been characterised and described using empirical models. However, for these models to be predictive there is a need to identify fruit attributes before storage that consistently indicate subsequent softening rates for fruit from different orchards and seasons.

Several studies have attempted to correlate poststorage firmness in fruit from different orchards and seasons with prestorage mineral concentrations, meteorological data, and/or maturity indices and indicators of climacteric development (Bramlage et al., 1985; Fallahi et al., 1985; Marmo et al., 1985; Johnson et al., 1987; Ingle and Morris, 1989; Knee and Farman, 1989; Knee and Smith, 1989; Blankenship et al., 1997; Johnson and Ridout, 1998; de Jager and de Putter, 1999; Ingle et al., 2000; Johnson, 2000). A common feature in most of these studies was a positive association between harvest firmness and firmness after storage, although correlation coefficients varied considerably between seasons and cultivars. For COP, the ability to predict poststorage firmness from harvest firmness was improved by inclusion of harvest date, or days between harvest and an internal ethylene concentration (IEC) of 0.1 $\mu l.l^{-1}$ (Knee and Farman, 1989). In general, mineral concentrations were poor predictors of poststorage firmness for several cultivars (Bramlage et al., 1985; Fallahi et al., 1985; de Jager and de Putter, 1999). However, correlations between mineral concentrations (P, Ca, Mg, B, Mn, Cu, Zn, K and Na) and poststorage firmness have been noted in some apple cultivars (Johnson et al., 1987; Johnson, 2000).

Although several studies have correlated variables measured at harvest with subsequent poststorage firmness, only Ingle and Morris (1989) correlated variables at harvest with postharvest softening rates. Harvest firmness in fruit from different orchards and seasons was positively associated with subsequent softening rates at 0°C (r = 0.8-0.95)

for 'Rome', but not for 'Delicious' apples (Ingle and Morris, 1989). However, this study implicitly assumed that softening was linear in storage with softening rates calculated from firmness data before and after storage. As no firmness measurements were made during low temperature storage, account could not be taken of the triphasic nature of softening should it have occurred in those cultivars.

The variable correlations outlined above have limited potential for predicting firmness at a storage time different to that used in model development, as these models require the assumption that softening was linear in storage. While small sections of the softening curve may appear linear, the overall softening curve in several apple cultivars is non-linear (Chapters 4 and 7). This non-linear nature of the softening curve may explain why inconsistent relationships occurred between variables measured at harvest and poststorage firmness for apples from different cultivars and seasons.

It is possible that more accurate quantification of softening rates through storage will improve both accuracy and consistency of softening predictions derived from models based on attributes measured at harvest. This could be achieved by measuring firmness at regular intervals through storage, and then fitting the appropriate models to estimate the rate of firmness change (k) for fruit from different orchards. Thus, this study characterised the extent of softening rate variation between orchards for the rapid softening RG and COP cultivars, and sought to account for this variation by measuring specific fruit attributes before storage.

8.3 Materials and methods

8.3.1 Fruit supply and treatments

Export quality COP apples were harvested at commercial export maturity from 5 Hawkes Bay and 10 Nelson orchards in 1999, and from 1 Hawkes Bay and 7 Nelson orchards in 2000. RG apples were harvested from 6 Nelson and 9 Hawkes Bay orchards in 1999, and from 1 Palmerston North (Fruit Crops Unit, Massey University), and from 7 Hawkes Bay orchards in 2000. Fruit were transported to Massey University, Palmerston North, New Zealand within 72 hours of harvest. Fruit weights used in the experiments were 170 ± 10 g for RG and 140 ± 10 g for COP.

For each orchard, 100 fruit were evenly distributed among 10 perforated polyethylene bags (35 μm thickness; 50 x 5 mm diameter perforations per m^2), that in turn were packed into commercial cardboard cartons and placed at 0.5±0.5°C (3.0±0.5°C for COP). In addition, 100 fruit from each of 6 orchards in 1999, and 8 orchards in 2000 were packed as above and stored at 20°C.

A sample of 20 fruit from each orchard had respiration rate (r_{CO_2}), fresh weight, density, skin greenness (hue°; COP only), internal ethylene concentration (IEC), firmness, total soluble solids (TSS), and starch pattern index (SPI) measured before storage. In addition a longitudinal cylinder of outer cortex tissue was removed from each fruit with a 10 mm core-borer, snap-frozen in liquid nitrogen, stored at –80°C until required, then lyophilised and stored at –20°C until analysis of carbohydrate and mineral content. A separate longitudinal wedge from each fruit was juiced in an electronic juicer, and the juice stored at –80°C until analysis of titratable acidity (TA). Flesh firmness was measured on 10 fruit (1 fruit randomly removed from each bag) after 2 to 3 days in storage, and thereafter at 20 to 30 day intervals at 0-3°C, and 2-5 day intervals at 20°C. Flesh firmness was measured at the storage temperature of the fruit. Fruit with rots and disorders were omitted from datasets during data analysis.

8.3.2 At harvest and storage measurements

Flesh firmness, IEC, density, hue° and r_{CO_2} were measured as previously described (Chapter 5), as was TSS, TA and SPI (Chapter 7). Dry matter content in the outer cortex was estimated by dividing dry weight of the tissue sample after freeze-drying, by the fresh weight before freezing in liquid nitrogen. Freeze-dried tissue was then ground to a fine powder for carbohydrate and mineral analysis.

Calcium (Ca), magnesium (Mg) and potassium (K) concentrations were determined by digesting ~150 mg of freeze-dried tissue in 4 ml of concentrated nitric acid at 150°C until the reaction mixture cleared, with residual acid then evaporated at 250°C. Digest residues were resuspended in 10 ml of quenching solution (1000 $mg.l^{-1}$ Sr and Cs in 0.2

M HCl), and concentrations determined using atomic flame absorption (Ca and Mg in both years, and K in 2000), or emission (K in 1999) spectrometry (Technicon, 1973).

Nitrogen (N) and phosphorus (P) concentrations were determined by digesting ~350 mg of freeze-dried tissue in 4 ml of Kjeldahl digest solution (250 g potassium sulphate and 2.5 g selenium powder in 2.5 l of concentrated sulphuric acid) at 350°C until the solution cleared. Once cool, digests were diluted to 50 ml with deionised water, and N and P concentrations were determined colorimetrically (Twine and Williams, 1971).

Soluble sugars (fructose, glucose and sucrose) were extracted from ~40 mg of freeze-dried tissue in 2 ml of 62.5% methanol at 60°C for 1 hour, then centrifuged, and the supernatant decanted into clean tubes. The remaining tissue pellet was re-extracted in 2 ml of 62.5% methanol at room temperature for 15 minutes, centrifuged, and the supernatants combined for measurement of soluble sugars. The remaining tissue pellet was held at -20°C until starch measurement. The soluble sugar extract was diluted in deionised water, and the sugar concentrations determined using a colorimetric enzyme kit (Boehringer Mannheim, Germany, catalogue number 716260).

Starch in the residual tissue pellet was solubilised in 0.5 ml of 8 M HCl and 2 ml of dimethylsulphoxide at 60°C for 1 hour. The solution was cooled before addition of 0.5 ml of 8 M NaOH, and then made up to 10 ml with 0.112 M sodium citrate buffer (pH 4). Starch concentration in the extract was determined using a colorimetric enzyme kit (Boehringer Mannheim, Germany, catalogue number 207748).

8.3.3 Data analysis

Firmness (f, N) after different times (t, day) at 0°C, 3°C or 20°C was fitted with the following empirical sigmoidal function (Chapter 4) using non-linear regression:

$$f = f_{-\infty} - (f_{-\infty} - f_{+\infty}) \cdot \left(1 - \left(1 + \exp\frac{t + k^{-1} \cdot \ln(31) - (5.2 \cdot k^{-1.0168})}{k^{-1}}\right)^{-0.2}\right) \qquad \text{(Eq. 8-1)}$$

where model parameters were an initial firmness asymptote ($f_{-\infty}$, N), a minimum firmness asymptote ($f_{+\infty}$, N), and rate of firmness change (k, day^{-1}). Simultaneous

analysis of softening data from all orchards initially indicated that $f_{+\infty}$ could be fixed to 41.15 N for RG and 37.81 N for COP, with $f_{+\infty}$ values determined by the statistical software. However, fixing of k or $f_{-\infty}$ resulted in a poor description of softening data from different orchards, and consequently both parameters were kept variable in subsequent data analysis.

Equation 8-1 was rearranged so that time (t, days) to soften to a specified firmness (f, N) could be calculated from the following equation:

$$t = \ln\left(\left(-1\cdot\left(\frac{f-f_{-\infty}}{f_{+\infty}-f_{-\infty}}-1\right)^{-5}\right)-1\right)\cdot\frac{1}{k}+5.2\cdot k^{-1.0168}-\frac{1}{k}\cdot\ln(31) \qquad \text{(Eq. 8-2)}$$

where f was substituted with 65 or 55 to calculate time to 65 N (t_{65N}) and 55 N (t_{55N}) respectively, for fruit from each orchard.

Multiple linear regression was used to determine if the variables measured at harvest were associated with the subsequent softening parameters (k and $f_{-\infty}$) for fruit from different orchards. The R^2 selection procedure was used to identify important predictor variables in both seasons, with variables only considered if they significantly ($P < 0.05$) contributed to the regression model in both seasons.

Non-linear regression was performed using the NLIN procedure, and both multiple linear regression and simple linear regression performed using the REG procedure, in the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

8.4 Results

Softening of COP at 3°C and RG at 0.5°C was triphasic (data not shown), with softening data fitted with Eq. 8-1 to estimate k and $f_{-\infty}$ for fruit from each orchard. Furthermore, Eq. 8-2 and the parameter estimates from Eq. 8-1 were used to calculate t_{65N} for fruit from each orchard (Fig's 8-1 and 8-2). The $f_{-\infty}$ values for RG at 0.5°C ranged from 65-85 N in 1999, and 60-85 N in 2000, with the mode values in 1999 being 70-75 N and 65-75 N in 2000 (Fig. 8-1). The $f_{-\infty}$ values for COP at 3°C were more variable than for RG at 0.5°C, ranging from 55-85 N in 1999, and from 60-90 N in 2000, with the mode

$f_{-\infty}$ value being 70-75 N in both seasons (Fig. 8-2). The range in harvest firmness (f_H) values for both cultivars was slightly greater than the range in $f_{-\infty}$ values in both seasons, with the upper limit and mode value being 5 N greater for f_H than $f_{-\infty}$ (Fig's 8-1 and 8-2). The range in t_{65N} values was greater for RG at 0.5°C (0-120 days), than for COP at 3°C (0-50 days), with the mode value for t_{65N} being 40-60 days for RG in 1999, 20-40 days for RG in 2000, and 10-20 days for COP in both seasons (Fig's 8-1 and 8-2).

Values of k were greater for all COP fruit at 3°C than for RG fruit at 0.5°C, with mode values for RG being 0.04-0.06 day^{-1} (Fig. 8-1), and 0.15-0.175 day^{-1} for COP (Fig. 8-2). The range in k values for COP at 3°C was similar in both seasons (Fig. 8-2), while the upper limit for k was lower in 1999 than 2000 for RG at 0.5°C (Fig. 8-1). Estimates of k at 0.5°C or 3°C were proportional to k estimates at 20°C for fruit from the same orchard, with k being 8.5 and 5.2 times greater at 20°C than at 0.5°C and 3°C for RG and COP respectively (Fig. 8-3).

Values of k for COP at 3°C were strongly ($r > 0.69$) negatively associated with at harvest measurements of firmness, skin greenness and starch concentration, and strongly positively associated with SPI (Table 8-1). Values of k for this cultivar were also moderately ($r = 0.5$ to 0.69) positively associated with TSS, sucrose concentration and r_{CO_2}, and moderately negatively associated with TA. Values of k for RG at 0.5°C were strongly negatively associated with at harvest measurements of firmness, strongly positively associated with TSS, moderately negatively associated with density and starch concentration, and moderately positively associated with IEC, SPI, fructose, and sucrose concentrations (Table 8-2). While k was poorly correlated with Ca concentration between orchards for either cultivar, Ca concentrations in COP were consistently lower, and k values greater at 3°C, than in RG at 0.5°C (Fig. 8-4).

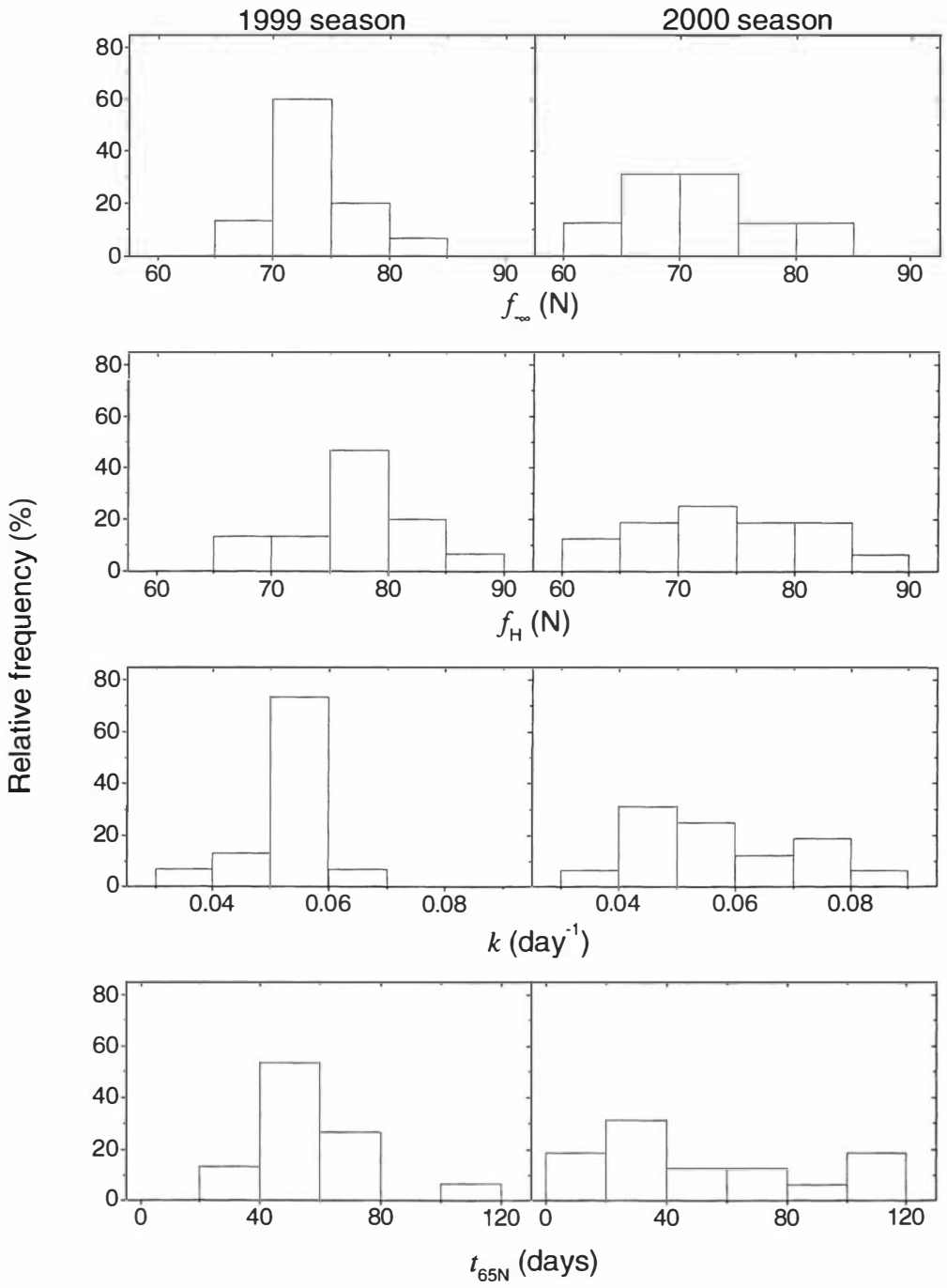

Fig. 8-1 Frequency distribution of initial firmness ($f_{-\infty}$), rate of firmness change (k), time to 65N (t_{65N}) at 0.5°C, and firmness at harvest (f_H), for 'Royal Gala' (RG) fruit from 15 orchards in 1999 and 8 orchards in 2000. k, $f_{-\infty}$ and t_{65N} values were estimated from softening data at 0.5°C using Eq's 8-1 and 8-2.

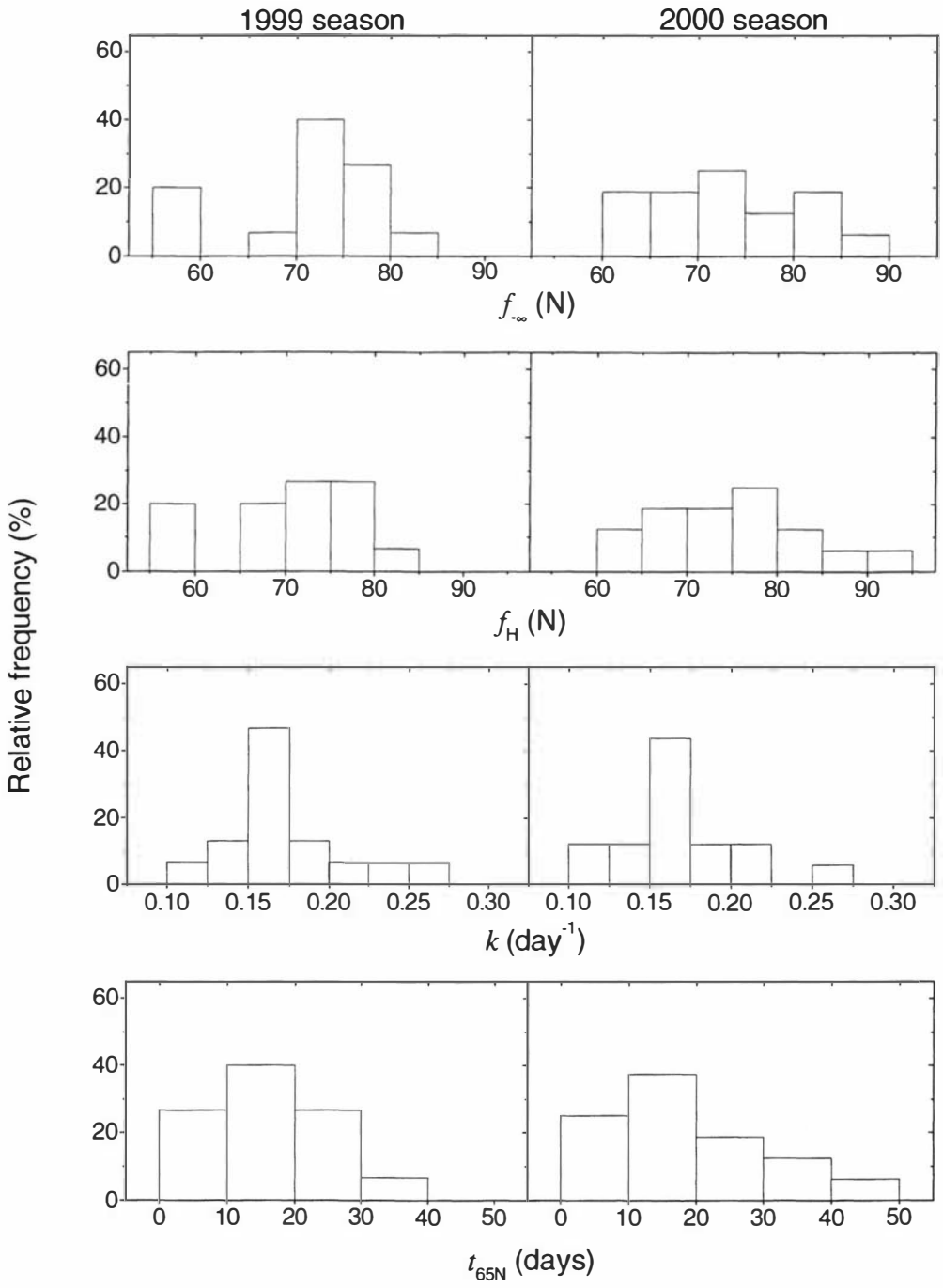

Fig. 8-2 Frequency distribution of initial firmness ($f_{-\infty}$), rate of firmness change (k), time to 65N (t_{65N}) at 3°C, and firmness at harvest (f_H), for 'Cox's Orange Pippin' (COP) fruit from 15 orchards in 1999 and 8 orchards in 2000. k, $f_{-\infty}$ and t_{65N} values were estimated from softening data at 3°C using Eq's 8-1 and 8-2.

