The Influence of Breaks in Optimal Storage Conditions on 'Cripps Pink' Apple Physiology and Quality

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Andrew Richard East

BE (Hons) (Food Engineering)

Abstract

Apples stored onshore in Australia and New Zealand, are maintained at optimal storage conditions with the aid of low temperatures; controlled atmospheres (CA) and new technologies that retard the production or effect of ethylene (AVG and 1-MCP respectively). These technologies allow distribution of the highest quality apples to local and export markets on a year round basis. However, during distribution, maintenance of optimal storage conditions may be lost due to refrigeration system breakdown, operational constraints or management decisions. This thesis quantifies the influence of commercially realistic breaks in optimal storage conditions (temperature and CA) on fruit physiology and quality, both at the time of the break and in subsequent optimal storage conditions. The 'Cripps Pink' ('Pink Lady™') apple cultivar was chosen for consideration in this thesis because it is a high value cultivar that is of considerable importance to the Australian apple industry.

The knowledge of the behaviour of 'Cripps Pink' apples in coolstorage conditions (in air and CA) was confirmed through comparison of physiological and quality change behaviour of fruit from three harvests collected in this research and those reported recently by other authors. The investigation of the influence of breaks in temperature control during storage in air at 0°C, revealed that preclimacteric apples exposed to a break in temperature control, were advanced towards the establishment of the climacteric. Postclimacteric apple, responded by doubling ethylene production a short time after return to coolstorage. Harvest maturity, timing of break during coolstorage, length of break of temperature control and multiple breaks in temperature control, had little influence on the increase ethylene production response. Quality factors (firmness, background hue angle, and titratable acidity) were all reduced as a result of exposure to warmer temperatures, but on return to coolstorage temperatures rates of loss in these quality factors were not influenced by the increased ethylene production.

Short-term (3-day) breaks in CA while fruit remained at refrigerated temperatures were shown to have no substantial effect on fruit physiology or quality, either during the period of the break in CA or in subsequent CA storage. Breaks in temperature control in combination with breaks in CA were observed to cause a doubling of ethylene production on CA stored apples regardless of being returned to 0°C in air or CA. Those apples that were exposed to a break in temperature control and returned to air storage at refrigerated temperature lost quality (firmness and background hue angle) more rapidly than apples not

exposed to breaks in temperature control and transferred to air storage. This result strengthened the knowledge of the influence of ethylene on changes in apple quality, as found for many other apple cultivars.

The influence of the decision to transport fruit in CA or air atmosphere shipping containers was initially investigated with a laboratory simulation. Physiology (respiration rate and ethylene production) of air shipped fruit was found not only to be more rapid, but more variable between fruit, than for apples shipped in CA. This more rapid and larger variation of possible fruit physiologies, suggests that in addition to losing quality at a faster rate, the variation in the quality of fruit shipped in air will also enlarge during shipment. This hypothesis was confirmed with data pooled from treatments subjected to 0°C and 3°C, simulating the likely temperature variability within a shipping container. Validation of the influence of shipping atmosphere on delivered fruit quality, was conducted in the commercial environment. This trial found that the length of time to ship fruit from Australia and New Zealand to European markets was not sufficient to induce commercially significant differences between 'Cripps Pink' apples shipped in the two atmospheres.

Finally, as ethylene production was influenced by fluctuations in temperature control and subsequently affected quality of apples previously stored in CA, an investigative attempt to model ethylene production in temperature variable scenarios was conducted. Published models of ethylene production in apples were adapted to the variable temperature storage scenario and a new model was proposed. Unfortunately, none of the models investigated were able to predict all of the consistent behaviours of ethylene production observed during the experimental work, indicating that more knowledge of the ethylene production pathway is required, before modelling of ethylene production and subsequently apple quality can be conducted successfully.

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Contents

1. Thesis Outline

The Australian and New Zealand apple industries have been a traditional strength of the horticultural sector of both countries, generating AUS$41M and NZ$560M in export earnings for each country respectively (Anon, 2004). The 'Cripps Pink' cultivar represents 23% of all apple plantings of the AUS$220M Australian apple industry, while only representing 2% of the apple export industry in New Zealand.

Apples that are harvested between March and May, are stored onshore in Australia and New Zealand, with the aid of low temperatures; controlled atmospheres and new ethylene retarding (AVG) and blocking (1-MCP) technologies. This allows distribution of the apples to both local and export markets on a year round basis. Considerable effort is made to maintain optimal storage conditions, to allow the product to be stored for as long as possible, and to ensure that the highest quality product is delivered to consumers after removal from storage. However, during distribution, optimal storage conditions may be lost because of refrigeration system breakdown, operational constraints (e.g. portside genset facilities) or management decisions (e.g. use of nonrefrigerated curtainsiders for short journeys). This research attempts to quantify the influence of breaks in optimal storage conditions on fruit physiology and quality, both at the time of the break and in subsequent optimal storage conditions.

This research was conducted as a joint project between Food Science Australia and Massey University, New Zealand. Subsequently, the experimental research was conducted in both countries, while a further period was spent at the Catholic University of Leuven, Belgium in order to gain postharvest modelling knowledge. As a fruit subject, the 'Cripps Pink' ('Pink Lady™') apple cultivar was chosen, as it allowed comparison to the well documented behaviour of other apple cultivars, and the generation of knowledge of a high value cultivar with future importance to the Australian apple industry.

The second chapter provides insight to current knowledge on apple physiology and quality, and the influence of temperature, controlled atmospheres and ethylene; and the modelling of postharvest physiological and quality changes. The third chapter establishes the behaviour of 'Cripps Pink' apples in constant optimal storage conditions, both in air and in controlled atmospheres, and compares these to the finding for 'Cripps Pink' and other apple cultivars, in order to clearly establish cultivar specific behaviour.

The forth chapter, reveals the results of the investigation of the influence of breaks in temperature control during storage of 'Cripps Pink' apple in air at 0°C, on apple physiology and quality during the temperature break and in subsequent coolstorage. The influence of harvest maturity, timing of break, length of break and influence of multiple breaks were all investigated, over a two season period. The rate of production of the plant ripening hormone ethylene was found to be consistently influence by breaks in temperature control, whereas respiration rate and quality indices (firmness, colour, and titratable acidity) showed little influence.

The fifth chapter investigates the influence of the effect of breaks in controlled atmospheres while remaining in coolstorage and in combination with breaks in temperature control, through simulation experiments conducted in the laboratory environment. Additionally, the influence of atmosphere and small temperature variations (0-3°C) on return to coolstorage was investigated, in order to determine the influence of the decision to transport fruit in CA and air atmospheres shipping containers. Breaks in temperature control were observed to have the same influence in CA stored apples as that previously found in air stored apples, while significant physiological differences were measured during the simulated shipped period which resulted in quality differences between treatments after a long period of time.

An experiment conducted in commercial conditions, in which two 40 foot containers, one each of air and controlled atmospheres, were monitored during shipment from Western Australia to the United Kingdom, and the quality of the fruit evaluated at the destination, is detailed in chapter 6. This experiment was used to validate the results obtained in the laboratory environment, and allowed more accurate quantification of the influence of atmosphere selection during shipping on quality outcomes.

In the seventh chapter, the work turns to the attempted modelling of ethylene production, the physiological response found to be dependent on temperature fluctuation in the experimental work, and a hormone that has well established links to affecting apple quality changes. Recently published models of ethylene production in apples are adapted to the variable temperature storage scenario and compared to the performance of a new proposed model. None of the models presented are able to predict all of the consistent behaviours of ethylene production observed during the experimental work, indicating that more

knowledge of the ethylene production pathway is required, before modelling of ethylene and subsequently apple quality can be conducted successfully.

In the final chapter, overall conclusions and suggestions for future research are formulated.