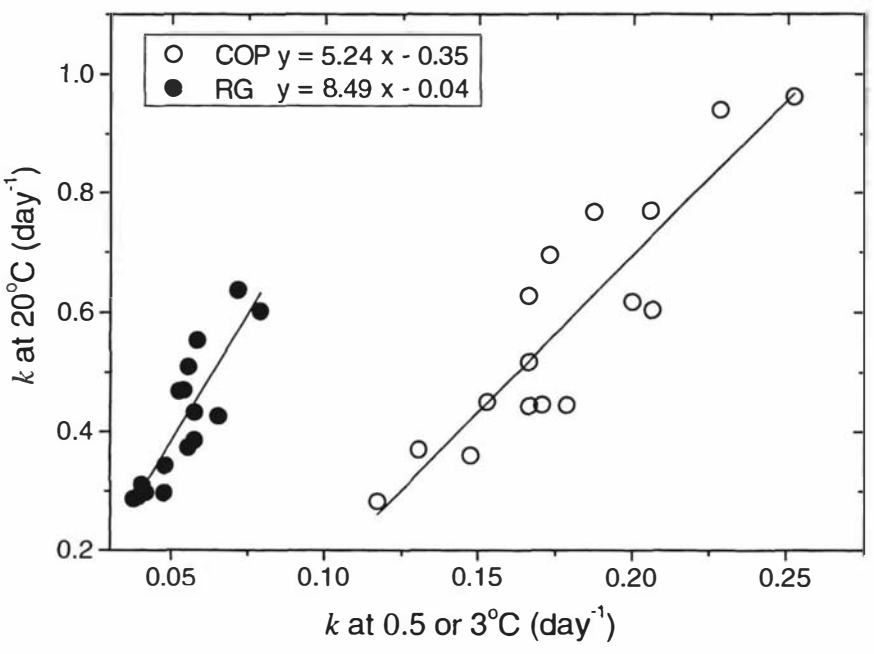

Fig. 8-3 Rate of firmness change (k) at 20°C for 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples with different k values at 0.5°C (RG) or 3°C (COP). Values of k were estimated from softening data for each orchard at 0.5°C (3°C for COP) and 20°C using Eq. 8-1.

Values of f_{∞} for COP fruit from different orchards were strongly positively associated with at harvest measurements of firmness, starch concentration and TA, strongly negatively associated with SPI and glucose concentration, moderately positively associated with skin greenness, and moderately negatively associated with sucrose concentration, TSS and r_{CO_2} (Table 8-1). The f_{∞} values for RG from different orchards were strongly positively associated with firmness and starch concentration, strongly negatively associated with SPI, TSS and sucrose concentration, moderately positively associated with TA, and moderately negatively associated with IEC (Table 8-2). The correlations between t_{65N} and at harvest variables were similar to those obtained for f_{∞} (Tables 8-1 and 8-2).

Table 8-1 Correlation coefficients between variables measured at harvest and subsequent softening parameters at 3°C for 'Cox's Orange Pippin' apples from different orchards in 1999 and 2000. Softening parameters were rate of firmness change (k), initial firmness ($f_{-\infty}$), and time to 65N (t_{65N}), which were estimated from each orchard's softening data using Eq's 8-1 and 8-2.

At harvest variable	Range	Correlation coefficients		
		k (day^{-1})	$f_{-\infty}$ (N)	t_{65N} (days)
Firmness (N)	58.9 - 90.7	-0.85	0.96	0.95
Total soluble solids (%)	10.1 – 14.4	0.55	-0.68	-0.59
Skin greenness (Hue°)	99.4 – 113.2	-0.73	0.69	0.71
Internal ethylene (µl.l^{-1})	0.01 – 9.7	0.17	-0.47	-0.45
Starch pattern index	0.1 – 4.8	0.82	-0.88	-0.89
Density (g.ml^{-1})	0.83 – 0.87	-0.36	0.34	0.41
Fructose (µmol.gFW^{-1})	249.8 – 426.7	0.20	-0.44	-0.38
Sucrose (µmol.gFW^{-1})	100.1 – 177.7	0.62	-0.66	-0.64
Starch (mg.gFW^{-1})	4.0 – 30.3	-0.70	0.85	0.81
Dry matter (g.gFW^{-1})	0.138 – 0.170	-0.07	0.07	0.09
Potassium (µmol.gFW^{-1})	25.9 – 42.3	-0.02	0.09	0.17
Respiration rate (nmol.kg.s^{-1})	108.1 – 262.0	0.51	-0.61	-0.49
Titratable acidity (mmol.l^{-1})	40.6 – 77.5	-0.65	0.71	0.69
Magnesium (µmol.gFW^{-1})	1.5 – 1.9	-0.01	-0.17	-0.07
Nitrogen (µmol.gFW^{-1})	25.8 – 77.6	0.06	0.04	-0.04
Glucose (µmol.gFW^{-1})	8.7 – 64.5	0.41	-0.72	-0.65
Calcium (µmol.gFW^{-1})	0.5 – 1.1	-0.07	-0.03	-0.02
Phosphorus (µmol.gFW^{-1})	8.9 – 20.1	-0.23	0.27	0.19

Table 8-2 Correlation coefficients between variables measured at harvest and subsequent softening parameters at 0.5°C for 'Royal Gala' apples from different orchards in 1999 and 2000. Softening parameters were rate of firmness change (k), initial firmness ($f_{-\infty}$), and time to 65N (t_{65N}), which were estimated from each orchard's softening data using Eq's 8-1 and 8-2.

At harvest variable	Range	Correlation coefficients		
		k (day^{-1})	$f_{-\infty}$ (N)	t_{65N} (days)
Firmness (N)	61.7 - 88.7	-0.78	0.92	0.91
Total soluble solids (%)	9.6 – 12.2	0.70	-0.83	-0.82
Internal ethylene (µl.l^{-1})	0.01 – 4.1	0.63	-0.68	-0.67
Starch pattern index	0.2 – 5.5	0.60	-0.83	-0.79
Density (g.ml^{-1})	0.84 – 0.88	-0.53	0.38	0.44
Fructose (µmol.gFW^{-1})	345.1 – 524.2	0.55	-0.23	-0.38
Sucrose (µmol.gFW^{-1})	60.4 – 131.0	0.55	-0.72	-0.67
Starch (mg.gFW^{-1})	1.1 – 23.8	-0.50	0.70	0.68
Dry matter (g.gFW^{-1})	0.125 – 0.148	0.42	-0.19	-0.25
Potassium (µmol.gFW^{-1})	21.6 – 33.2	-0.38	0.40	0.42
Respiration rate (nmol.kg.s^{-1})	83.5 – 222.9	0.39	-0.46	-0.44
Titratable acidity (mmol.l^{-1})	15.0 – 31.1	-0.29	0.51	0.46
Magnesium (µmol.gFW^{-1})	1.2 – 1.8	-0.24	0.25	0.23
Nitrogen (µmol.gFW^{-1})	17.3 – 54.4	-0.24	0.25	0.27
Glucose (µmol.gFW^{-1})	26.3 – 80.4	0.23	-0.19	-0.25
Calcium (µmol.gFW^{-1})	0.8 – 1.7	-0.14	0.23	0.23
Phosphorus (µmol.gFW^{-1})	7.6 – 17.1	0.05	-0.10	-0.06

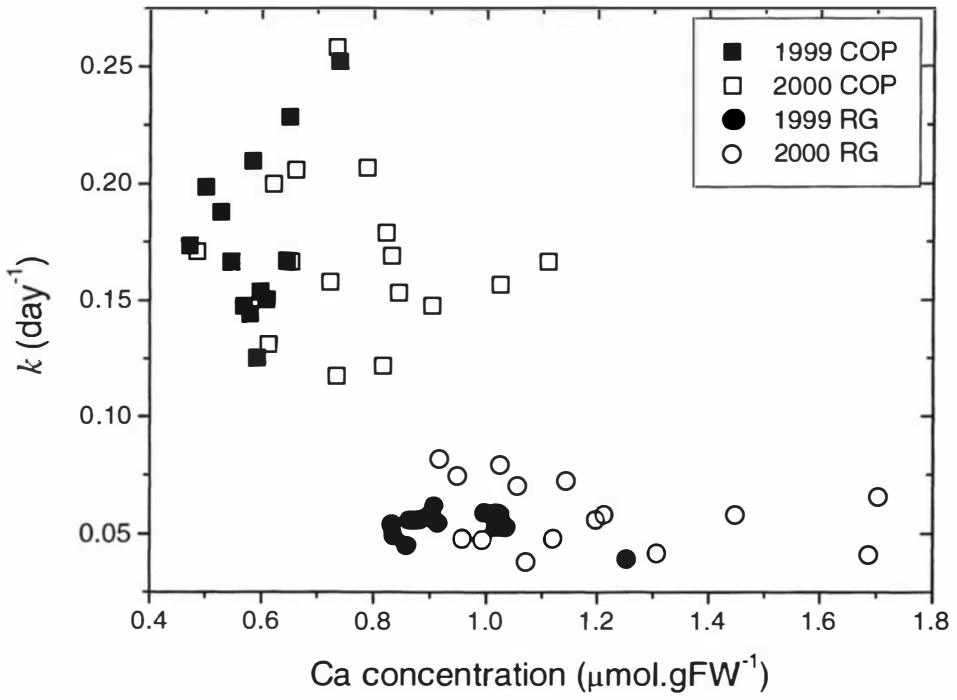

Fig. 8-4 Rate of firmness change (k) for 'Royal Gala' (RG) apples at 0.5°C, and 'Cox's Orange Pippin' (COP) apples at 3°C, for fruit with different cortical calcium (Ca) concentrations. k values were estimated from softening data at 0 to 3°C using Eq. 8-1. Mean Ca concentrations (n = 20 fruit) for each orchard are shown.

Further data analysis was undertaken to determine if a larger proportion of the variation in k and $f_{-\infty}$ and from different orchards could be accounted for by multiple regression models. The proportion of variation in k between COP orchards that was accounted for by firmness at harvest increased from 0.72 to 0.75-0.76 when combined with either IEC or glucose concentration, and to 0.79 when combined with both of these variables (Table 8-3). Individually, both IEC and glucose concentrations were poor predictors of k for COP at 3°C. For RG at 0.5°C, the proportion of variation in k between orchards accounted for by firmness at harvest increased from 0.59 to 0.71 when combined with cortical dry matter content, and to 0.83 when both variables were combined with glucose concentration (Table 8-4). As for COP, glucose concentration and dry matter alone were poor predictors of k for RG at 0.5°C. Addition of other variables to the multiple regression models for k did not significantly improve the R^2 values for either cultivar.

Table 8-3 Proportion of variation (R^2) in 'Cox's Orange Pippin' softening at 3°C explained by multiple variables measured at harvest, for fruit from different orchards in 1999 and 2000. Softening parameters were rate of firmness change (k) and initial firmness ($f_{-\infty}$), which were estimated from each orchard's softening data using Eq's 8-1 and 8-2.

At harvest variable combination	R^2	P value
k (day^{-1})		
Internal ethylene concentration	0.03	0.3488
Glucose	0.17	0.0278
Firmness	0.72	0.0001
Firmness + internal ethylene concentration	0.75	0.0001
Firmness + glucose	0.76	0.0001
Firmness + internal ethylene concentration + glucose	0.79	0.0001
$f_{-\infty}$ (N)		
Firmness	0.93	0.0001

Table 8-4 Proportion of variation (R^2) in 'Royal Gala' softening at 0.5°C explained by multiple variables measured at harvest, for fruit from different orchards in 1999 and 2000. Softening parameters were rate of firmness change (k) and initial firmness ($f_{-\infty}$), which were estimated from each orchard's softening data using Eq's 8-1 and 8-2.

At harvest variable combination	R^2	P value
k (day^{-1})		
Glucose	0.05	0.2395
Dry matter	0.17	0.0245
Firmness	0.59	0.0001
Firmness + glucose	0.63	0.0001
Firmness + dry matter	0.71	0.0001
Firmness + dry matter + glucose	0.83	0.0001
$f_{-\infty}$ (N)		
Total soluble solids	0.66	0.0001
Starch pattern index	0.67	0.0001
Firmness	0.81	0.0001
Firmness + starch pattern index	0.87	0.0001
Firmness + total soluble solids	0.88	0.0001

A considerable proportion of the variation in $f_{-\infty}$ values from different orchards was explained by firmness at harvest alone in both COP (Table 8-3) and RG (Table 8-4). Addition of other variables to the regression model did not significantly improve the R^2 value for $f_{-\infty}$ in COP, while addition of TSS to firmness increased the R^2 value for $f_{-\infty}$ from 0.81 to 0.88 for RG. Individually, both SPI and TSS alone were moderate predictors of $f_{-\infty}$ for RG at 0.5°C.

Regression equations based on harvest firmness alone (model A), and multiple-variable equations with highest R^2 values in Tables 8-3 and 8-4 (models B and C), were used for both cultivars to predict k and $f_{-\infty}$ values for fruit from each orchard in 1999 using 2000 season model coefficients, and in 2000 orchards using 1999 season coefficients. Predicted k and $f_{-\infty}$ values were substituted into Eq. 8-2 to calculate t_{65N} and t_{55N} for each orchard (Fig. 8-5). A comparison of t_{65N} calculations from k and $f_{-\infty}$ predictions at harvest, versus t_{65N} and t_{55N} calculations from non-linear regression of actual softening data, indicated that softening variation between orchards could be predicted from variables measured at harvest. While the RG model based on firmness alone (model A) was a good predictor of t_{65N} and t_{55N}, prediction accuracy was improved by 10-15% when firmness was combined with TSS, dry matter and glucose concentration (model B). However, addition of glucose concentration and IEC to firmness (model C) did not improve the accuracy for prediction of t_{65N} and t_{55N} for COP at 3°C when compared to the model based on firmness alone (model A).

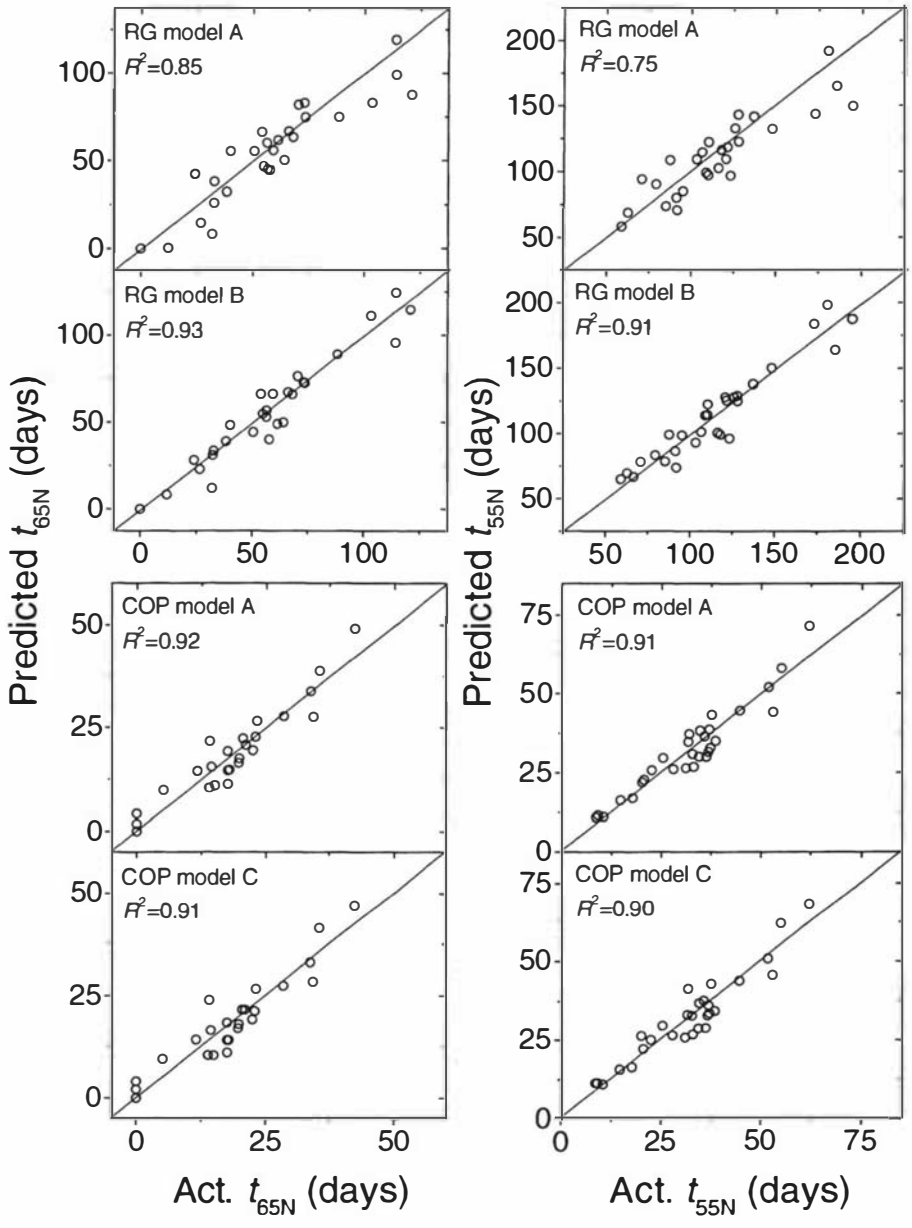

Fig. 8-5 Prediction of times to 65N (t_{65N}) and 55N (t_{55N}) for 'Royal Gala' (RG) at 0.5°C and 'Cox's Orange Pippin' (COP) at 3°C, for fruit from different orchards. Values for t_{65N} and t_{55N} were estimated using Eq. 8-2, with the rate of firmness change (k) and initial firmness (f_{∞}) parameters being predicted for each orchard from variables measured at harvest. Prediction variables were: firmness alone for both COP and RG (model A); firmness, total soluble solids, dry matter and glucose concentration for RG (model B); and firmness, internal ethylene concentration and glucose concentration for COP (model C). The same variables were used in predictions in both seasons, with 1999 predictions being from variable coefficients estimated from 2000 data, and 2000 predictions from 1999 coefficients.

8.5 Discussion

Consistent with previous studies (Chapters 4 and 7), postharvest softening in COP and RG fruit from several orchards was triphasic during low temperature storage, and could be described by an empirical sigmoidal function. The range in estimates of k, $f_{-\infty}$ and t_{65N} for fruit from different orchards indicates that preharvest factors such as grower management systems, climate, and orchard characteristics have a strong influence on postharvest softening in both cultivars.

The finding that k was about 8.5 times greater at 20°C than at 0.5°C in RG, and approximately 5.2 times greater at 20°C than at 3°C in COP, suggests that softening was more strongly inhibited by low-temperatures in RG than in COP. It is possible that the difference in k between cultivars occurred because comparisons were made on cultivars stored at different low temperatures. However, k was only slightly lower at 0.5°C than at 3°C for COP, and when compared at 0.5°C the k values were still greater for COP than for RG (Chapter 4). The observation that k values for any one orchard were proportional at different temperatures, suggests that orchards that produce rapid softening fruit at low temperatures also produce fruit that soften rapidly at shelf-life temperatures.

Models for predicting k and $f_{-\infty}$ before storage for either cultivar were largely dominated by maturity-related variables. Positive associations of k with soluble sugar concentrations (TSS, fructose and sucrose) and IEC, and negative associations with starch concentration and firmness in RG, indicated that fruit from orchards with greater k values were generally more mature at harvest than fruit from orchards with lower k values. Likewise in COP, positive associations of k with soluble sugar concentrations (TSS and sucrose), and negative associations with TA, skin greenness, firmness and starch concentration, indicated that across orchards fruit harvested at a later maturity softened faster than fruit harvested at an earlier maturity. Thus, much of the variation in softening between orchards could be attributed to differences in fruit maturity at harvest for both cultivars.

In general, fruit mineral concentrations at harvest were poor predictors of subsequent k values in both cultivars. Poor correlations between poststorage firmness and mineral concentrations in fruit or leaves were also found for 'McIntosh' (Bramlage et al., 1985) and 'Starkspur Golden Delicious' (Fallahi et al., 1985) apple cultivars. However, P was found to be positively associated with poststorage firmness for COP (Johnson et al., 1987; de Jager and de Putter, 1999), 'Red Pippin' (Johnson, 2000), 'Jonagold' and 'Elstar' (de Jager and de Putter, 1999), although correlation coefficients varied substantially between seasons and cultivars (r = 0.27-0.72). Furthermore, Johnson (2000) found that poststorage firmness was associated with at harvest fruit concentrations of Zn, Mn and B for 'Red Pippin', and Ca, K and B for 'Gala'. Thus, the importance of certain minerals in predicting apple softening during low-temperature storage may be cultivar and season specific (Johnson, 2000).

Calcium concentration could not explain the observed variation in k between orchards for either cultivar. However, Ca concentration may account for cultivar differences in k, as COP generally had lower Ca concentrations, and greater k values, than RG at 0.5-3°C. Ca is thought to maintain, or increase, the structural stability of cell walls and membranes, and consequent textural quality of many fruits during ripening (Poovaiah et al., 1988). Accordingly, softening was reduced in several apple cultivars when Ca was applied as a preharvest spray (Watkins et al., 1989; Raese and Drake, 1993) or postharvest dip (Mason et al., 1974; Sams and Conway, 1984; Abbott et al., 1989; Saftner et al., 1998). Apart from the cell wall and membrane, large quantities of Ca are also sequestered into organelles to regulate the Ca concentration in the cytoplasm (Poovaiah et al., 1988). Thus, improved correlations between k values and Ca may have been attained if apoplastic Ca had been measured, instead of the total concentrations used herein.

Harvest firmness was an important determinant of softening potential in fruit from different orchards for both cultivars, as it was strongly negatively associated with k, and strongly positively associated with f_{∞} and t_{65N}. These strong correlations indicate that the market life (fruit with firmness >65N) of both cultivars was largely determined by firmness at harvest. In general, fruit with lower firmness at harvest had a lower f_{∞} value and higher k value at 0.5-3°C, resulting in a lower t_{65N}, than in fruit with greater

firmness at harvest. Harvest firmness was also correlated with poststorage firmness in 'York' (Ingle et al., 2000), COP (Knee and Smith, 1989; Johnson and Ridout, 1998; de Jager and de Putter, 1999), 'Elstar', 'Boskoop', 'Jonagold' (de Jager and de Putter, 1999), 'Rome' (Ingle and Morris, 1989), 'McIntosh' (Marmo et al., 1985) 'Red Pippin' and 'Gala' (Johnson, 2000) apple cultivars. However, correlation coefficients varied between cultivars and seasons (r = 0.32 to 0.93), indicating that firmness may need to be combined with some other attribute for accurate and consistent predictions.

Addition of dry matter, TSS and glucose concentration to firmness models improved the accuracy of softening predictions at 0.5°C for RG. In contrast, while IEC and glucose concentration slightly improved the ability to predict k at 3°C for COP, addition of these variables to the harvest firmness model did not improve the overall ability to predict t_{65N} and t_{55N} at 3°C. Addition of harvest date, and days between harvest and an IEC of 0.1 $\mu l.l^{-1}$, have also inconsistently improved the ability of harvest firmness based models to predict firmness of COP after controlled atmosphere storage (R^2 = 0.68 to 0.95) in several seasons (Knee and Farman, 1989). Multiple regression models based on different combinations of commercial maturity indices were poor (R^2 < 0.36) predictors of poststorage firmness in 'Fuji' (Blankenship et al., 1997) and 'York' (Ingle et al., 2000) apples. Thus, the ability of certain combinations of variables to predict postharvest softening rates in apples appears to be cultivar specific.

Cross validation of the multiple regression models between 1999 and 2000 season datasets, indicated that softening rates in RG at 0.5°C and COP at 3°C could be predicted before storage by measuring specific fruit attributes at harvest. Prediction of COP softening would require measurement of firmness before storage, while prediction of RG softening would require measurement of firmness, TSS, dry matter, and glucose concentrations. Of these variables, firmness and TSS are currently routinely measured in most commercial packhouses. Dry matter could also be easily measured in these facilities, while glucose concentration may have to be measured in a specialised laboratory.

Alternatives to specialised laboratories for measuring glucose could include the use of TSS or near infrared spectroscopy. However, TSS was only able to predict sucrose

concentrations in fruit, and was a poor predictor of glucose and fructose concentrations (Appendix B). Currently near infrared spectroscopy can measure TSS non-destructively in apples (Peirs et al., 2000), and individual sugar concentrations in fruit juice (Rambla et al., 1997), and could potentially measure individual sugars non-destructively in fruits in real time. Furthermore, near infrared spectroscopy has also been used to measure dry matter and firmness in kiwifruit (McGlone and Kawano, 1998), and firmness in apples (Peirs et al., 2000). Thus, near infrared spectroscopy could be a commercially viable option for non-destructive determination of softening potential during on-line sorting of individual fruit in packhouses.

In summary, considerable variation in postharvest fruit softening between orchards was identified for RG at 0.5°C and COP at 3°C. This variation could largely be accounted for in both cultivars by differences in firmness at harvest. The ability of harvest firmness to predict softening of RG at 0.5°C was improved with the additional measurements of TSS, glucose concentration and dry matter, while additional measurements did not improve the ability to predict softening of COP at 3°C. Fruit mineral concentrations were poor predictors of softening within either cultivar. However, Ca concentration may explain differences between cultivars, as COP generally had lower Ca, and faster softening rates, than RG at 0.5-3°C. These results demonstrate that variation in softening between orchards in two seasons could be largely attributed to differences in maturity at harvest. They also confirm the importance of harvesting rapid softening apple cultivars early in the commercial harvest period to maximise firmness before and after storage, but not too early so that other aspects of quality are compromised.

8.6 References

Abbott, J.A., Conway, W.S., Sams, C.E., 1989. Postharvest calcium chloride infiltration affects textural attributes of apples. J. Am. Soc. Hortic. Sci. 114, 932-936.

Blankenship, S.M., Parker, M., Unrath, C.R., 1997. Use of maturity indices for predicting poststorage firmness of 'Fuji' apples. HortScience 32, 909-910.

Bramlage, W.J., Weis, S.A., Drake, M., 1985. Predicting the occurrence of poststorage disorders of 'McIntosh' apples from preharvest mineral analyses. J. Am. Soc. Hortic. Sci. 110, 493-498.

de Jager, A., de Putter, H., 1999. Preharvest factors and postharvest quality decline of apples. Acta Hortic. 485, 103-110.

Fallahi, E., Righetti, T.L., Richardson, D.G., 1985. Predictions of quality by preharvest fruit and leaf mineral analyses in 'Starkspur Golden Delicious' apple. J. Am. Soc. Hortic. Sci. 110, 524-527.

Ingle, M., D'Souza, M.C., Townsend, E.C., 2000. Fruit characteristics of 'York' apples during development and after storage. HortScience 35, 95-98.

Ingle, M., Morris, J.C., 1989. Predicting firmness changes of 'Rome' apples in refrigerated storage. J. Am. Soc. Hortic. Sci. 114, 90-94.

Johnson, D.S., 2000. Mineral composition, harvest maturity and storage quality of 'Red Pippin', 'Gala' and 'Jonagold' apples. J. Hortic. Sci. Biotechnol. 75, 697-704.

Johnson, D.S., Marks, M.J., Pearson, K., 1987. Storage quality of Cox's Orange Pippin apples in relation to fruit mineral composition during development. J. Hortic. Sci. 62, 17-25.

Johnson, D.S., Ridout, M.S., 1998. Prediction of storage quality of 'Coxs Orange Pippin' apples from nutritional and meteorological data using multiple regression models selected by cross validation. J. Hortic. Sci. Biotechnol. 73, 622-630.

Knee, M., Farman, D., 1989. Sources of variation in the quality of CA stored apples. Proceedings of the Fifth International Controlled Atmosphere Research Conference, Wenatchee, Washington, USA, pp. 255-261.

Knee, M., Smith, S.M., 1989. Variation in quality of apple fruits stored after harvest on different dates. J. Hortic. Sci. 64, 413-419.

Marmo, C.A., Bramlage, W.J., Weis, S.A., 1985. Effects of fruit maturity, size, and mineral concentrations on predicting the storage life of 'McIntosh' apples. J. Am. Soc. Hortic. Sci. 110, 499-502.

Mason, J.L., Drought, B.G., McDougald, J.M., 1974. Effect of a calcium chloride dip on senescent breakdown, firmness and calcium concentration in 'Spartan' apple. HortScience 9, 596.

McGlone, V.A., Kawano, S., 1998. Firmness, dry-matter and soluble solids assessment of postharvest kiwifruit by NIR spectroscopy. Postharvest Biol. Technol. 13, 131-141.

Peirs, A., Lammertyn, J., Ooms, K., Nicolai, B.M., 2000. Prediction of the optimal picking date of different apple cultivars by means of VIS/NIR-spectroscopy. Postharvest Biol. Technol. 21, 189-199.

Poovaiah, B.W., Glenn, G.M., Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hortic. Rev. 10, 107-152.

Raese, J.T., Drake, S.R., 1993. Effects of preharvest calcium sprays on apple and pear quality. J. Plant Nut. 16, 1807-1819.

Rambla, F.J., Garrigues, S., de la Guardia, M., 1997. PLS-NIR determination of total sugar, glucose, fructose and sucrose in aqueous solutions of fruit juices. Anal. Chim. Acta 344, 41-53.

Saftner, R.A., Conway, W.S., Sams, C.E., 1998. Effect of postharvest calcium chloride treatments on tissue water relations, cell wall calcium levels and postharvest life of 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 123, 893-897.

Sams, C.E., Conway, W.S., 1984. Effect of calcium infiltration on ethylene production, respiration rate, soluble polyuronide content, and quality of 'Golden Delicious' apple fruit. J. Am. Soc. Hortic. Sci. 109, 53-57.

Technicon, 1973. Potassium, calcium and magnesium are determined by atomic emission (K) and absorption (Ca and Mg) spectroscopy following digest in nitric acid. Industrial method 98/70w. Technicon, New York.

Twine, J.R., Williams, C.H., 1971. The determination of phosphorus in Kjeldahl digests of plant material by automated analysis. Commun. Soil Sci. Plant Anal. 2, 485-489.

Watkins, C.B., Hewett, E.W., Bateup, C., Gunson, A., Triggs, C.M., 1989. Relationships between maturity and storage disorders in 'Cox's Orange Pippin' apples as influenced by preharvest calcium or ethephon sprays. New Zeal. J. Crop Hortic. Sci. 17, 283-292.

Chapter 9 Characterisation of apple softening in controlled atmospheres.

9.1 Abstract

The effect of controlled atmosphere (CA) storage on the three phases of apple (*Malus domestica* Borkh.) softening is not known. This study sought to characterise the softening curve of the rapid softening 'Royal Gala' and 'Cox's Orange Pippin' apple cultivars stored in CA. Fruit in CA had a triphasic softening curve during storage similar to that of fruit in regular air atmosphere (RA). However, fruit in CA had a longer initial slow softening phase, and a slower rapid softening phase than fruit in RA. In addition, fruit in CA required more time to exceed an internal ethylene concentration (IEC) of 1.5 $\mu l.l^{-1}$, and had a lower maximum IEC than fruit in air. Thus, CA may extend the initial slow softening phase, and reduce softening in the rapid softening phase, by decreasing ethylene production in both cultivars. The longer the time in RA prior to transfer to CA, the softer the fruit were on transfer to CA, and the faster the softening rates subsequently were in CA. In a reciprocal experiment, duration of CA storage generally had a minimal effect on initiation and rate of rapid softening once transferred to RA storage at 0.5-3°C or 20°C. However, CA storage for 50 to 80 days temporarily induced a period of slow softening for 10 days when Cox's Orange Pippin were transferred to RA at 3°C, and a period of slow softening for 2 days when both cultivars were transferred to RA at 20°C. These results demonstrate that the softening curve for both COP and RG cultivars were similar for fruit maintained in both RA and in CA, and suggests that ethylene may play an important role in regulating the onset of rapid phase softening regardless of the storage atmosphere.

Keywords: Malus domestica (Borkh.); Firmness; Softening rate; Ethylene; CA; Temperature; Empirical modelling; Quality

9.2 Introduction

Controlled atmosphere (CA) storage is used to slow loss of postharvest quality in many fruits and vegetables. Pioneering studies on CA found that apples softened slower in CA than in a regular air atmosphere (RA) (Magness and Diehl, 1924; Kidd and West, 1936). However, in these and subsequent studies, firmness was usually measured 1 to 5

times only during storage, which is insufficient to accurately characterise the softening curve for apples in CA. Detailed knowledge about shape of the softening profile in CA could be used to identify critical times in CA when important physiological and biochemical changes occur that influence the storage potential of apples. In addition, characterisation of softening in CA could enable development of models for predicting firmness changes during postharvest handling.

'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) are important early season apple cultivars in New Zealand that soften rapidly after harvest. Softening of these cultivars was characterised in RA, and found to have an initial slow softening phase, followed by a phase of more rapid softening, and a final slow softening phase (Chapters 4 and 7). While this triphasic softening curve occurred in RA regardless of temperature (Chapter 4), fruit size or harvest maturity (Chapter 7), such factors did influence duration of the initial slow softening phase and rate of rapid phase softening. It is possible that softening in CA may also be triphasic for these cultivars, and like these other factors, CA may influence duration and/or the rate of softening in these phases.

Despite CA being effective in reducing apple softening, it is logistically difficult to maintain apples in CA throughout the entire postharvest handling chain. Freshly harvested apples are often stored in RA until a sufficient volume of fruit is obtained to fill a CA store. Once delivered to wholesale or retail outlets fruit are usually removed from CA. Thus the influence of time in RA before CA storage, and time in CA before RA storage, needs to be characterised in relation to the apple softening curve.

Reducing the time between harvest and establishment of CA conditions in storage improved poststorage firmness in several apple cultivars (Smock and Blanpied, 1963; Lau and Looney, 1982; Lau, 1983; Fica et al., 1985; Liu, 1986; Dilley et al., 1989), including COP (Sharples and Munoz, 1974). Furthermore, softening rates in CA increased as prior time in RA increased for 'McIntosh', 'Empire' (Dilley et al., 1989) and 'Golden Delicious' (Lau and Looney, 1982; Lau et al., 1984) apples. The softening rate of COP in CA also increased as preceding time in RA increased from 0 to 20 days, but then decreased as time in RA increased further to 40 and 80 days (Sharples and Munoz, 1974). In these studies firmness was measured 1-5 times only during storage,

making it difficult to determine the effect of time in RA on the subsequent shape of the apple softening curve in CA.

Limited information is available on the effect of time in CA on subsequent apple softening in RA. Softening rates of 'McIntosh' apples in RA at 0°C were similar after different times at 3.0%O_2:5.0%CO_2, but were slower after increased time at 1.0%O_2:1.5%CO_2 (Lidster, 1982). Further research is required to determine if time in CA influences the subsequent softening profile of apples in RA at both low and shelf-life temperatures.

It has been suggested that CA improves poststorage firmness in apples predominantly by reducing the biosynthesis and action of ethylene (Fica et al., 1985; Dilley et al., 1989), and/or by reducing the respiration rate (Beaudry, 1999). Apples in atmospheres with reduced O_2 concentrations were firmer, induced rapid ethylene production later, and had lower ethylene production after prolonged storage, than apples stored in air (Knee, 1980; Stow, 1989; Fan et al., 1997). Likewise, fruit in atmospheres with higher CO_2 concentrations were firmer and had lower IEC's than fruit in lower CO_2 concentrations (Ben-Arie et al., 1993). Increased time between harvest and establishment of CA conditions in storage reduced both poststorage firmness and time before induction of rapid ethylene production in several cultivars (Fica et al., 1985; Liu, 1986). Evidence for the role of ethylene in promoting apple softening was strengthened following studies using inhibitors of ethylene action, as softening was reduced in several apple cultivars when treated with 1-methylcyclopropene at harvest (Fan et al., 1999; Watkins et al., 2000). Thus, the effects of CA on loss of firmness in each phase of the apple softening curve may reflect changes in endogenous ethylene concentrations.

Despite extensive and numerous studies on the effects of CA on poststorage firmness in many apple cultivars, the effects of CA on softening in each of the three phases of apple softening is currently not known. This study sought to characterise the effect of constant CA, time in RA before CA storage, and time in CA before RA storage, on softening of the RG and COP apple cultivars. It was also determined if softening responses to CA and/or RA treatments were related to changes in IEC for these cultivars.

9.3 Materials and methods

9.3.1 Fruit supply, treatments and measurements

COP and RG were harvested from orchards in Hawkes Bay (RG) and Nelson (COP) at commercial maturity, then graded, packed and transported to Palmerston North, New Zealand, within 72 hours of harvest. Fruit sizes utilised were 160-180 g for RG, and 130-150 g for COP.

RG fruit were randomly allocated to each of the following treatments (120 fruit per treatment): constant RA at 0.5±0.5°C and 20.0±0.5°C; constant CA at 0.5±0.5°C; RA at 0.5±0.5°C for 7, 20, 50 and 80 days before transfer to CA at 0.5±0.5°C; and CA at 0.5±0.5°C for 7, 20, 50 and 80 days before transfer to RA at 0.5±0.5°C (70 fruit) and 20.0±0.5°C (40 fruit). The same treatments were used for COP, except that 3.0±0.5°C was used instead of 0.5±0.5°C.

CA treatments were performed using a flow-through system in 50 l plastic barrels. Atmospheres of 2.0±0.2% O_2 and 1.8±0.3% CO_2 were generated using cylinders of N_2 (O_2-free grade; B.O.C. Gases New Zealand Ltd) and CO_2 (food grade; B.O.C. Gases New Zealand Ltd), and compressed air. The barrels were purged with N_2 for 2 hours after sealing to rapidly reduce O_2 concentrations, after which the humidified (~95% RH) gas mixture was then applied at a flow-rate of 10 $ml.min^{-1}$ per barrel. Initially, 'Soda-lime' was required to reduce CO_2 concentrations, and 'Purafil' required to reduce ethylene concentrations, in barrels containing fruit recently transferred from air treatments (fruit with high respiration rates and IEC). Concentrations of O_2 and CO_2 were equilibrated in each barrel for about 18 hours after sealing, and concentrations were monitored together with ethylene concentration every 1-2 days.

Apples in RA were stored in perforated polyethylene bags (35 µm thickness; 50 x 5 mm diameter perforations per m^2) that in turn were packed into commercial cardboard cartons and placed into the coolstores containing the CA barrels. An additional RA control was stored in a barrel with similar conditions (temperature, RH and flow-rate) to those in CA barrels, except that only humidified air was passed through the barrel.

Softening and IEC in fruit from the barrel RA control was similar to fruit from the static RA control for both cultivars (data not shown), allowing direct comparison between fruit in flow-through CA and fruit in static RA.

Concentrations of CO_2 and O_2 were determined by injecting 1 ml headspace gas samples from each barrel into a gas chromatograph (Shimadzu GC-8A) fitted with a thermal conductivity detector (set at 60°C; current of 90 mA), a CTR1 column containing activated molecular sieve and porous polymer mixture (set at 30°C with H_2 as the carrier gas at 30 ml.min^{-1}), and a Hewlett Packard integrator (model 3390A) calibrated with external O_2 and CO_2 standards (certified as β-standard by B.O.C. Gases New Zealand Ltd). Ethylene concentrations were determined by injecting 1 ml headspace gas samples from each barrel into a gas chromatograph (Pye Unicam GCD) fitted with a flame ionisation detector (set at 140°C with H_2 and air flow rates of 30 ml.min^{-1} and 300 ml.min^{-1}, respectively), an activated alumina column (set at 100°C with N_2 as the carrier gas at 30 ml.min^{-1}), and a Hewlett Packard integrator (model 3390A) calibrated with external ethylene standards (certified as β-standard by B.O.C. Gases New Zealand Ltd).

For each treatment, flesh firmness and IEC were measured on 20 fruit at harvest, and subsequently on 10 fruit immediately before and after transfer to different atmospheres, and at 20-30 day intervals in RA fruit at 0.5-3°C, 2-5 day intervals in RA fruit at 20°C, and 30-50 day intervals in CA fruit. Flesh firmness and IEC were measured as previously described (Chapter 4.3).

9.3.2 Data analysis

Firmness (f, N) after different times (t, day) in constant RA and CA at 0.5-3°C, and constant RA at 20°C, were fitted with the following empirical sigmoidal function (Chapter 4.3) using non-linear regression:

$$f = f_{-\infty} - (f_{-\infty} - f_{+\infty}) \cdot \left(1 - \left(1 + \exp \frac{t + k^{-1} \cdot \ln(31) - (5.2 \cdot k^{-1.0168})}{k^{-1}} \right)^{-0.2} \right) \qquad \text{(Eq. 9-1)}$$

where model parameters were an initial firmness asymptote ($f_{-\infty}$, N), a minimum firmness asymptote ($f_{+\infty}$, N), and rate of firmness change (k, day^{-1}). Softening rates of

fruit in CA that had different times in RA were estimated using linear regression. Non-linear regression was performed using the NLIN procedure, and linear regression performed using the REG procedure, in the SAS statistical software (version 8.0, SAS Institute Inc, Cary, NC 27513, USA).

9.4 Results

Fruit in CA had a longer initial slow softening phase, and a slower rapid softening phase, than fruit in RA for both cultivars (Fig. 9-1). The rate of firmness change (k) was approximately 10 and 2 times lower in CA than in RA for COP and RG respectively (Table 9-1). Values of k for RG were consistently lower than for COP in both atmospheres, with k for RG in RA being similar to that for COP in CA.