2. Literature Review

2.1. THE INFLUENCE OF STORAGE CONDITIONS ON POSTHARVEST FRUIT QUALITY

A feature of fresh fruit as a food commodity is that the product remains a living biological system to the point of consumption. After the point of harvest fruit continue the processes of respiration and transpiration, taking in oxygen (O_2), and producing carbon dioxide (CO_2) and heat, and losing water. When attached to the plant the energy and water lost during metabolic activities can be replaced by the plant via the xylem and phloem. However, once removed, the fruit is reliant on its own limited resources for existence. Subsequently, from the point of harvest all fruit deteriorate. This deterioration causes changes in the fruit appearance, flavour and texture, significantly influencing the acceptance of the fruit to the consumer (Hoehn et al., 2003).

The lifespan of a fruit can be divided in to three phases; growth, maturation and senescence, although clear distinction between each of the phases can be difficult. The growth phase occurs while on the tree and involves cell division, subsequent fruit enlargement and ultimately the definition of the final fruit size. Maturation usually begins before the completion of the growth phase, and causes different responses depending on the fruit and cultivar (Wills et al., 1998). Ripening is the process of transforming physically mature but inedible fruit, into a fresh food product, through a vast range of biochemical changes (Table 2.1). Senescence is defined as the time in which catabolic (degredative) processes dominate anabolic (synthetic) processes. Ripening and senescence of fruit may occur on or off the plant.

Table 2.1, Changes that may occur during ripening. (Adapted from: Wills et al, 1998)

Seed Maturation
Colour changes
- Chlorophyll degradation (loss of green)
- Lycopene accumulation
- Carotenoid accumulation
- Anthocyanins
Increased Respiration rate and ethylene production
Increased Tissue Permeability
Cellular compartmentalisation
Softening
Carbohydrate composition changes
Organic acids reduction
Protein changes
Production of flavour volatiles
Development of wax on skin

Postharvest science encompasses the understanding and manipulation of the natural ripening and senescent processes of fruit in order to reduce wastage and deliver more acceptable products to consumers and hence provide financial benefits to fresh fruit and vegetable industries. The manipulation of temperature, atmosphere and plant hormone ethylene in postharvest systems are commonly used methods for obtaining shelf life and quality benefits (Johnston et al., 2002a).

2.1.1. Temperature

Temperature is the single most important factor governing the maintenance of postharvest quality of fruits and vegetables (Kays, 1991; Wills et al., 1998). The use of cool temperatures (-1°C to 15°C) for fresh produce enables preservation of a product, creating opportunities for the products to be transported to distant markets and be marketed for extended periods of time.

The mechanism of preservation by cool storage is to slow the metabolic processes (respiration rate and ethylene production) within the product and hence reduce the rate of senescence. Consequently, the rates of change of firmness, colour, acidity and nutritional quality are greatly reduced (Paull, 1999). Lower temperatures provide further benefit by reducing the growth of plant pathogenic organisms and hence reducing product loss by spoilage (Wills et al., 1998).

The stress of being stored at low temperatures can have negative effects on the product quality (Paull, 1999). A vast range of cultivar specific discolourations and textural changes can occur and are all classified under the term of chilling injuries. Chilling injuries are defined as disorders that reduce product quality as a result of prolonged cool storage and are due to imbalances in fruit metabolism at the lower temperatures. Products from sub-tropical and tropical environments are extremely prone to the development of chilling injuries and are subsequently stored above 13°C (Wills et al., 1998). Even fruit from more temperate environments, such as apples and kiwifruit can develop chilling injuries, although these are usually more sporadic in occurrence and affect only certain cultivars and growing regions.

2.1.1.1. The Influence of Temperature Variation on Produce Quality

Studies conducted at constant temperature conditions can indicate optimal storage temperature for extension of postharvest shelf life. However,

investigations into the use of fluctuating temperatures (Artes et al., 1998), pre-storage heat treatments (Klein and Lurie, 1992) or dual temperature storage regimes (Taylor et al., 1994) have found that further quality benefits can be derived from applying variable temperature regimes. Additionally, warm or cold temperature treatments can be used as a method of non-chemical disinfestations (Hoffman et al., 2003; Heather et al., 2002), which is becoming more popular, as the industry looks for alternatives to chemicals disinfestation. There is a greater realisation that despite industry best practice, maintenance of a "perfect" coolchain which provide optimal storage temperatures to products at every step is unrealistic (Tanner at al., 2003). Hence, the study of the influence of fluctuating temperature scenarios on postharvest perishables physiology and quality is an emerging field.