The duration of the experiment was too short to accurately characterise the effect of CA on the final slow softening phase in either cultivar (Fig. 9-1). However, the softening curve for COP fruit in CA appeared to be converging with the final slow softening phase for fruit in RA after about 200 days at 3°C, with the final firmness reading in CA being only about 7N higher than that in RA. In contrast, RG fruit in CA had only softened by half of that in RA at the completion of the experiment (275 days), making it difficult to extrapolate the effect of CA on the final slow softening phase in this cultivar.

CA affected the rate at which IEC increased at the beginning of storage, and the maximum IEC attained thereafter, for RG fruit at 0.5°C and COP fruit at 3°C (Fig. 9-1). IEC of COP fruit in RA increased rapidly from a low concentration (<1 $\mu l.l^{-1}$) to 50 $\mu l.l^{-1}$ after 10 days at 3°C, whereas in CA, IEC remained <1 $\mu l.l^{-1}$ for about 20 days after which it increased rapidly to 40 $\mu l.l^{-1}$ after 60 days. CA also reduced the maximum IEC attained at 3°C for COP fruit, from 90 $\mu l.l^{-1}$ after 80 days in RA to 50 $\mu l.l^{-1}$ after 140 days in CA. Similarly for RG fruit, IEC exceeded 1 $\mu l.l^{-1}$ later in CA (60 days) than in RA (20 days), and the maximum IEC attained in fruit was lower in CA (20 $\mu l.l^{-1}$) than in RA (90 $\mu l.l^{-1}$).

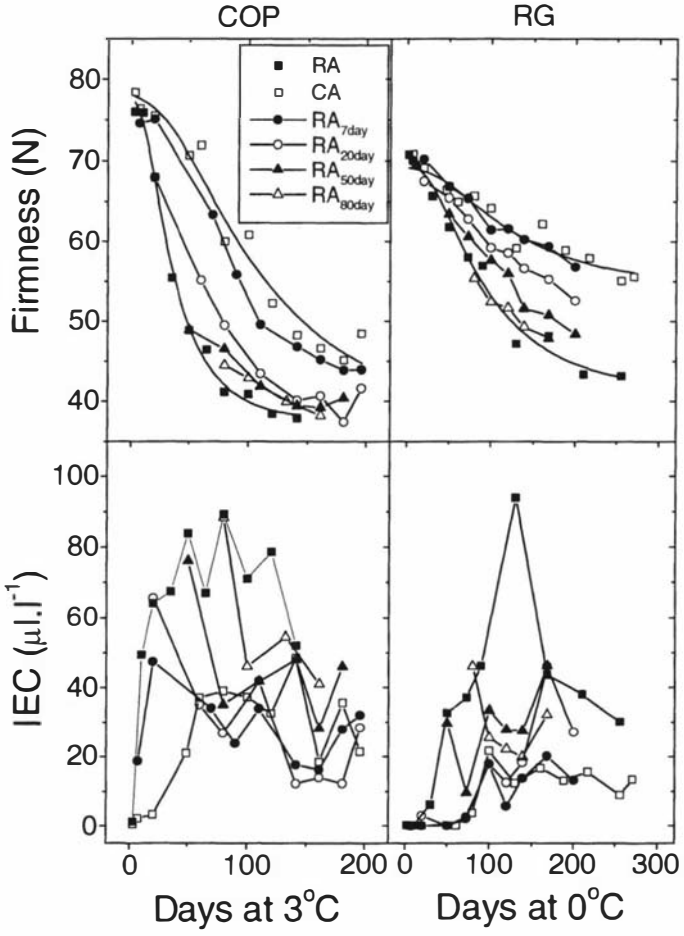

Fig. 9-1 Flesh firmness and internal ethylene concentration (IEC) of 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples at 0.5°C or 3°C respectively in regular atmosphere (RA), controlled atmosphere (CA), and in RA for 7, 20, 50 and 80 days before transfer to CA. CA conditions were 2.0%O_2:1.8%CO_2. Treatment means (n=10) are shown. Firmness data from continuous RA and CA treatments were fitted with Eq. 9-1 using non-linear regression.

Table 9-1 Rate of firmness change (k) in 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) in regular atmosphere (RA) and a controlled atmosphere (CA) of 2.0%O_2:1.8%CO_2 at 0.5°C or 3°C. Estimates of k and associated standard errors were obtained from non-linear regression analysis of firmness data (Fig. 9-1) with Eq. 9-1.

Cultivar	Temperature (°C)	Atmosphere	k (day^{-1})	
			Estimate	Standard error
COP	3	RA	0.560	0.147
COP	3	CA	0.055	0.002
RG	0.5	RA	0.066	0.007
RG	0.5	CA	0.033	0.003

The effectiveness of CA in reducing softening at 0.5-3°C was progressively reduced in fruit previously stored in RA at 0.5-3°C for increased time (Fig. 9-1). In both cultivars, fruit were softer at the beginning of CA storage, and thereafter in CA, as previous time in RA increased. COP and RG fruit that were transferred to CA after 0 and 7 days in RA were placed into CA when in the initial slow softening phase, while COP fruit transferred to CA after 20 days in RA, and RG fruit transferred to CA after 20 to 80 days in RA, were placed into CA after rapid phase softening phase had commenced. COP fruit that were transferred to CA after 50 and 80 days in RA, were placed into CA when in the final slow softening phase. Once in CA, the softening rates of COP fruit increased slightly as prior time in RA increased from 0 to 20 days, but then decreased thereafter as expected for fruit placed into CA when in the final slow softening phase (Fig. 9-2). Similarly, RG fruit in CA softened faster as prior time in RA increased from 0 to 50 days, but then slowed as prior time in RA increased to 80 days.

The commercial implications of time in RA before CA storage were determined by estimating time to soften to 65N (t_{65N}) for each prior RA treatment (Fig. 9-2). A reduction in time that COP fruit were in RA before transfer to CA from 20 days to 0 days increased t_{65N} from 28 days to 73 days. Similarly, reducing the time that RG fruit were in RA before transfer to CA from 38 days to 0 days increased t_{65N} from 39 to 76 days. Application of CA to COP fruit after 20 days in RA, and to RG after 40 days in RA, had no beneficial increase in t_{65N} over continuous RA storage.

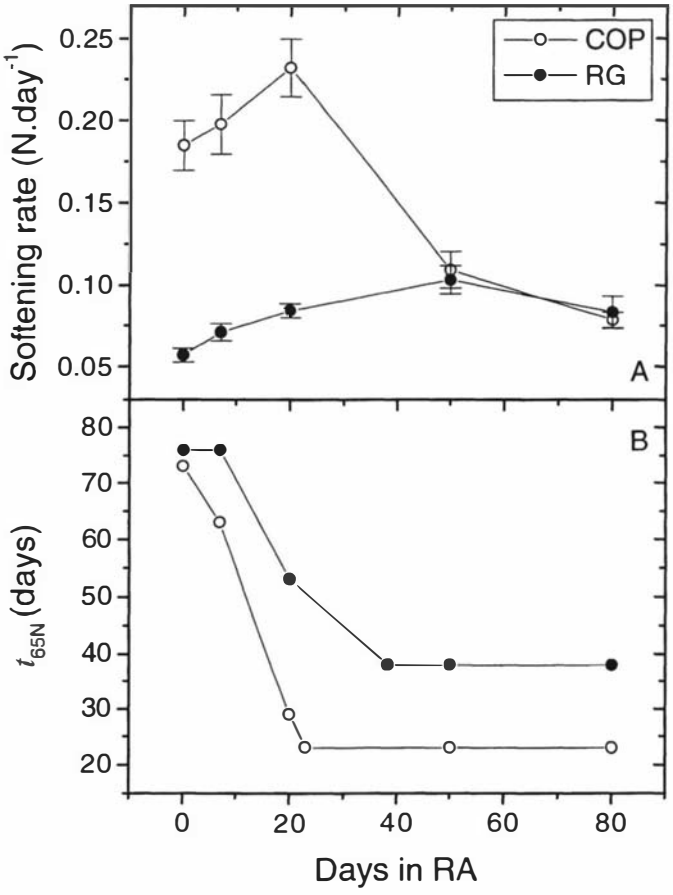

Fig. 9-2 Softening rates in controlled atmospheres (CA), and time to soften to 65N (t_{65N}), for 'Royal Gala' (RG) apples at 0.5°C and 'Cox's Orange Pippin' (COP) apples at 3°C after different times in regular atmosphere (RA) before transfer to CA. CA conditions were 2.0%O_2:1.8%CO_2. Softening rates and associated standard errors were estimated from linear regression of firmness data in CA, and t_{65N} estimated from firmness data in Fig. 9-1.

Time in RA also influenced IEC at the commencement of, and throughout CA storage (Fig. 9-1). Fruit from both cultivars had greater IEC's at the beginning of CA storage as prior time in RA increased. For COP fruit that previously had 20-80 days in RA, IEC decreased to approximately 40 $\mu l.l^{-1}$ once in CA, while IEC in CA increased by 30 $\mu l.l^{-1}$ for fruit that previously had 7 days in RA. For RG, IEC was low (<1 $\mu l.l^{-1}$) at the time of transfer to CA for fruit that had 0 and 7 days in RA, while fruit from longer preceding RA treatments were transferred to CA during the rapid increase in IEC that occurred in RA. The change in IEC through CA storage was similar for RG fruit that had 0 and 7 days in RA. IEC's of RG fruit from the longer RA treatments decreased by 5-20 $\mu l.l^{-1}$ once transferred to CA, but then attained greater maximum IEC's in CA than fruit that previously had less time in RA.

In the reciprocal experiment, time in CA slightly influenced subsequent softening of both cultivars once transferred to RA at 0.5-3°C (Fig. 9-3) and 20°C (Fig. 9-4). Once both cultivars were transferred from CA to RA, softening was initiated either immediately or was delayed 2-10 days. Rapid softening occurred immediately in RA at 0.5-3°C for COP fruit that had less than 50 days in CA, and RG fruit that had 0 to 80 days in CA (Fig. 9-3). In contrast, COP fruit that had 50 and 80 days in CA softened slowly in RA at 3°C for about 10 days, but then softened more rapidly than all other RA treatments. The softening rates of RG in RA at 0.5°C were similar regardless of prior CA treatment. COP fruit exposed to CA for 80 days, and RG fruit that had been in CA for 50 and 80 days, softened slowly for about 2 days once transferred to shelf life conditions in RA at 20°C (Fig. 9-4). In contrast, rapid softening was initiated immediately at 20°C for fruit exposed to CA for shorter times (7-20 days). Regardless of whether softening was delayed 1-2 days or initiated immediately on transfer from CA to RA, the subsequent softening rates of fruit at 20°C were similar.

The duration of CA storage also affected subsequent IEC's of both cultivars once transferred to RA storage at 0.5-3°C (Fig. 9-3) and 20°C (Fig. 9-4). Storage of both cultivars in CA for a longer time slightly increased the IEC of both cultivars at the time

of transfer to RA at 0.5-3°C and 20°C. Regardless of duration of CA storage, IEC then increased immediately to 80-100 $\mu l.l^{-1}$ for COP once transferred to RA at 3°C (Fig. 9-3), and to 400-500 $\mu l.l^{-1}$ for COP, and 250-350 $\mu l.l^{-1}$ for RG once transferred to RA at 20°C (Fig. 9-4). However, the longer that RG fruit were in CA before transfer to RA at 0.5°C, the lower the peak IEC attained; fruit with 0-20 days in CA had a peak IEC of 80-95 $\mu l.l^{-1}$, while those with 50 and 80 days in CA had peak IEC's of 40 and 20 $\mu l.l^{-1}$ in RA respectively (Fig. 9-3).

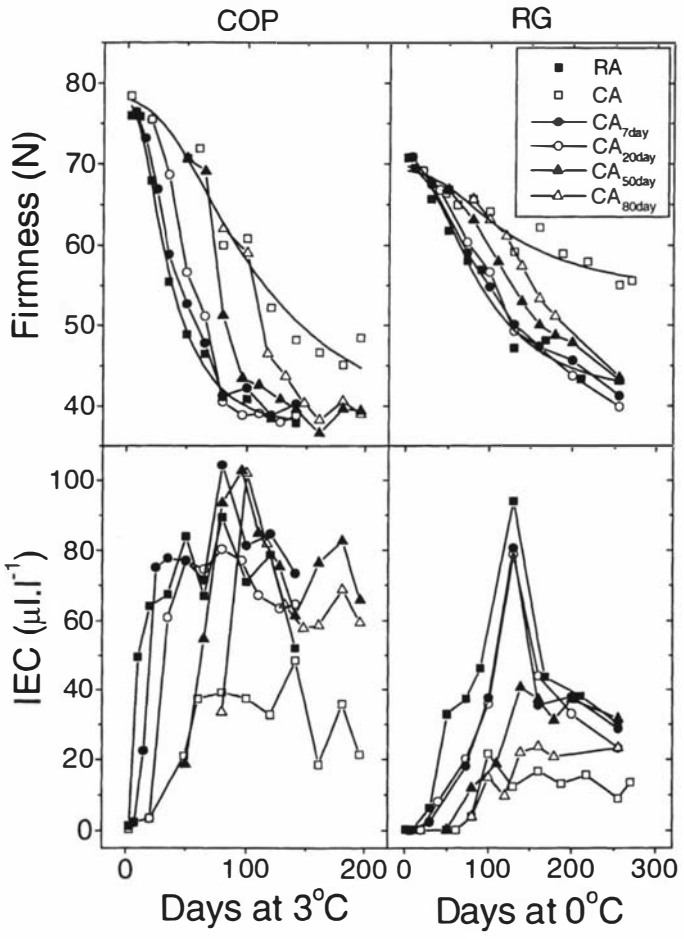

Fig. 9-3 Flesh firmness and internal ethylene concentration (IEC) of 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples at 0.5°C or 3°C respectively in regular atmosphere (RA), controlled atmosphere (CA), and in CA for 7, 20, 50 and 80 days before transfer to RA at 0.5°C or 3°C. CA conditions were 2.0%O_2:1.8%CO_2. Treatment means (n=10) are shown. Firmness data from continuous RA and CA treatments were fitted with Eq. 9-1 using non-linear regression.

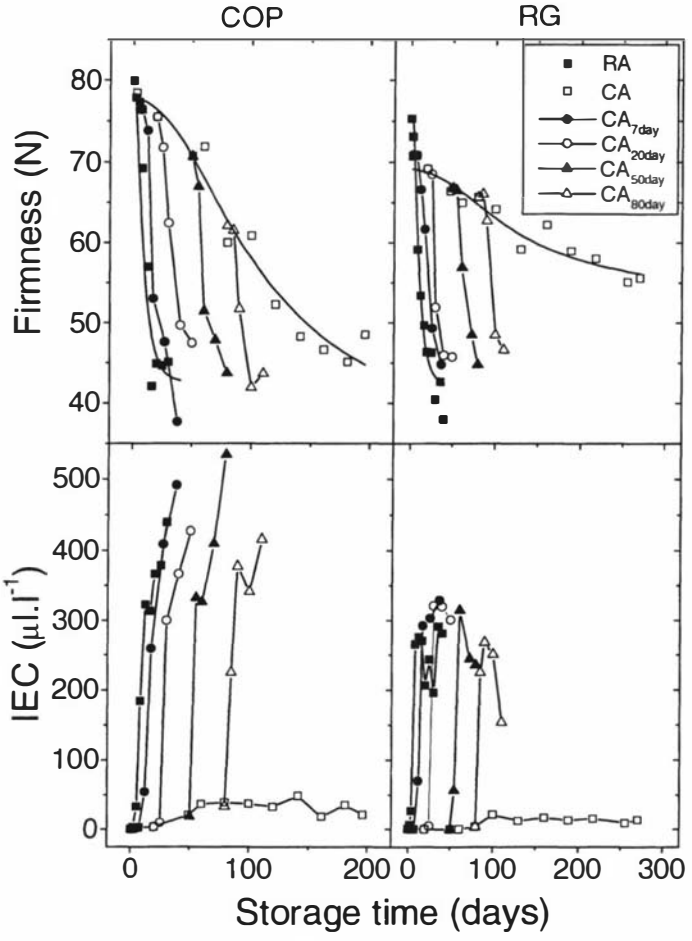

Fig. 9-4 Flesh firmness and internal ethylene concentration (IEC) of 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples at 0.5°C or 3°C respectively in regular atmosphere (RA), controlled atmosphere (CA), and in CA for 7, 20, 50 and 80 days before transfer to RA at 20°C. CA conditions were 2.0%O_2:1.8%CO_2. Treatment means (n=10) are shown. Firmness data from continuous RA and CA treatments were fitted with Eq. 9-1 using non-linear regression.

9.5 Discussion

Softening of RG and COP apples in CA was characterised, and found to have a similar triphasic softening curve to that previously found in RA for these cultivars (Chapters 4 and 7). However, apples in CA had a longer initial slow softening phase, and slower rapid softening phase, than fruit in RA. Firmness data from Lau and Looney (1982)

indicated that 'Golden Delicious' fruit in CA also had a longer initial slow softening phase, and slower rapid softening phase, than fruit in RA. This effect also occurred in other fruits such as kiwifruit (McDonald and Harman, 1982; Arpaia et al., 1984) and avocados (Meir et al., 1995).

The mechanism by which CA slowed softening of RG and COP apples at 0.5-3°C was probably mediated by ethylene, as apples from both cultivars had a slower increase in IEC, and a lower maximum IEC in CA than in RA. Ethylene probably promotes apple softening, as softening was reduced in apples treated with inhibitors of ethylene action at harvest (Fan et al., 1999; Watkins et al., 2000). It has been suggested that if the IEC of apples are maintained below 0.1 $\mu l.l^{-1}$ (Stow et al., 2000), and external ethylene concentrations are below 1 $\mu l.l^{-1}$ (Liu, 1977), then apple softening is reduced in CA. In previous softening studies on RG and COP in RA, onset of rapid phase softening in both cultivars was associated with IEC exceeding 1.5 $\mu l.l^{-1}$, regardless of temperature (Chapter 4) or harvest date (Chapter 7). A similar response occurred for both RG and COP in CA, as both duration of the initial slow softening phase, and time before IEC exceeded 1.5 $\mu l.l^{-1}$, were longer for fruit in CA than in RA. Thus, an important effect of ethylene in these apple cultivars may be to initiate rapid phase softening under both RA and CA conditions. Stow et al. (2000) found that ethylene appeared to initiate but not regulate the rate of COP softening in CA. Thus, a slower rapid softening phase in CA than in RA could be due a reduced respiration rate and/or reduced activity of cell wall degrading enzymes, rather than a direct effect of reduced IEC (Siddiqui et al., 1996; Beaudry, 1999).

Lower IEC's for RG and COP fruit in CA than in RA, could be due to the antagonistic effects of low O_2 and elevated CO_2 on ethylene biosynthesis and action. CO_2 is a competitive inhibitor of ethylene action, and O_2 is required for ethylene action to progress (Burg and Burg, 1967). Preclimacteric and climacteric 'Golden Delicious' apples stored in elevated CO_2 ($17\%O_2$:$20\%CO_2$) or low O_2 ($0.25\%O_2$:$0\%CO_2$) had lower ethylene production and reduced expression of the key regulatory ethylene biosynthetic enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, when compared to fruit in RA (Gorny and Kader, 1997). Expression of ACC oxidase, an

enzyme essential for the final step in ethylene biosynthesis, was also lower in these CA treatments than in RA, although it was not reduced to a level considered rate limiting for ethylene biosynthesis in apples (Gorny and Kader, 1997). Apples stored at lower CO_2 concentrations (<10%) had more ACC oxidase activity than those at higher CO_2 concentrations (Levin et al., 1993), and CO_2 increased the activity of ACC oxidase extracts from apple fruit when included in assays for this enzyme (Poneleit and Dilley, 1993). Thus, reduced IEC of RG and COP fruit in CA was more likely due to a reduction in ACC synthase activity, rather than a change in ACC oxidase activity.

Reduced softening of RG and COP apples in CA compared with RA at 0.5-3°C was probably due to slower disassembly of cell walls. 'Golden Delicious' apples in CA were firmer, and had slower loss of total pectin and hemicellulose from the cell wall, than fruit in RA (Siddiqui et al., 1996). Furthermore, the increase in free pectin and decrease in covalently bound pectin in the cell wall of 'Golden Delicious' fruit was slower in CA than in RA (Siddiqui et al., 1996). Tijskens (1979) suggested that loss of texture consisted of rapid degradation of some cellular components in both CA and RA, slower degradation of a second component in CA than in RA, and the slow decay of a third component in both CA and RA. It was subsequently suggested that some of these components could be different forms of pectin, which may degrade in either the presence or absence of oxygen (Tijskens et al., 1999). More research is required to determine what biochemical changes occur in the cell wall in relation to the three phases of softening for apples in CA and RA.

The shorter the time that fruit were maintained in RA before transfer to CA the firmer they were at the beginning of, and throughout CA storage. This was reflected in the market life (t_{65N}) of fruit in CA being reduced by about 2 days for COP, and 1 day for RG, with each additional day in RA at 0.5-3°C before CA storage. Poststorage firmness was also greater in several other apple cultivars when CA conditions were established rapidly after harvest (Smock and Blanpied, 1963; Lau and Looney, 1982; Lau, 1983; Fica et al., 1985; Liu, 1986; Dilley et al., 1989). However, when COP were stored in ultra-low O_2 (1.25%) CA, rapid establishment of CA did not improve poststorage firmness when compared to an establishment delay of 10-15 days (Stow, 1986; Stow and Genge, 1990). Stow (1986) suggested that long-term storage of COP in ultra-low

O_2 (1.25%) may nullify the effect of rate of CA establishment after harvest, as ultra-low CA was more effective in retaining firmness than atmospheres (O_2 > 1.5%) used in the studies referred to above. Softening of 'McIntosh' apples was completely inhibited in ultra-low CA (Dewey and Bourne, 1982), indicating that ultra-low CA may inhibit or substantially delay the onset of rapid phase softening.

Softening rates in CA varied depending on the phase of softening at the time of transfer from RA to CA. RG and COP fruit transferred to CA after 0 and 7 days in RA were in the initial slow softening phase when placed in CA, and subsequently softened slower in CA than fruit that had previously been in RA for longer time. COP fruit transferred to CA after 20 days in RA, and RG fruit transferred to CA after 20 to 80 days in RA, were placed in CA when in the rapid softening phase, and subsequently softened faster in CA than fruit that had previously been in RA for less time. COP fruit transferred to CA after 50 and 80 days in RA, were placed in CA when in the final slow softening phase, and as expected had the slowest softening rates in CA. Similar results were found in another study on COP, where rate of softening in CA increased as the preceding time in RA increased from 0 to 20 days, and then decreased as time in RA increased further to 40 and 80 days (Sharples and Munoz, 1974). The softening rates of 'Golden Delicious' (Lau and Looney, 1982), 'McIntosh' and 'Empire' (Dilley et al., 1989) cultivars in CA also increased when previously stored in RA for increased time. From a commercial perspective, these results indicate that maximum firmness benefits are attained from CA when applied to fruit in the initial slow softening phase, with minimal firmness benefits attained if CA is applied in the later rapid softening phase.

The effect of increasing time in RA on subsequent softening rates in CA may have also been mediated by ethylene, as the longer the fruit were in RA before transfer to CA, the greater the IEC's were at commencement of CA storage. Rapid establishment of CA conditions after harvest also reduced ethylene production and softening in 'McIntosh' (Fica et al., 1985; Liu, 1986; Dilley et al., 1989) and 'Golden Delicious' (Lau and Looney, 1982) apples, and reduced the increase of ACC in CA (Lau et al. 1984; Fica et al. 1985). Thus, improved retention of firmness by rapid establishment of CA conditions after harvest may be mediated by a reduction in ethylene biosynthesis in CA (Lau and Looney, 1982; Fica et al., 1985; Liu, 1986) the consequence of which is to

extend the initial slow softening phase and slow loss of firmness in the rapid softening phase.

CA storage for 50-80 days had a slight residual effect on softening of COP when transferred to RA at 3°C, and for both cultivars when transferred to RA at 20°C. A short period (1 to 3 days) of slow softening also occurred for pears after transfer from CA to RA at 18°C, with the period of slow softening increasing from 1 to 3 days as O_2 concentration in the prior CA treatment decreased from 21% to 0.5% (Stow, 1984). In contrast, 'McIntosh' apples had no period of slow softening in RA at 0°C immediately after transfer from 1.0%O_2:1.5%CO_2 nor 3.0%O_2:5.0%CO_2 (Lidster, 1982). Once rapid softening was initiated in COP and RG in RA at 0-3°C and 20°C, softening rates were similar regardless of prior time in CA. This also occurred for 'McIntosh' apples in RA at 0°C after different times in 3.0%O_2:5.0%CO_2, although increased time in 1.0%O_2:1.5%CO_2 slowed the subsequent softening rates of this cultivar in RA at 0°C (Lidster, 1982). In contrast, the softening rates of kiwifruit in RA at 0°C were faster when previously stored in CA for increased time (Arpaia et al., 1984). The residual effects of CA on reduction of respiration and ethylene production of strawberries in RA were more pronounced when O_2 concentrations decreased and CO_2 concentrations increased in CA treatments (Li and Kader 1989). Thus, storage of COP and RG in ultra-low O_2 (<1.25% O_2) may invoke a more pronounced residual effect on subsequent softening in RA, than was seen herein for these cultivars in 2.0%O_2:1.8%CO_2.

In summary, it has been demonstrated that softening of COP and RG was triphasic in CA, similar to that in RA. CA improved firmness retention in both cultivars by extending duration of the initial slow softening phase, and slowing the rate of rapid phase softening. This effect may have been mediated by ethylene, as CA also delayed the onset of rapid ethylene production, and the maximum ethylene concentration attained at 0.5-3°C. The effectiveness of CA in reducing softening and ethylene production was less pronounced when previously stored in RA for increased time. Results from this study demonstrated that a similar softening pattern occurred in both RA and CA, and that ethylene may have an important role in regulating the onset of rapid phase softening of both cultivars in either atmosphere. It should also be possible

to use these results to develop models that predict or describe the effects of CA on softening rates of these cultivars during postharvest handling.

9.6 References

Arpaia, M.L., Mitchell, F.G., Mayer, G., Kader, A.A., 1984. Effects of delays in establishing controlled atmospheres on kiwifruit softening during and following storage. J. Am. Soc. Hortic. Sci. 109, 768-770.

Ben-Arie, R., Levin, A., Sonego, L., Zutkhi, Y., 1993. Differential effects of CO_2 at low and high O_2 on the storage quality of two apple cultivars. Acta Hortic. 326, 165-174.

Beaudry, R.M., 1999. Effect of O_2 and CO_2 partial pressure on selected phenomena affecting fruit and vegetable quality. Postharvest Biol. Technol. 15, 293-303.

Burg, S.P., Burg, E.A., 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42, 144-152.

Dewey, D. H., Bourne, M.L., 1982. Low oxygen CA storage of McIntosh apples. Proc. Third Nat. CA Res. Conf., 22-24 July 1981, Oregon State University, USA, pp. 101-107.

Dilley, D.R., Lange, E., Tomala, K., 1989. Optimizing parameters for controlled atmosphere storage of apples. Proc. Fifth Int. CA Res. Conf., 14-16 June 1989, Wenatchee, Washington, USA, pp. 221-236.

Fan, X., Blankenship, S.M., Mattheis, J.P., 1999. 1-Methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124, 690-695.

Fan, X., Mattheis, J.P., Patterson, M., Fellman, J.K., 1997. Optimum harvest date and controlled atmosphere storage potential of 'Fuji' apples. Proc. Seventh Int. CA Res. Conf., July 13-18 1997, University of California, Davis, USA, pp. 42-49.

Fica, J., Skrzynski, J., Dilley, D.R., 1985. The effect of delayed cooling and delayed application of CA storage of McIntosh apples under low and high ethylene levels. Proc. Fourth Nat. CA Res. Conf., 23-26 July 1985, Raleigh, North Carolina, USA, pp. 82-94.

Gorny, J.R., Kader, A.A., 1997. Low oxygen and elevated carbon dioxide atmospheres inhibit ethylene biosynthesis in preclimacteric and climacteric apple fruit. J. Am. Soc. Hortic. Sci. 122, 542-546.

Kidd, F., West, C., 1936. Gas storage of fruit IV. Cox's Orange Pippin apples. J. Pomol. Hortic. Sci. 14, 276-294.

Knee, M., 1980. Physiological responses of apple fruits to oxygen concentrations. Ann. Appl. Biol. 96, 243-253.

Lau, O.L., 1983. Storage responses of four apple cultivars to a rapid CA procedure in commercial controlled-atmosphere facilities. J. Am. Soc. Hortic. Sci. 108, 530-533.

Lau, O.L., Liu, Y., Yang, S.F., 1984. Influence of storage atmospheres and procedures on 1-aminocyclopropane-1-carboxylic acid concentration in relation to flesh firmness in 'Golden Delicious' apple. HortScience 19, 425-426.

Lau, O.L., Looney, N.E., 1982. Improvement of fruit firmness and acidity in controlled atmosphere stored 'Golden Delicious' apples by a rapid O_2 reduction procedure. J. Am. Soc. Hortic. Sci. 107, 531-534.

Levin, A., Sonego, L., Zutkhi, Y., Ben-Arie, R., 1993. Effects of CO_2 on ethylene production by apples at low and high O_2 concentrations. In: Pech, J.C., Latche, A. , Balague, C. (Eds.), Cellular and molecular aspects of the plant hormone ethylene. Kluwer Academic Publishers, Dordrecht, pp. 150-151.

Li, C., Kader, A.A., 1989. Residual effects of controlled atmospheres on postharvest physiology and quality of strawberries. J. Am. Soc. Hortic. Sci. 114, 629-634.

Lidster, P.D., 1982. Low oxygen atmospheres to maintain apple quality in storage. Proc. Third Nat. CA Res. Conf., 22-24 July 1981, Oregon State University, pp. 109-120.

Liu, F.W., 1977. Varietal and maturity differences of apples in response to ethylene in controlled atmosphere storage. J. Am. Soc. Hortic. Sci. 102, 93-95.

Liu, F.W., 1986. Effects of delayed cooling and delayed low-ethylene CA storage on the keeping quality of 'McIntosh ' apples. J. Am. Soc. Hortic. Sci. 111, 719-723.

Magness, J.R., Diehl, H.C., 1924. Physiological studies on apples in storage. J. Agric. Res. 27, 1-38.

McDonald, B., Harman, J.E., 1982. Controlled atmosphere storage of kiwifruit. I Effect on fruit firmness and storage life. Sci. Hortic. 17, 113-123.

Meir, S., Akerman, M., Fuchs, Y., Zauberman, G., 1995. Further studies on the controlled atmosphere storage of avocados. Postharvest Biol. Technol. 5, 323-330.

Poneleit, L.S., Dilley, D.R., 1993. Carbon dioxide activation of 1-aminocyclopropane-1-carboxylate (ACC) oxidase in ethylene biosynthesis. Postharvest Biol. Technol. 3, 191-199.

Sharples, R.O., Munoz, G.C., 1974. The effects of delays in the period taken to cool and establish low oxygen conditions on the quality of stored Cox's Orange Pippin apples. J. Hortic. Sci. 49, 277-286.

Siddiqui, S., Brackmann, A., Streif, J., Bangerth, F., 1996. Controlled atmosphere storage of apples: cell wall composition and fruit softening. J. Hortic. Sci. 71, 613-620.

Smock, R.M., Blanpied, G.D., 1963. Some effects of temperature and rate of oxygen reduction on the quality of controlled atmosphere stored McIntosh apples. Proc. Am. Soc. Hortic. Sci. 83, 135-138.

Stow, J., 1984. The controlled atmosphere storage of Conference pears. J. Hortic. Sci. 59, 507-513.

Stow, J., 1986. Effects of rate of establishment of storage conditions and ethylene removal on the storage performance of 'Cox's Orange Pippin' apples. Sci. Hortic. 28, 369-378.

Stow, J.R., 1989. The response of apples cv. Cox's Orange Pippin to different concentrations of oxygen in the storage atmosphere. Ann. Appl. Biol. 114, 149-156.

Stow, J., Dover, C.J., Genge, P.M., 2000. Control of ethylene biosynthesis and softening in 'Cox's Orange Pippin' apples during low-ethylene, low-oxygen storage. Postharvest Biol. Technol. 18, 215-225.

Stow, J.R., Genge, P.M., 1990. Low-ethylene storage of apples cv. 'Cox's Orange Pippin': effects of 'Rapid CA' and ultra-low oxygen. Ann. Appl. Biol. 117, 673-681.

Tijskens, L.M.M., 1979. Texture of Golden Delicious apples during storage. Lebensm. Wiss. Technol. 12, 138-142.

Tijskens, L.M.M., Van Schaik, A.C.R., de Jager, A., Hertog, M.L.A.T.M., 1999. Modelling the firmness of 'Elstar' apples during storage and transport. Acta. Hortic. 485, 363-371.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Chapter 10 General discussion.

Consumers are increasingly demanding apples that have good texture, flavour, appearance and nutritional value, and are free from disorders, defects and pesticide residues. Texture is regarded as a quality attribute of primary importance in apples, where consumers often demand apples that are crisp and crunchy, and not dry or mealy. Consequently, markets are imposing stringent firmness standards on apple producers. However, the rapid softening characteristic of commercially important cultivars such as 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP), make it difficult for producers to meet firmness specifications. Failure to meet these standards can result in shipment rejections, reduced returns to growers, and a damaged reputation as a producer of top quality apples. The quality perception of these cultivars is of particular importance to New Zealand growers, as these cultivars are early maturing and often "set the scene" in the marketplace for cultivars to follow. Thus for New Zealand growers to maintain a pre-eminent position in an extremely competitive and congested world apple market, knowledge is required on the relative influence of different pre- and postharvest factors on firmness of fruit in the marketplace.

10.1 Change in softening rates with time.

Given that rapid softening of RG and COP is a serious commercial problem, it is surprising that the softening curve for these cultivars, and apples in general, has not been previously characterised. This is probably due to most studies being interested in the firmness before and after storage, and either not being interested in determining the softening rates of apples through storage, or implicitly assuming that loss of firmness during storage is linear. The research presented herein is the first study that has characterised the softening curves for several commercially important apple cultivars in New Zealand. For the first time this research has allowed:

- quantification of the relative influence of different at harvest and postharvest factors on the market life of apples in relation to firmness standards utilised in international markets (section 10.2)
- accurate comparisons between rapid and slow softening cultivars for softening responses to different temperatures (sections 10.3 and 10.5)

- identification of critical times during storage when important physiological and biochemical events occur that influence softening (i.e. ethylene; sections 10.4 and 10.6)
- development of a prototype mathematical model that can describe softening of apples as affected by temperature during the postharvest handling chain (section 10.7).

Each of these aspects will be discussed in more detail in the indicated sections.

This research clearly indicated that harvested apples had a triphasic softening curve at 0-5°C (Chapters 4, 7 and 9), with an initial slow softening phase (I), a second phase of more rapid softening (II), and a third and final slow softening phase (III) (Fig. 10-1). The shape of the softening curve for apples (Fig. 10-1) had some similarities to that of several melon varieties (Aggelis et al., 1997), tomatoes (Sozzi et al., 1998) and early harvested kiwifruit (MacRae et al., 1989), as all these fruits had three phases of softening. However, most of these fruits are melting-type fruits and soften by 75-100% during ripening (MacRae et al., 1989), while apples only soften by 25-50% so that the final slow softening phase occurs at 38-60 N rather than at 5-10 N. Other fruits such as quinces, cranberries, and non-melting pear and peach cultivars are also considered partially ripening fruits (Bourne, 1979; Karakurt et al., 2000). In contrast to apples, melons, tomatoes and early harvested kiwifruit, the softening curves of late harvested kiwifruit (MacRae et al., 1989), pears (Bourne, 1968) and nectarines (King et al., 1989) appeared biphasic with no discernible initial slow softening phase. However, it is possible that these fruits also had triphasic softening curves, and that the initial slow softening phase could have occurred on the tree before harvest as was found for apples harvested at a late maturity (Chapter 7). A study such as that undertaken for apples in Chapter 7 could determine if pears and nectarines also have an initial slow softening phase on the tree, and if this phase occurs postharvest when fruit are harvested at an early maturity.

The softening profile of apples was influenced by fruit temperature at the time of firmness measurement. Apples were physically firmer at harvest, and physically softer after prolonged storage, when firmness was measured at a fruit temperature of 20°C instead of 0-3°C (Chapter 3). This meant that the softening curve for fruit stored at 0-

3°C was triphasic when the fruit temperature was 0-3°C at the time of firmness measurement, but was biphasic with no discernible initial slow softening phase, when the fruit temperature was 20°C at the time of firmness measurement. Apple firmness is usually measured when the fruit are equilibrated to a standard temperature of 20°C, which could explain why the initial slow softening phase has not been reported previously for apples stored at low temperatures.

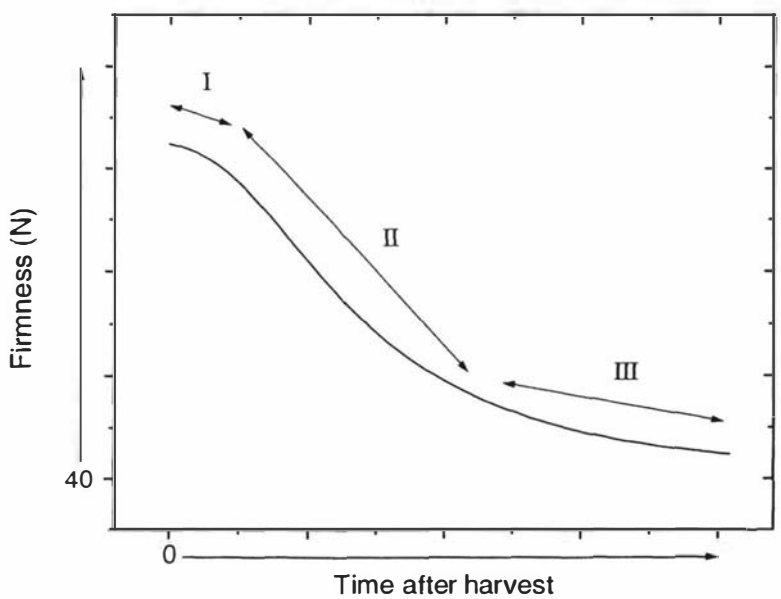

Fig. 10-1 A typical softening profile for apple fruit at 0-5°C.

10.2 Influence of postharvest and at-harvest factors on the apple softening profile.

The influence of preharvest, at harvest, and postharvest factors on firmness before and after storage has been studied extensively for apples (Harker et al., 1997; DeEll et al., 1999; Sams, 1999). However, until softening curves for apples were determined as in this research, it was not previously possible to accurately quantify the relative importance of each factor on the market life of apples. It was also not possible to qualitatively determine times during storage when individual factors exerted their influence on postharvest softening rates and hence the market life of apples.

An interesting aspect of determining the softening curves of RG and COP apples in relation to different at- and post-harvest factors was that regardless of the factor studied, the softening curve was always triphasic. However, within each curve, individual factors affected firmness at the beginning of storage, duration of the initial slow softening phase, rate of rapid phase softening, and/or the firmness at which the minimum firmness asymptote occurred. Delaying the harvest date reduced both firmness at harvest, and duration of the subsequent initial slow softening phase at low and shelf-life temperatures (Chapter 7). Maturity differences at harvest could also largely explain differences in softening rates between fruit from different orchards (Chapter 8). Fruit size had no effect on firmness at harvest, or on the subsequent softening profile at 0.5-3°C, when fruit were harvested at early and mid maturity (Chapter 7). However, small fruit picked at a late maturity had a longer initial slow softening phase (both RG and COP) and a slower rapid softening phase at 0.5-3°C (COP only) than larger fruit picked at the same maturity. Increasing the storage temperature from 0°C to 22°C progressively reduced duration of the initial slow softening phase, and increased the rate of rapid phase softening (Chapter 4). However, rate of rapid phase softening then decreased as temperature increased from 22°C through 35°C, with the rate of softening at 35°C being similar to that at 12°C. Controlled atmospheres (CA) had a similar effect to low temperatures, where CA extended the initial slow softening phase and reduced the rate of rapid phase softening at 0.5-3°C (Chapter 9).

The time fruit were maintained at ambient temperatures before cooling to 0.5-3°C (Chapter 6), and the time fruit were stored in air at 0.5-3°C before establishment of CA (Chapter 9), both influenced the subsequent softening profiles of RG and COP. A longer delay before cooling to 0.5-3°C or establishing CA, reduced firmness at the beginning of storage and shortened the subsequent initial slow softening phase. The rate of rapid phase softening at 0.5-3°C was slightly slower after longer cooling delays than after rapid cooling. In contrast, the longer that fruit was in air at 0.5-3°C prior to transfer to CA at the same temperature, the faster the rate of rapid phase softening in CA. Thus, the effect of delayed cooling on firmness slowly diminished during storage, while the effect of delayed CA became more pronounced.

The relative influences of harvest maturity, storage temperature and atmosphere on the market life of RG and COP apples are summarised in Table 10-1. Market life is defined as the time to soften to 65 N (t_{65N}), and was determined from softening data using a sigmoidal equation. Benefits in t_{65N} were obtained for both cultivars when the storage temperature was reduced from 20°C to 0°C. However, the benefit of storage at 0.5-3°C was progressively reduced with increasing delays between harvest and cooling, with the market life being reduced by 10-30% for every additional day at 20°C before cooling. Utilisation of CA at 0.5-3°C was also extremely effective at increasing t_{65N} in both cultivars. But like delayed cooling, the benefit of CA in increasing the market life of both cultivars was reduced by 2-3% with each additional day in air at 0.5-3°C before CA storage, and any benefit disappeared if the delay into CA was longer than 30 days.