Due to the vast quantity and largely poorly understood mechanisms of senescence in perishable products and the variation in senescent processes due to preharvest conditions, time of harvest, species, cultivar and product maturity the study of the effects of variable temperatures on the physiology and quality of produce is conducted empirically. As a result a large body of work exists in which produce exposed to variable temperatures is compared to fruit stored at a constant (and often considered optimal) temperature at the end of a cool storage period and/or after a simulated shelf life. In general this vast body of work can be classified into the investigation of the following effects:

a. Delay times prior to cool storage.
b. Applying an elevated heat treatment before cool storage.
c. Intermittent warming during storage.
d. Cold-shock treatments before storage.
e. Intermittent cooling during storage.
f. Low temperature conditioning before storage.
g. Dual temperature storage.
h. Fluctuating temperatures around a set point temperature.

Each of the scenarios is reviewed with focus on the research conducted with apples and pears as the fruit used in this research is the 'Cripps Pink' apple.

As the focus of this study is the influence of breaks in temperature control, the information from the studies of delay times before storage (a) and intermittent

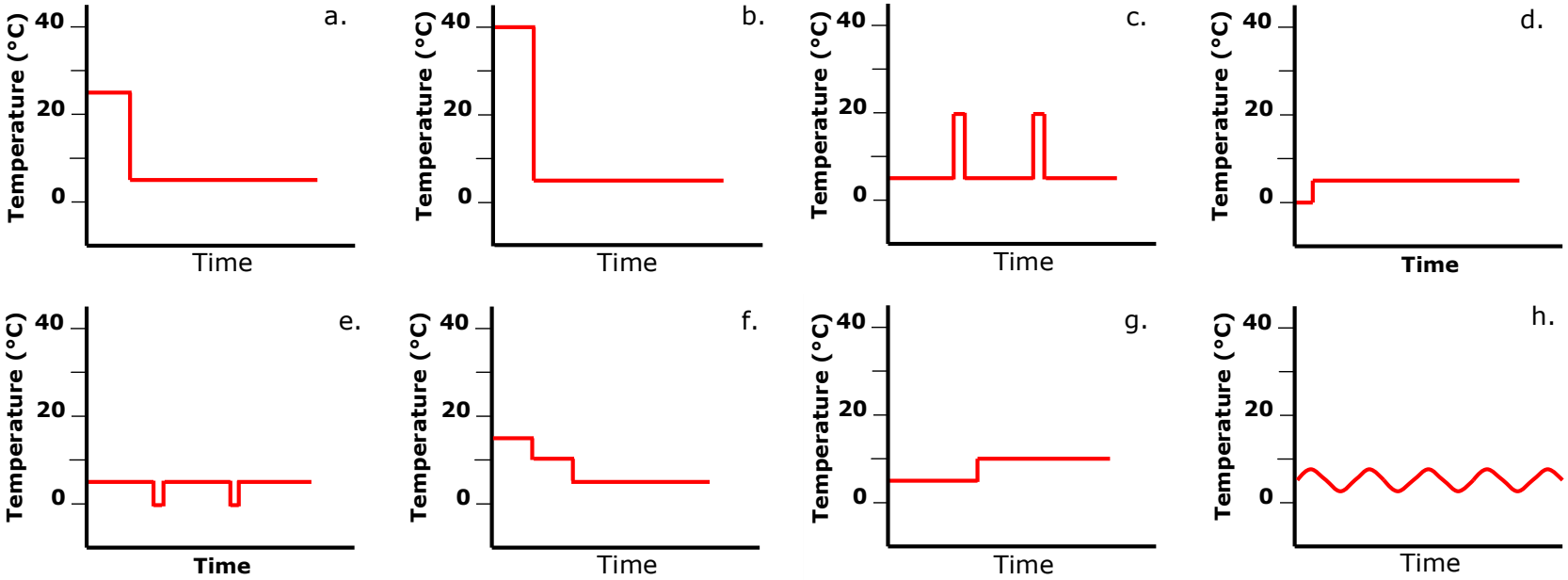

Figure 2.1, Diagrammatic representation of the variable temperature scenarios investigated in postharvest horticulture