Harvest date also influenced t_{65N}, with the market life being reduced by 1-5% with each additional day on the tree before harvest. These results confirm the importance of harvesting these cultivars early in the commercial harvest period to maximise firmness at harvest and after storage. However, while harvesting the fruit at an early maturity is beneficial for firmness retention, this stage of maturity could have adverse effects on other aspects of quality, including reduced red colouration of the skin, increased incidence of certain storage disorders, and reduced flavour (Blankenship, 1987; Kader, 1999). Thus, the choice of harvest maturity is a compromise between different aspects of fruit quality. Interestingly, the reduction in t_{65N} from delaying the harvest by 10 days was similar to the reduction in market life that occurred following a cooling delay of 1 day after harvest (Table 10-1). Thus, while it is important to optimise the storage potential of the fruit by harvesting at an early maturity, this true potential will not be realised unless the fruit are rapidly cooled to 0.5-3°C and placed in CA.

The relative effectiveness of different pre-, at- and post-harvest factors on the market life of apples, as depicted in Table 10-1, has not been presented in this form previously. This is because most previous studies only measured firmness a limited number of times during storage, making it difficult to estimate the time required to soften to a specified firmness. Furthermore, these different factors were often studied individually, and fruit from these different studies were often stored for different times before assessment of firmness, making it difficult to accurately estimate the relative influence of each factor

on the poststorage firmness of a particular apple cultivar. Despite this inability to accurately quantify the relative influence of different factors, it is generally accepted that harvesting fruit at an early to mid maturity, rapidly cooling fruit after harvest, and immediately placing fruit into CA storage following cooling, are all important commercial practises for firmness retention in apples (Blanpied, 1985; Fica et al., 1985; Dilley et al., 1989). It should also be noted that these practises are not restricted to apples, and that these techniques have been adopted in several other horticultural crops to maintain postharvest quality.

Table 10-1 Comparison of the effects of different at harvest and postharvest factors on the change in market life[1] of 'Royal Gala' and 'Cox's Orange Pippin' apples.

Change in at harvest or postharvest factor		Relative market life[2]	
Factor	Change	'Royal Gala'	'Cox's Orange Pippin'
Harvest date	Early harvest and stored in air at 0.5-3°C[3]	1.0	1.0
	Harvest delayed by 10 days	0.8-0.9	0.6-0.8
	Harvest delayed by 20 days	0.6	0.5-0.6
	Harvest delayed by 30 days	0.3	0.0-0.3
Temperature	Storage temperature of 0.5°C	1.0	1.0
	Storage temperature of 2.5°C	0.8	0.7
	Storage temperature of 5°C	0.7	0.4
	Storage temperature of 12°C	0.4	0.2
	Storage temperature of 20°C	0.1	0.1
	1 day at 20°C before storage at 0.5-3°C	0.8	0.8
	2 days at 20°C before storage at 0.5-3°C	0.5	0.6
	4 days at 20°C before storage at 0.5-3°C	0.2	0.4
Atmosphere	Air (0.5-3°C)	1.0	1.0
	CA (2.0%O_2:1.8%CO_2 at 0.5-3°C)	2.0	3.2
	7 day delay in air at 0.5-3°C before CA	2.0	2.7
	20 day delay in air at 0.5-3°C before CA	1.4	1.3

Abbreviations: controlled atmospheres (CA).
[1]Time to soften to a firmness of 65 N (minimum firmness accepted in most markets).
[2]Reduction (<1.0) or increase (>1.0) in market life relative to optimum market life in air at 0.5-3°C.
[3]'Cox's Orange Pippin' were stored at 3°C, while 'Royal Gala' were stored at 0.5°C.

Results in this thesis indicated that the market life of apples, when based solely on firmness, was largely determined by duration of the initial slow softening phase. Firmness benefits attained from harvesting fruit at an early maturity (Chapter 7), by CA storage (Chapter 9), and by cooling the fruit rapidly after harvest (Chapter 6), were largely achieved by extending this phase of softening. However, once fruits had initiated rapid phase softening in air storage, the application of CA could not reduce the rate of softening in this phase. Similarly, despite an inhibitor of ethylene of ethylene action being effective in reducing softening when applied at harvest, this same inhibitor could not reduce softening when applied to actively softening fruit (Blankenship and Sisler, 1993a). This suggests that it becomes increasingly difficult to maintain the firmness of apples once rapid phase softening has been initiated, and that most effort should be directed at preventing the onset of rapid phase softening by extending the initial slow softening phase. This effect is probably due to the rapid softening phase being driven by system II ethylene production and the associated climacteric; a stage of development that occurs once, is irreversible, and difficult to slow once initiated. Thus, the most effective methods of increasing the market life of apples for firmness are often technologies that maintain the fruit in a preclimacteric stage of development for longer time (Fica et al., 1985; Dilley et al., 1989). Further discussion on the potential role of ethylene in regulating softening of apples is located in section 10.4.

10.3 Differential cultivar responses differences to temperature.

This research has shown for the first time that apple cultivars have at least three different responses in relation to softening at different temperatures (Chapters 4 to 6). Softening of RG and COP at 0 to 35°C could be described by a modified Arrenhius equation, where the rate of softening increased from 0 to 22°C, and decreased thereafter through 35°C (Chapter 4). In contrast, 'Granny Smith' (GS) and 'Pacific Rose™' (PR) softened at similar rates from 0 to 12°C, and softened slowly without initiating rapid phase softening at 20°C through 35°C. Further research showed that rapid phase softening could occur in GS at 20°C, but only when previously exposed to ethylene or storage at 0.5°C (Chapter 5). Interestingly, rapid phase softening in GS at 20°C was not initiated immediately after ethylene treatment, but was delayed some 15-20 days. Likewise, initiation of rapid phase softening of GS at 20°C was delayed after cold

treatment, with the delay reduced as prior time at 0.5°C increased. PR was unique amongst these four cultivars, as this cultivar was apparently incapable of initiating rapid phase softening at 20°C, regardless of prior exposure to ethylene or cold treatment.

The softening rate of RG and COP at any given temperature between 0-20°C was not influenced by prior exposure to another temperature between 0 and 20°C (Chapter 6). Furthermore, time at 0.5-3°C had no influence on the subsequent rate of softening of either cultivar at 20°C. However for GS, the shorter the time at 0.5°C before transfer to 20°C, the greater the rate of rapid phase softening once initiated at 20°C (Chapter 5). Thus, prior exposure to different temperatures had a strong influence on both initiation and rate of rapid phase softening in GS, but not in RG or COP. The different softening response of GS could be due to the underlying chilling requirement for this cultivar to rapidly initiate autocatalytic ethylene production at shelf life temperatures, whereas the ethylene production of COP and RG at shelf life temperatures are not chilling responsive (Knee et al., 1983; Jobling et al., 1991; Larrigaudiere et al., 1997).

10.4 Role of ethylene in apple softening.

Experiments with transgenic fruits and inhibitors of ethylene action have been used to identify which ripening pathways are ethylene dependent and independent in fruit. Using transgenic melons, the ripening pathways of rind yellowing, softening, aroma volatiles and climacteric respiration were determined as ethylene dependent, while flesh colouration, and changes in sugars and acids were ethylene independent (Pech et al., 1999). Similarly, the softening of tomatoes was reduced in transgenic fruits with decreased ethylene biosynthesis (Klee, 1993; Sozzi et al., 1998). Studies with 1-methylcyclopropene, an inhibitor of ethylene action, have also shown that softening is ethylene dependent in several fruits, including kiwifruit, peaches and apples (Hewett et al., 1999; Watkins et al., 2000; Mathooko et al., 2001). Thus, it is clear that ethylene is an important promoter of softening in several fruits, including apples. However, it was not clear how changes in endogenous ethylene concentrations relate to changes in softening rates through apple ripening. Furthermore, it is not known if the effects of different pre- and postharvest factors on softening are mediated by ethylene.

This research has showed for the first time that onset of rapid phase softening in RG and COP was consistently associated with the time when internal ethylene concentrations (IEC's) rapidly increased from low (<1.5 $\mu l.l^{-1}$) basal concentrations, regardless of the at harvest or postharvest factor studied. Thus, most of the effects of the different factors on the softening curve for RG and COP can be explained by changes in endogenous ethylene concentration (Fig. 10-2). From these results it is suggested that the initial slow softening phase was associated with system I ethylene production, and that the rapid softening phase was associated with system II ethylene production. System I ethylene production occurs in preclimacteric fruits when ethylene production is low, while system II ethylene production occurs during the respiratory climacteric when rates of ethylene production are high (McMurchie et al., 1972; Oetiker and Yang, 1995). However, system II ethylene production is normally considered autocatalytic, and no attempts were made to confirm whether the increased IEC during storage in the above experiments was autocatalytic in nature.

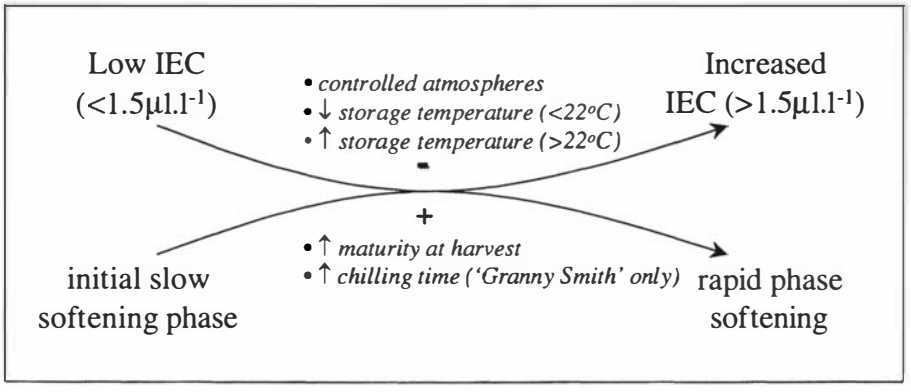

Fig. 10-2 Diagram summarising the influence of different at harvest and postharvest factors on increased internal ethylene concentration (IEC) and transition between the initial slow and rapid softening phases. This transition was either promoted (+) or inhibited (-) by a change (increase, ↑; decrease, ↓) in each factor.

Results in Chapter 7, in relation to results in Chapter 4, indicated that on-tree softening was approximately 3 to 7 times slower than rapid phase softening of harvested apples at comparable temperatures for RG and COP. Thus, it was concluded that the initial slow softening phase commenced while apples remained on the tree and continued temporarily in harvested fruit during the early phases of storage, with the postharvest

longevity depending on maturity of fruit at harvest. Sfakiotakis and Dilley (1973) found that IEC's of attached 'Red Delicious' apples remained low (<1 $\mu l.l^{-1}$) for about 20 days longer than in detached apples, which could explain the slow on-tree softening. However, the time required before autocatalytic ethylene production occurs in attached fruit is cultivar dependent. Autocatalytic ethylene production occurred during the commercial harvest period for RG and GS, midway through the harvest period for 'Delicious' strains, after the commercial harvest period for COP and 'Braeburn', and did not occur during the sampling period for 'Golden Delicious' (Watkins et al., 1989). Cultivar differences in timing of autocatalytic ethylene production relative to the commercial harvest were also observed in several other apple cultivars (Chu, 1988). Data in Chapter 7 agreed with ethylene production results from Watkins et al. (1989), where the IEC's in COP remained low for the first three harvests and then increased to 1-2 $\mu l.l^{-1}$ by harvest five, while the IEC's in RG progressively increased with each harvest date to be about 4 $\mu l.l^{-1}$ at harvest five. However, these on-tree concentrations were relatively low in comparison to those in detached fruits, where the IEC's can be as high as 50-100 $\mu l.l^{-1}$ and 400-600 $\mu l.l^{-1}$ when stored at 0-3°C and 20°C respectively (Chapter 7). Studies by Lau et al. (1986) and Blankenship and Unrath (1988) showed that on-tree softening occurred without an increase in IEC, suggesting that ethylene may not regulate on-tree softening. However, it is likely that a low basal rate of ethylene production was present that may have been sufficient to promote and sustain the slow softening that occurred on the tree and during the initial slow softening phase. This has also been suggested for kiwifruit and for some non-climacteric fruits, as ethylene production remains low in these fruits during softening (Lelievre et al., 1997; Kim et al., 1999). It is likely that the sensitivity of fruits to ethylene may have a more important role in regulating the rate of softening than the actual ethylene concentration *per se*, especially when ethylene concentrations are low as in the initial slow softening phase of apples, or during most phases of softening in kiwifruit and in non-climacteric fruits. On-tree softening could also have been influenced by the physical consequences of cell expansion and associated fruit growth, and/or by the occurrence of other plant growth regulators.

Exceptions to the relationship between increased IEC's and the onset of rapid phase softening for apples were observed for GS and PR when stored at 20-35°C (Chapters 4 and 5). Yet at temperatures less than 20°C, the onset of rapid phase softening was associated with onset of system II ethylene production as previously described for RG and COP. As indicated in section 10.3, GS initiated rapid phase softening at 20°C when previously exposed to ethylene or time at 0.5°C. However, initiation of rapid phase softening in this cultivar at 20°C was delayed relative to the cold induced increase in IEC from a low basal concentration (Chapter 5). Furthermore, this delay was less pronounced in GS when previously stored at 0.5°C for increased time before transfer to 20°C. Thus, the sensitivity of GS to ethylene may have a more immediate role in regulating the softening of GS at shelf life temperatures than the actual ethylene concentration. Similarly, the non-occurrence of rapid phase softening in PR at shelf life temperatures, despite having IEC's in excess of 100 $\mu l.l^{-1}$, indicated that lack of sensitivity to ethylene may be more important than ethylene concentration *per se* for regulating softening of PR at shelf life temperatures.

Studies using different concentrations of exogenous ethylene have shown that pears, kiwifruit and apples all have increased sensitivity to ethylene during maturation (Harkett et al., 1971; Wang et al., 1972; Sfakiotakis and Dilley, 1973; Knee et al., 1987; Kim et al., 1999). Increased sensitivity can be due to an increased number of ethylene receptors, increased affinity between ethylene and the receptor, and increased response capacity of signal transduction pathways following ethylene binding to the receptor (Firn, 1986). There is some evidence that the number of ethylene receptors do not change, and that the affinity of ethylene for receptors is reduced during the maturation of apples (Blankenship and Sisler, 1989; 1993b). However in tomato, five ethylene receptors have been found (Klee et al., 1999), with at least one of these receptors having increased expression during ripening (Payton et al., 1996). The signal transduction pathways following ethylene binding to the receptor has been partially characterised (Bleecker et al., 1999). However it not known whether the different ethylene dependent ripening pathways have different signal transduction pathways and different ethylene receptors, or whether they have one or both of these components in common. Different signal transduction pathways and/or ethylene receptors for each ripening pathway could explain why skin yellowing, respiration and ethylene biosynthesis occurred at a different

time to initiation of rapid phase softening in PR and cold treated GS fruit at 20°C (Chapter 5).

10.5 Cultivar differences in softening rates.

Apple cultivars not only varied in their softening response to different temperatures (section 10.3), but also had different softening rates at any one temperature. COP tended to soften the fastest, followed by RG, GS, and then PR (Chapter 4 and Fig. 10-3). In New Zealand, RG and COP mature early in the season, while GS and PR mature late in the apple season. Early season cultivars are generally considered to have greater rates of ethylene production and respiration than late season cultivars (Kidd and West, 1939; Hansen, 1945; Watkins et al., 1989). COP had the highest IEC's and fastest softening rate, RG had lower IEC's and slower softening rates than COP, and PR the lowest IEC's and slowest softening rates of all cultivars (Fig. 10-3). However, GS softened slower than RG despite having higher IEC's during the early phases of storage. This indicates that cultivar differences in softening rates could not be consistently explained by differences in IEC. A study comparing the rates of softening and ethylene production from different apples cultivars came to the same conclusion (Gussman et al., 1993). However, cultivar differences in softening rates relative to IEC could be interpreted in terms of cultivar differences in ethylene sensitivity. It is possible that RG is more sensitive than GS to ethylene at 0°C, resulting in greater softening rates in RG than in GS, despite GS having greater IEC's than RG during the first 60 days at 0°C. Similarly, COP may be the most sensitive cultivar to ethylene resulting in the fastest softening rates, and PR the least sensitive cultivar to ethylene resulting in the slowest softening rates at 0°C.

Another factor to be considered is whether these cultivars are in fact all climacteric fruits. Climacteric fruits differ from non-climacteric fruits in that they typically have a burst of increased respiration and autocatalytic ethylene production during ripening, have ripening irreversibly hastened by application of exogenous ethylene when unripe, and that the magnitude of the climacteric is not influenced by different concentrations of exogenous ethylene (Biale, 1964; Lelievre et al., 1997). The slow ripening behaviour of PR, and the observation that it did not respond to ethylene treatment, suggests that it

may be a non-climacteric fruit, especially at shelf life temperatures. However, PR did have a burst of respiratory activity, and peak IEC's in excess of 100 $\mu l.l^{-1}$ at 20°C (Chapter 5), which suggests that PR, like the other cultivars, is probably climacteric in nature. Thus, the slow softening of PR at shelf life temperatures, regardless of prior exposure to ethylene or cold treatment, is more likely due to a reduction in ethylene sensitivity at these temperatures.

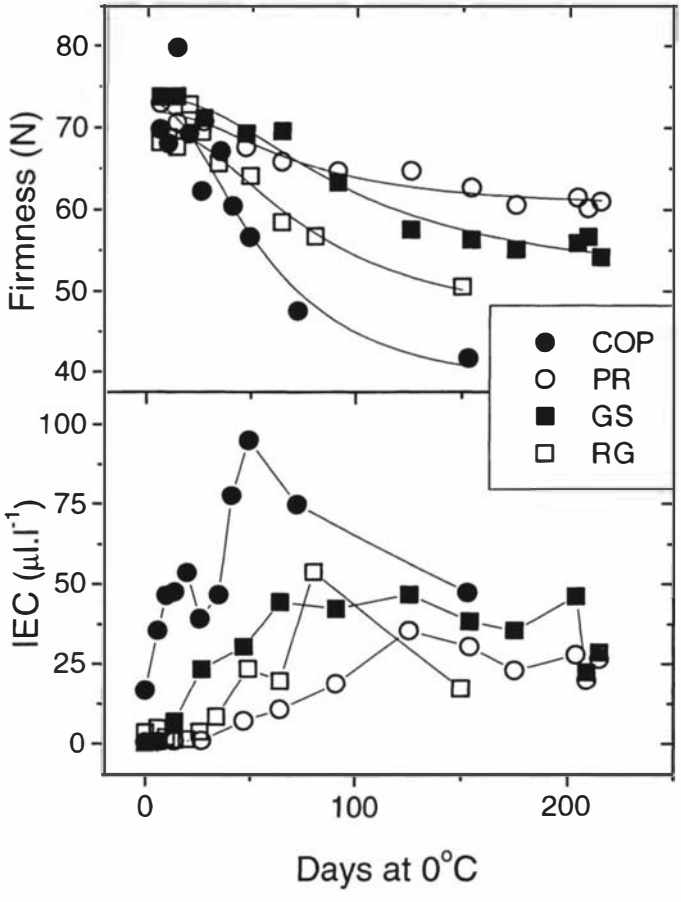

Fig. 10-3 Firmness and internal ethylene concentration (IEC) of 'Cox's Orange Pippin' (COP), 'Royal Gala' (RG), 'Granny Smith' (GS) and 'Pacific Rose™' (PR) at 0°C (redrawn from Chapter 4).

In Chapter 5 it was suggested that PR maybe a ripening mutant with reduced capacity for ethylene biosynthesis and action, and hence softening. While apple cultivars such as

'Honeycrisp', 'NJ55' and 'PA14-238' also soften slowly at 0–4°C (Gussman et al., 1993; Tong et al., 1999), it is not known if these cultivars are like PR in that they cannot initiate rapid phase softening at shelf-life temperatures. Thus, until further research is done on these other slow softening apple cultivars to prove otherwise, the softening characteristics of PR appear to be unique for apples. Ripening mutants have been identified in several fruits, including nectarines and tomatoes (Tigchelaar et al., 1978; Brecht et al., 1984). Of the well-studied tomato ripening mutants of never-ripe (*Nr*), non-ripening (*nor*) and ripening-inhibitor (*rin*), PR has most similarities with *Nr*. Like PR, *Nr* has reduced, but not completely inhibited, ethylene production and respiration rates during the climacteric, and it softens slowly relative to normal ripening genotypes (Tigchelaar et al., 1978). In contrast, *nor* and *rin* are regarded as non-climacteric fruits as they do not have a detectable respiratory and ethylene climacteric (Tigchelaar et al., 1978). Interestingly, *Nr* has reduced sensitivity to ethylene (Lanahan et al., 1994); the same mechanism previously speculated as limiting occurrence of rapid phase softening in PR at shelf-life temperatures. Molecular evidence suggests that reduced ethylene sensitivity in *Nr* is probably due to presence of an ethylene receptor isoform that negatively regulates the ethylene signal transduction pathway (Tieman et al., 2000). Regardless of the mechanism by which PR softens slowly after harvest, this trait is commercially desirable, as this cultivar rarely have textural quality problems in the market place. This suggests that breeding programs aimed at producing novel apple cultivars with high textural quality should be screening future selections for the presence of this slow-softening trait.

Results from Chapter 8 indicated that faster softening rates in COP as compared to RG could be attributed to lower calcium concentrations in COP than in RG. Calcium is important for stabilising membranes and improving the strength of the cell wall (Poovaiah et al., 1988), as well as being able to reduce ethylene production (Sams and Conway, 1984). However, calcium concentrations were not measured in GS and PR fruit, making it difficult to determine if these cultivars softened slower than RG and COP as a result of having higher calcium concentrations.

Although not measured in this research, differences in cell turgor, activity of cell wall degrading enzymes, cell wall chemistry, and cell packing could also affect cultivar

differences in softening rates. A study using several apple cultivars showed that some of the variation in poststorage firmness between apple cultivars was explained by cell turgor, where less turgid cultivars were softer than more turgid cultivars after storage (Tong et al., 1999). Kovacs et al. (1999) showed that differences in activities of PG and β-galactosidase, and cell size and packing existed between cultivars. However, as no firmness data were presented it is difficult to determine how much these characteristics may have influenced softening. In another study, a slower softening cultivar lost less arabinose and uronic acid from the cell wall, lost less cell wall material in total, and had less visual disruption of the middle lamella and the cell membrane when compared with faster softening cultivars (Tong et al., 1999). Nara et al. (2001) also demonstrated that the loss of terminal arabinose residues and increase in terminal galactose residues in pectin during softening were more pronounced in cultivars that were more susceptible to the development of mealiness. Thus, it is likely that softening rate differences between cultivars could be mediated by several differences in cellular characteristics.

10.6 A schematic model of apple softening.

Characterisation of the softening curves for apples in relation to different pre-, at- and post-harvest factors has allowed the development of an improved schematic model of apple softening (Fig. 10-4). The primary model of apple softening that was proposed in section 1.6 from a survey of the literature had a number of shortcomings, including:

- no information about changes in softening rates through storage
- nothing about the effects of different pre-, at- and post-harvest factors on softening rates
- no distinct roles were specified for ethylene and enzymes involved in cell wall disassembly and loss of membrane integrity.

Most of these shortcomings have now been rectified in the new model, as:

- characterisation of the softening curves of apples in relation to different at harvest and postharvest factors has generated information on changes in softening rates through storage
- the relation of changes in IEC with loss of firmness has identified a specific role for ethylene in regulating softening.

This new model also depicts the apparent chilling requirement of GS before rapid phase softening is induced at 20-35°C, and the non-occurrence of rapid phase softening in PR at 20-35°C despite having IEC's in excess of 100 $\mu l.l^{-1}$. However, because no enzymatic studies were undertaken on apples during this project, it is not possible to identify specific roles in this model for cell wall degrading and membrane modifying enzymes. However, a number of other studies have been done on specific enzymes and their changes during storage, so some inferences can be drawn as to their specific role and the way in which they may be associated with loss of firmness and timing of increases in ethylene concentrations within fruit.

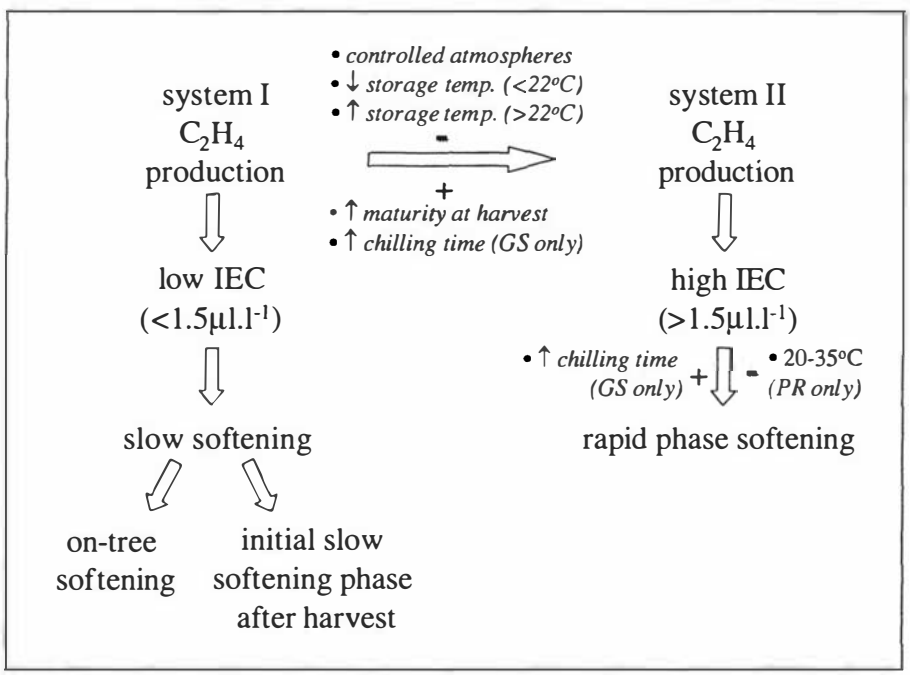

Fig. 10-4 Proposed schematic diagram for regulation of initiation of rapid phase softening in apple fruit. Abbreviations: 'Granny Smith' (GS); 'Pacific Rose™' (PR); internal ethylene concentration (IEC); ethylene (C_2H_4); inhibit the process (-); promote the process (+); increase (↑); decrease (↓).

The activity of endo-PG in apples increased in parallel with the decline in firmness during storage (Wu et al., 1993). Correlative evidence indicates that expression of endo-polygalacturonase (PG) in apples increased during the early stages of ripening, where levels of endo-PG increased markedly at IEC's from 0.7 to 2.1 $\mu l.l^{-1}$ in RG, from 0.2 to 2.7 $\mu l.l^{-1}$ for GS, and from 11 to 46 $\mu l.l^{-1}$ for 'Braeburn' (Atkinson et al., 1998).

Interestingly, the IEC's at the onset of rapid phase softening that were measured on COP and RG in the present study were about 1.5 $\mu l.l^{-1}$, which are comparable with the IEC ranges associated with increased endo-PG expression in Atkinson et al. (1998). Thus, it is possible that ethylene induced rapid phase softening in apples is mediated by increased expression of endo-PG. The ethylene dependence of endo-PG has been confirmed in tomatoes and melons (Sirit and Bennett, 1998; Pech et al., 1999), although it is yet to be confirmed for apples.

Other cell wall degrading enzymes that have increased activity in parallel with a reduction in firmness of apples during storage include β-galactosidase and α-L-arabinofuranosidase (Bartley, 1974; 1977; Wallner, 1978; Berard et al., 1982; Yoshioka et al., 1995). Pectin methyl esterase (PME), exo-PG and rhamnogalacturonase activity has also been measured in ripe apples (Bartley, 1978; Gross et al., 1995; Klein et al., 1995). The role that each of these enzymes play in each phase of apple softening is not known. However, knowing that the activity of β-galactosidase and α-L-arabinofuranosidase were both ethylene dependent in melons (Pech et al., 1999), suggests that these enzymes may also have important roles in mediating rapid phase softening in apples. Similarly, the finding that exo-PG and PME were both ethylene independent enzymes in melons (Pech et al., 1999), suggests that these enzymes may have roles in all phases of apple softening. Until similar transgenic studies to those performed on melons and tomatoes (Sirit and Bennett, 1998; Pech et al., 1999), have also been performed on apples, it will be difficult to allocate definitive roles for each of these enzymes in each phase of apple softening.

10.7 Can softening rates be predicted before storage?

The ability to describe/predict softening rates of apples before storage would have a number of commercial advantages, as outlined in section 1.7. Results in Chapter 4 showed that a modified Arrenhius equation and a sigmoidal softening function could describe softening of RG and COP at 0 to 35°C. Consequently, these equations were combined to form a mathematic model (FirmCalc) that could describe softening in relation to changes in temperature during postharvest handling. FirmCalc required the assumption that the softening rate at a given temperature was not influenced by prior

exposure to another temperature, which was subsequently proven true for RG and COP in Chapter 6.

FirmCalc could be used to quantify the loss of firmness for several different postharvest handling chain scenarios, and for fruit accidentally stored at incorrect temperatures during on and offshore storage. Thus, FirmCalc could be used commercially:

- to optimise the postharvest coolchain for RG and COP
- as a training tool for industry personnel to demonstrate the consequences of not following industry coolchain guidelines
- to estimate the consequences of unintended periods of intermittent warming that may arise from refrigeration malfunctions during storage and transportation.

An example of inputs and outputs from the FirmCalc model for RG exposed to a typical postharvest handling chain is depicted in Table 10-2, with the outputs from different coolchain scenarios depicted in Fig. 10-5.

Table 10-2 Theoretical firmness of 'Royal Gala' apples after different phases of postharvest handling using interactive FirmCalc model. Text and numerals in italics are numbers that can be changed by the user, and non-italic numerals represent the models output.

Postharvest handling chain	Temperature details				Cumulative time (days)	Firmness (N)
	IT (°C)	FT (°C)	t_{IT-FT} (days)	t_{FT} (days)		
Picking & packing	*20*	*20*	*0*	*1*	1	76.4
Coolstorage	*20*	*0.5*	*3*	*14*	18	72.2
Ship loading	*0.5*	*12*	*0.5*	*0*	18.5	71.5
Transportation	*12*	*0.5*	*3*	*21*	42.5	63.6
Ship unloading	*0.5*	*12*	*0.5*	*0*	43	62.9
Final firmness at destination market						62.9

Abbreviations: Initial fruit temperature (IT); final fruit temperature (FT); days to warm/cool from initial to final temperature (t_{IT-FT}); days at final temperature (t_{FT}).

A problem with FirmCalc is that it is currently limited to being descriptive, as it does not consider harvest maturity, orchard and seasonal effects on postharvest softening rates of COP and RG. However, it should be possible to correct for harvest maturity

and orchard effects by incorporating at harvest measurements of firmness, total soluble solids, glucose and dry matter for RG, and similarly at harvest measurements of firmness for COP (Chapter 8). It should also be possible to incorporate the effects of CA that were quantified in Chapter 9. An updated model with all of these factors should enable FirmCalc to be predictive, rather than its currently descriptive status. This would then create the potential for industry to segregate batches of fruit before storage for differences in softening potential, as well as provide a tool for optimising the use of CA and the coolchain during postharvest handling.

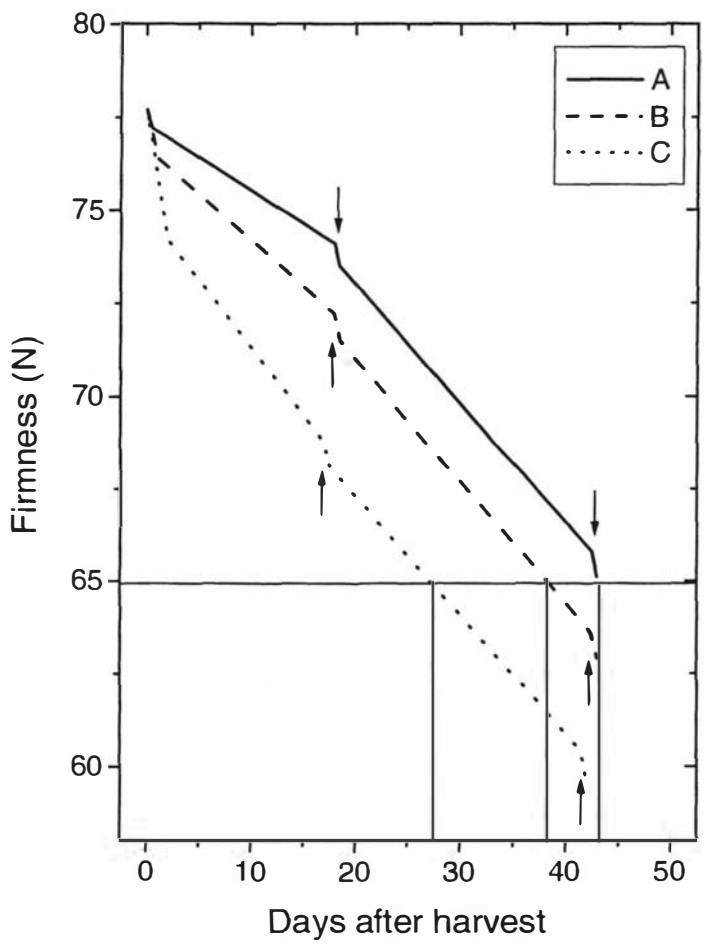

Fig. 10-5 Theoretical firmness of 'Royal Gala' apples exposed to different postharvest coolchain scenarios using the FirmCalc model. Coolchain scenarios were: A) held at 20°C for 12 hours, then cooled to 0.5°C within 12 hours; B) held at 20°C for 24 hours, then cooled to 0.5°C within 3 days; C) held at 20°C for 48 hours, then cooled to 0.5°C within 5 days. Arrows denote a 12 hour period at 12°C for ship loading and unloading. The horizontal line depicts the minimum firmness accepted by most international markets for this cultivar.

Interestingly, FirmCalc is not the only model that has been developed to describe the softening rates of apples, as another model (Q-apple) has been developed that describes softening of the 'Elstar' cultivar during postharvest handling (van Schaik and Hertog, 1997; Tijskens et al., 1999). However, Q-apple was based on theoretical concepts, and was not based on raw data (Tijskens et al., 1999). Thus, FirmCalc is unique in that was developed from raw data. There is also potential to use data in this thesis to develop mechanistic models that describe apple softening as a function of endogenous ethylene concentration. This approach has been used to describe loss of quality in several fruits in relation to respiration rate or exogenous ethylene concentration (Hertog, per. com.).

The accuracy of such models in predicting postharvest softening rates of apples is dependent on the ability to measure firmness accurately. As indicated in section 1.2, the handheld penetrometer is prone to operator variation regardless of whether it is mounted in a drill-press or not (Harker et al., 1996). Furthermore, firmness readings from different puncture test devices also vary for both firm and soft fruit (Abbott et al., 1976; Harker et al., 1996; Lehman-Salada, 1996; DeLong et al., 2000). Results in Chapter 3 indicated that environmental conditions such as fruit temperature also affect firmness readings. Thus, to optimise the accuracy of firmness predictions, apple industries need to adopt a standard testing device that is not prone to operator variation, and set a standard fruit temperature to be used during firmness assessment. However, given that the magnitude of the physical change in firmness due to temperature was not affected by orchard or harvest date, data presented in Chapter 3 could be used to standardise firmness readings for fruit measured at different temperatures.

10.8 Future research.

The main areas of research that are needed to significantly further knowledge on apple softening are an increased understanding of the role of ethylene sensitivity in regulating softening in different cultivars, determination of the enzymes that mediate softening in each phase of softening, and characterisation of the cellular characteristics that influence the objective and sensory textural quality of different cultivars. Further research is also required to incorporate orchard, maturity and CA effects into the FirmCalc model, and then validate the model with commercial trials.

In sections 10.4 and 10.5 the concept of the fruits sensitivity to ethylene in relation to regulation of rapid phase softening was discussed. Experiments are required to determine whether sensitivity to ethylene changes during ripening, and how these changes relate to the onset of system II ethylene production and rapid phase softening for several apple cultivars. These experiments may explain the apparent disassociation between induction of system II ethylene production and the onset of rapid phase softening in GS fruit at 20°C following cold treatment, and may also explain why PR fruit had no rapid softening phase at 20°C despite having IEC's in excess of 100 $\mu l.l^{-1}$. It is likely that molecular experiments are the most appropriate for this form of study, as these studies could determine changes in expression of specific ethylene receptors and components of the signal transduction pathway(s) in relation to onset of system II ethylene production and rapid phase softening. There is also the need to undertake binding studies to determine whether the affinity of the ethylene receptor(s) changes during ripening, perhaps using techniques described in Blankenship and Sisler (1993b) and Harpham et al. (1996). These studies may identify if PR is deficient or has a mutation in a particular receptor isoform, or component of the signal transduction pathway, that may cause this cultivar to soften slowly relative to other apple cultivars.

Research has shown that the genetic altering of fruits to suppress ethylene biosynthesis (Klee, 1993; Sozzi et al., 1998; Pech et al., 1999), and the treatment of fruits with inhibitors of ethylene action (Watkins et al., 2000), are both effective techniques for reducing fruit softening. However, these techniques often have adverse effects on other aspects of quality, such as suppressing the biosynthesis of aroma and flavour volatiles (Fan and Mattheis, 1999; Pech et al., 1999). Thus, it would be beneficial to adopt techniques that specifically reduce softening without affecting other aspects of quality. This could be achieved by genetic altering of fruit so that expression of a particular softening enzyme is reduced, as has been done with polygalacturonase in tomato (Smith et al., 1990). Before such transgenic experiments are performed, the appropriate enzyme(s) need to be identified that have important roles in mediating softening. In sections 1.3.2 and 10.6, a number of cell wall enzymes were identified that may have important roles in mediating apple softening. The importance of individual enzymes could be determined by relating the activity of certain enzymes to the softening curves

of apples, and in particular identifying those enzymes that increase in activity immediately prior to, or during the onset of rapid phase softening. Furthermore, the initiation of the rapid softening phase appears to be ethylene dependent, and inhibitors of ethylene action could be used to determine which enzymes are ethylene dependent or independent. An alternative and more robust approach would be to genetically suppress ethylene biosynthesis, and determine if certain cell wall degrading enzymes are expressed in the absence of ethylene as has been done for melons (Pech et al., 1999). However, the current consumer opposition to the production and sale of transgenic foods may temper the commercial acceptance of such experiments.

While it is important that further research is undertaken to determine the mechanisms by which apples soften, it is also equally important that the cellular basis of sensory and objective assessment of texture at a given stage of ripeness is known for different cultivars. It is likely that different cultivars are rated as having completely different sensory textural characteristics despite being objectively measured as having similar firmness. The cellular basis for these differences could be due to cultivar differences in cell packing (cell size and number, and cell to cell adhesion), cell wall chemistry, and cell turgor (Kovacs et al., 1999; Tong et al., 1999; Nara et al., 2001). The relation of these cellular characteristics to sensory perception of texture could be used in breeding programs to rapidly identify the textural properties of new selections. It is also possible that some of these texture-defining cellular characteristics are influenced by preharvest factors (cell number and cell size especially). Thus, it may be possible to manipulate the sensory textural quality of apples by altering a specific preharvest factor.

In section 10.7, the development, uses, and limitations of a prototype FirmCalc model were discussed. Once the model is updated to include the effects of orchard, maturity and CA, it should be at a status whereby it can be used to predict the softening rates of RG and COP before storage. However, further research would be required to validate this model, and if needed, update the model using new information from this trial. This validation trial should be undertaken using fruit from several orchards that are picked at different stages of maturity, and then be stored in air and CA. Following this trial it should be possible for the model to be released into the commercial environment on a limited basis for industry validation. Once the model has passed a commercial

validation step, the model should be ready for full commercial release. However, systems need to be implemented so that a central controlling agency can continuously monitor and validate the use of the model, so that ongoing improvements or refinements can be made. The data entered in FirmCalc could also be used as an industry database that records the softening curves of orchards across several seasons. This database could then be used to study seasonal effects, whereby attempts could be made to associate weather patterns with softening curves for different seasons. Information from this could be used as a warning system if it is known that a particular preharvest weather pattern exacerbates the production of rapid softening fruit.

10.9 Thesis conclusions.

Softening of harvested apples was determined to be triphasic, regardless of the storage temperature, atmosphere, harvest maturity, fruit size and cultivar. The three phases included an initial slow softening phase, followed by a phase of more rapid softening, and then a final slow softening phase. The initial slow softening phase, thought to be a continuation of the slow softening that occurred on the tree, is considered a crucially important phase that largely determines the market life of apples. The factors of temperature, atmosphere, harvest maturity and cultivar all had a pronounced influence on the duration of the initial slow softening phase. Temperature, and establishment of CA while fruit were in the initial slow softening phase, both affected the rate of rapid phase softening. The influence of these factors on these phases of softening may be mediated by ethylene, as the onset of rapid phase softening coincided with the time when the internal ethylene concentration began to increase rapidly from a low (<1.5 $\mu l.l^{-1}$) basal concentration for all factors. Thus, the initial slow softening phase may be regulated by system I ethylene production, and the rapid softening phase appears to be induced by system II ethylene production. However, the cold-requiring GS cultivar, and the extremely slow softening PR cultivar, provided exceptions to this observation when stored at shelf-life temperatures. Thus, further research is required to determine the role of sensitivity in ethylene-mediated regulation of apple softening. Further research is also required to determine which cell wall degrading and membrane modifying enzymes have important roles in each phase of apple softening. Results from this thesis could be used to develop models that predict the softening rates of apples before storage, which

then could be used commercially to improve management of the "soft fruit" problem for rapid softening cultivars.

10.10 References

Abbott, J.A., Watada, A.E., Massie, D.R., 1976. Effe-gi, Magness-Taylor, and Instron fruit pressure testing devices for apples, peaches, and nectarines. J. Am. Soc. Hortic. Sci. 101, 698-700.

Aggelis, A., John, I., Grierson, D., 1997. Analysis of physiological and molecular changes in melon (*Cucumis melo* L.) varieties with different rates of ripening. J. Exp. Bot. 48, 769-778.

Atkinson, R.G., Bolitho, K.M., Wright, M.A., Iturriagagoitia-Bueno, T., Reid, S.J., Ross, G.S., 1998. Apple ACC-oxidase and polygalacturonase: ripening specific gene expression and promoter analysis in transgenic tomato. Plant Mol. Biol. 38, 449-460.

Bartley, I.M., 1974. β-Galactosidase activity in ripening apples. Phytochem. 13, 2107-2111.

Bartley, I.M., 1977. A further study of β-galactosidase activity in apples ripening in store. J. Exp. Bot. 28, 943-948.

Bartley, I.M., 1978. Exo-polygalacturonase of apple. Phytochem. 17, 213-216.

Berard, L.S., Lougheed, E.C., Murr, D.P., 1982. β-Galactosidase activity of 'McIntosh' apples in storage. HortScience 17, 660-661.

Biale, J.B., 1964. Growth, maturation, and senescence in fruits. Science 146, 880-888.

Blankenship, S.M., 1987. Night-temperature effects on rate of apple fruit maturation and fruit quality. Sci. Hortic. 33, 205-212.

Blankenship, S.M., Sisler, E.C., 1989. Ethylene binding changes in apple and morning glory during ripening and senescence. J. Plant Growth Reg. 8, 37-44.

Blankenship, S.M., Sisler, E.C., 1993a. Response of apples to diazocyclopentadiene inhibition of ethylene binding. Postharvest Biol. Technol. 3, 95-101.

Blankenship, S.M., Sisler, E.C., 1993b. Ethylene binding site affinity in ripening apples. J. Am. Soc. Hortic. Sci. 118, 609-612.

Blankenship, S.M., Unrath, C.R., 1988. Internal ethylene levels and maturity of 'Delicious' and 'Golden Delicious' apples destined for prompt consumption. J. Am. Soc. Hortic. Sci. 113, 88-91.

Blanpied, G.D., 1985. Low ethylene CA storage for 'Empire' apples. Proc. Fourth Nat. C.A. Res. Conf., 23-26 July 1985, Raleigh, North Carolina, USA, 95-102.

Bleecker, A.B., Hall, A.E., Rodriguez, F.I., Esch, J.J., Binder, B., 1999. The ethylene signal transduction pathway. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech,

J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 51-57.

Bourne, M.C., 1968. Texture profile of ripening pears. J. Food Sci. 33, 223-226.

Bourne, M.C., 1979. Texture of temperate fruits. J. Text. Stud. 10, 25-44.

Brecht, J.K., Kader, A.A., Ramming, D.W., 1984. Description and postharvest physiology of some slow-ripening nectarine genotypes. J. Am. Soc. Hortic. Sci. 109, 596-600.

Chu, C.L., 1988. Internal ethylene concentration of 'McIntosh', 'Northern Spy', 'Empire', 'Mutsu', and 'Idared' apples during the harvest season. J. Am. Soc. Hortic. Sci. 113, 226-229.

DeEll, J.R., Saad, F., Khanizadeh, S., 1999. Factors influencing apple fruit firmness. Compact Fruit Tree 32, 56-58.

DeLong, J.M., Prange, R.K., Harrison, P.A., McRae, K.B., 2000. Comparison of a new apple firmness penetrometer with three standard instruments. Postharvest Biol. Technol. 19, 201-209.

Dilley, D.R., Lange, E., Tomala, K., 1989. Optimizing parameters for controlled atmosphere storage of apples. Proc. Fifth Int. C.A. Res. Conf., 14-16 June 1989, Wenatchee, Washington, USA, 221-236.

Fan, X., Mattheis, J.P., 1999. Impact of 1-methylcyclopropene and methyl jasmonate on apple volatile production. J. Agric. Food Chem. 47, 2847-2853.

Fica, J., Skrzynski, J., Dilley, D.R., 1985. The effect of delayed cooling and delayed application of CA storage of McIntosh apples under low and high ethylene levels. Proc. Fourth Nat. C.A. Res. Conf., 23-26 July 1985, Raleigh, North Carolina, USA, 82-94.

Firn, R.D., 1986. Growth substance sensitivity: the need for clearer ideas, precise terms and purposeful experiments. Physiol. Plant. 67, 267-272.

Gross, K.C., Starrett, D.A., Chen, H.J., 1995. Rhamnogalacturonase, α-galactosidase and β-galactosidase: potential roles in fruit softening. Acta Hortic. 398, 121-130.

Gussman, C.D., Goffreda, J.C., Gianfagna, T.J., 1993. Ethylene production and fruit softening rates in several apple fruit ripening variants. HortScience 28, 135-137.

Hansen, E., 1945. Quantitative study of ethylene production in apple varieties. Plant Physiol. 20, 631-635.

Harker, F.R., Maindonald, J.H., Jackson, P.J., 1996. Penetrometer measurement of apple and kiwifruit firmness: operator and instrument differences. J. Am. Soc. Hortic. Sci. 121, 927-936.

Harker, F.R., Redgwell, R.J., Hallett, I.C., Murray, S.H., 1997. Texture of fresh fruit. Hortic. Rev. 20, 121-224.

Harkett, P.J., Hulme, A.C., Rhodes, M.J.C., Wooltorton, L.S.C., 1971. The threshold value for physiological action of ethylene on apple fruits. J. Food Technol. 6, 39-45.

Harpham, N.V.J., Berry, A.W., Holland, M.G., Moshkov, I.E., Smith, A.R., Hall, M.A., 1996. Ethylene binding sites in higher plants. Plant Growth Reg. 18, 71-77.

Hewett, E.W., Kim, H.O., Lallu, N., 1999. Postharvest physiology of kiwifruit: the challenges ahead. Acta Hortic. 498, 203-216.

Jobling, J., McGlasson, W.B., 1991. Induction of ethylene synthesizing competency in Granny Smith apples by exposure to low temperature in air. Postharvest Biol. Technol. 1, 111-118.

Kader, A.A., 1999. Fruit maturity, ripening, and quality relationships. Acta Hortic. 485, 203-208.

Karakurt,Y., Huber, D.J., Sherman, W.B., 2000. Quality characteristics of melting and non-melting flesh peach genotypes. J. Sci. Food Agric. 80, 1848-1853.

Kidd, F., West, C., 1939. The climacteric in early and late varieties of apple on the tree. Rep. Food Investig. Board Year 1939 57-59.

Kim, H.O., Hewett, E.W., Lallu, N., 1999. The role of ethylene in kiwifruit softening. Acta Hortic. 498, 255-261.

King, G.A., Henderson, K.G., Lill, R.E., 1989. Ultrastructural changes in the nectarine cell wall accompanying ripening and storage in a chilling-resistant and chilling-sensitive cultivar. New Zeal. J. Crop Hortic. Sci. 17, 337-344.

Klee, H.J., 1993. Ripening physiology of fruit from transgenic tomato (*Lycopersicon esculentum*) plants with reduced ethylene synthesis. Plant Physiol. 102, 911-916.

Klee, H., Tieman, D., Lashbrook, C., 1999. Ethylene perception in tomato: lots of genes, lots of functions. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 351-356.

Knee, M., Hatfield, S.G.S., Bramlage, W.J., 1987. Response of developing apple fruits to ethylene treatment. J. Exp. Bot. 38, 972-979.

Knee, M., Looney, N.E., Hatfield, S.G.S., Smith, S.M., 1983. Initiation of rapid ethylene synthesis by apple and pear fruits in relation to storage temperature. J. Exp. Bot. 34, 1207-1212.

Klein, J.D., Hanzon, J., Irwin, P.L., Ben-Shalom, N., Lurie, S., 1995. Pectin esterase activity and pectin methyl esterification in heated golden delicious apples. Phytochem. 39, 491-494.

Kovacs, E., Sass, P., Al-Ariki, K., 1999. Cell wall analysis of different apple cultivars. Acta Hortic. 485, 219-224.

Lanahan, M.B., Yen, H-C., Giovannoni, J.J., Klee, H.J., 1994. The never ripe mutation blocks ethylene perception in tomato. Plant Cell 6, 521-530.

Larrigaudiere, C., Graell, J., Salas, J., Vendrell, M., 1997. Cultivar differences in the influence of a short period of cold storage on ethylene biosynthesis in apples. Postharvest Biol. Technol. 10, 21-27.

Lau, O.L., Liu, Y., Yang, S.F., 1986. Effects of fruit detachment on ethylene biosynthesis and loss of flesh firmness, skin color, and starch in ripening 'Golden Delicious' apples. J. Am. Soc. Hortic. Sci. 111, 731-734.

Lehman-Salada, L., 1996. Instrument and operator effects on apple firmness readings. HortScience 31, 994-997.

Lelievre, J-M, Latche, A., Jones, B., Bouzayen, M., Pech, J-C., 1997. Ethylene and fruit ripening. Physiol. Plant. 101, 727-739.

MacRae, E.A., Lallu, N., Searle, A.N., Bowen, J.H., 1989. Changes in the softening and composition of kiwifruit (*Actinidia deliciosa*) affected by maturity at harvest and postharvest treatments. J. Sci. Food Agric. 49, 413-430.

Mathooko, F.M., Tsunashima, Y., Owino, W.Z.O., Kubo, Y., Inaba, A., 2001. Regulation of genes encoding ethylene biosynthetic enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropene. Postharvest Biol. Technol. 21, 265-281.

McMurchie, E.J., McGlasson, W.B., Eaks, I.L., 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237, 235-236.

Nara, K., Kato, Y., Motomura, Y., 2001. Involvement of terminal-arabinose and -galactose pectic compounds in mealiness of apple fruit during storage. Postharvest Biol. Technol. 22, 141-150.

Oetiker, J.H., Yang, S.F., 1995. The role of ethylene in fruit ripening. Acta Hortic. 398, 167-178.

Payton, S., Fray, R.G., Brown, S., Grierson, D., 1996. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. Plant Mol. Biol. 31, 1227-1231.

Pech, J.C., Guis, M., Botondi, R., Ayub, R., Bouzayen, M., Lelievre, J.M., El Yahyaoui, F., Latche, A., 1999. Ethylene-dependent and ethylene-independent pathways in a climacteric fruit, the melon. In: Kanellis, A.K., Chang, C., Klee, H., Bleecker, A.B., Pech, J.C. , Grierson, D. (eds.), Biology and biotechnology of the plant hormone ethylene II. Kluwer Academic Publishers, Dordrecht, pp. 105-110.

Poovaiah, B.W., Glenn, G.M., Reddy, A.S.N., 1988. Calcium and fruit softening: physiology and biochemistry. Hortic. Rev. 10, 107-152.

Sams, C.E., 1999. Preharvest factors affecting postharvest texture. Postharvest Biol. Technol. 15, 249-254.

Sams, C.E., Conway, W.S., 1984. Effect of calcium infiltration on ethylene production, respiration rate, soluble polyuronide content, and quality of 'Golden Delicious' apple fruit. J. Am. Soc. Hortic. Sci. 109, 53-57.

Sfakiotakis, E.M., Dilley, D.R., 1973. Induction of autocatalytic ethylene production in apple fruits by propylene in relation to maturity and oxygen. J. Am. Soc. Hortic. Sci. 98, 504-508.

Sirit, Y., Bennett, A.B., 1998. Regulation of tomato fruit polygalacturonase mRNA accumulation by ethylene: a re-examination. Plant Physiol. 116, 1145-1150.

Smith, C.J.S., Watson, C.F., Morris, P.C., Bird, C.R., Seymour, G.B., Gray, J.E., Arnold, C., Tucker, G.A., Schuch, W., Harding, D., Grierson, D., 1990. Inheritance and effect on ripening of antisense polygalacturonase genes in transgenic tomatoes. Plant Mole. Biol. 14, 369-379.

Sozzi, G.O., Camperi, S.A., Cascone, O., Fraschina, A.A., 1998. Galactosidases in tomato fruit ontogeny: decreased galactosidase activities in antisense ACC synthase fruit during ripening and reversal with exogenous ethylene. Australian J. Plant Physiol. 25, 237-244.

Tieman, D.M., Taylor, M.G., Ciardi, J.A., Klee, H.J., 2000. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. Proc. Nat. Acad. Sci. USA. 97, 5663-5668.

Tigchelaar, E.C., McGlasson, W.B., Buescher, R.W., 1978. Genetic regulation of tomato fruit ripening. HortScience 13, 508-513.

Tijskens, L.M.M., van Schaik, A.C.R., de Jager, A., Hertog, M.L.A.T.M., 1999. Modelling the firmness of 'Elstar' apples during storage and transport. Acta Hortic. 485, 363-371.

Tong, C., Krueger, D., Vickers, Z., Bedford, D., Luby, J., El-Shiekh, A., Shackel, K., Ahmadi, H., 1999. Comparison of softening-related changes during storage of 'Honeycrisp' apple, its parents, and 'Delicious'. J. Am. Soc. Hortic. Sci. 124, 407-415.

van Schaik, A., Hertog, M.L.A.T.M., 1997. Q-apple can predict loss of hardness in Elstar. Fruitteelt 87, 16-17.

Wallner, S.J., 1978. Apple fruit β-galactosidase and softening in storage. J. Am. Soc. Hortic. Sci. 103, 364-366.

Wang, C.Y., Mellenthin, W.M., Hansen, E., 1972. Maturation of 'Anjou' pears in relation to chemical composition and reaction to ethylene. J. Am. Soc. Hortic. Sci. 97, 9-12.

Watkins, C.B., Bowen, J.H., Walker, V.J., 1989. Assessment of ethylene production by apple cultivars in relation to commercial harvest dates. New Zeal. J. Crop Hortic. Sci. 17, 327-331.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17-32.

Wu, Q., Szakacs-Dobozi, M., Hemmat, M., Hzrazdina, G., 1993. Endopolygalacturonase in apples (*Malus domestica*) and its expression during fruit ripening. Plant Physiol. 102, 219-225.

Yoshioka, H., Kashimura, Y., Kaneko, K., 1995. β-D-galactosidase and α-L-arabinofuranosidase activities during the softening of apples. J. Jap. Soc. Hortic. Sci. 63, 871-878.

Chapter 11 Appendices

11.1 Appendix A

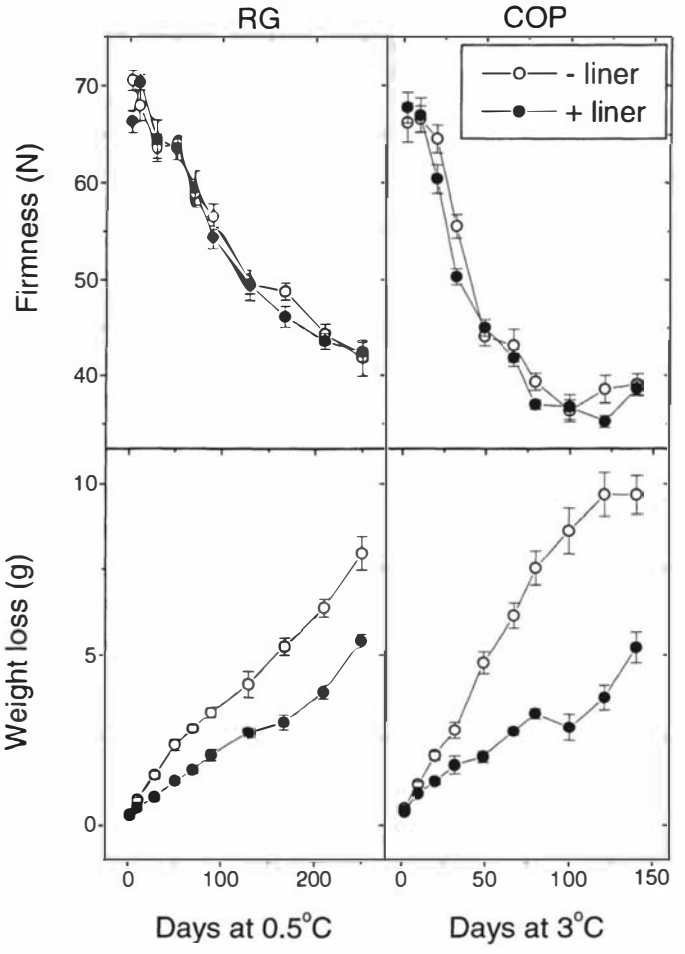

Fig. 11-1 Firmness and weight loss of 'Royal Gala' (RG) apples at 0.5°C and 'Cox's Orange Pippin' (COP) apples at 3°C stored in commercial cardboard cartons with (+) and without (-) commercial perforated polyethylene plastic liners. Means (n=10) and standard errors of the mean are shown. Firmness was measured as described in Chapter 3.

11.2 Appendix B

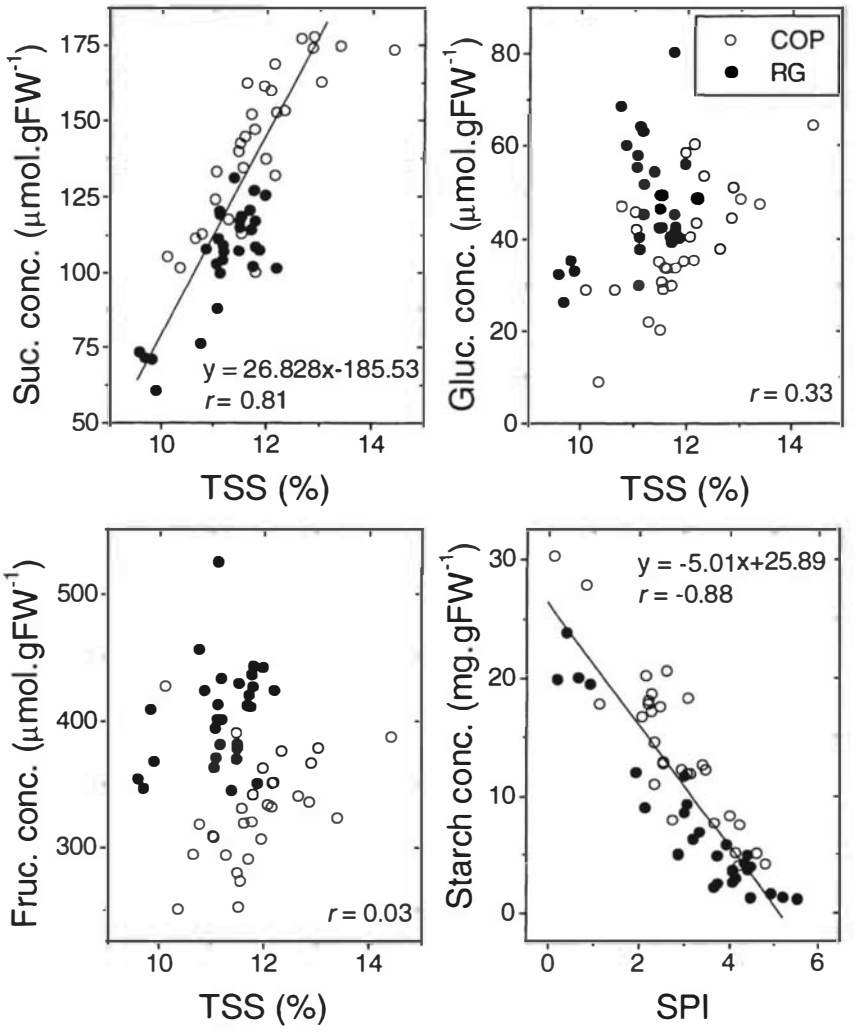

Fig. 11-2 Sucrose (suc.), glucose (gluc.) and fructose (fruc.) concentrations for 'Royal Gala' (RG) and 'Cox's Orange Pippin' (COP) apples with different total soluble solids (TSS), and starch concentrations for apples from these cultivars with different starch pattern index (SPI) ratings. Mean (n = 15 fruit) concentrations are shown for fruit from different orchards. Methods for measuring each variable are described in Chapter 8.3.