a. Delay times prior to cool storage.
b. Applying an elevated heat treatment before cool storage.
c. Intermittent warming during storage.
d. Cold-shock treatments before storage.
e. Intermittent cooling during storage.
f. Low temperature conditioning before storage.
g. Dual temperature storage.
h. Fluctuating temperatures around a set point temperature.

Other possible applied treatments were not investigated in this study, as they either were above the range of environmental temperatures (b); required a temperature below optimal (0°C) and hence would cause freezing (d and e); or involved non-optimal temperature exposure for long time periods (f, g and h). However, these treatments do provide information of the possible physiological and quality inducements that may occur, due to exposure to fluctuating temperature conditions.

2.1.1.1.1. Delay Times Prior to Cool Storage

Delays between the harvest of a product and cool storage can be caused by delays in transportation between field and storage facility, packing and sorting operations, and a limited capacity at cooling facilities (Figure 2.1a). This generally results in a reduced quality of the product at the completion of storage. The amount of delay that causes significant quality change is related to the inherent shelf life of the product. Broccoli (a product with a short shelf life), is affected by delays as short as 3 hours (Brennan and Shewfelt, 1989), where as pears (a long storing fruit) are not affected by delays of up to a week (Stow, 1988).

In a variety of fruit types a delay in cooling has been shown to cause increased ethylene production at cool storage (Liu, 1986), reduced fruit firmness (Lui, 1986; King and Henderson, 1988), more chlorophyll degradation (Stow, 1988; Downs et al., 1989; Crisosto et al, 1994), titratable acidity reduction (Lui, 1986; King and Henderson, 1988) greater weight loss (Nunes et al., 1995) and increases in microbial load (Harvey and Harris, 1986; Jackson et al., 1999). In some cases a delay can be beneficial in reducing subsequent pathological decay as a delay time allows development of protective layers on wounded sites (which are sites for infection) caused by picking (Pennycook and Manning, 1992).

2.1.1.1.2. Elevated Heat Treatments Before Cool Storage.

Heat treatments are applied to fruit before cool storage for the purpose of insect disinfestation, disease control and to reduce chilling injury development in cool storage (Figure 2.1b). The effect of the temperature applied and duration of the heat treatment is investigated with respect to the resulting effect on product quality at the end of a cool storage period. In general, heat treatments between the range of 35°C to 45°C for periods of time between 0.5 – 6 hours can result in significant benefits to product quality both at the end of cool storage and during a subsequent shelf life period. Temperature/time treatments below this generalised range are detrimental to product quality and can be treated as an identical

process to a delay in cooling of the product while more severe temperature/time treatments result in "cooked" product (Klein and Lurie, 1990).

Heat treatments have been shown to reduce chilling injury in apples and pears (Neven et al., 2000), avocados (Florissen et al., 1995; Woolf et al., 1995) and oranges (Wild and Hood, 1989). During heat treatment heat shock proteins that act to protect other proteins in the cell under stress conditions are produced. On cooling the product these heat shock proteins protect the cell from decompartmentalisation (a symptom of chilling injuries) even though the stress has changed from heat to cold (Florissen et al., 1995; Woolf et al., 1995). The heat shock proteins decay, but because the product is at low temperatures and hence has a low metaboloism, the decay of the proteins is slow. Eventually chilling injury occurs after extended cool storage periods, once the heat shock proteins have decayed (Sabehat et al., 1996).

The respiration rate of fruit increases during heat treatment and subsequently declines to near or less than that of non-heated fruit (Klein and Lurie, 1990). Conversely, ethylene synthesis (and response) is inhibited during heat treatments but also returns to levels similar to that of untreated fruit (Klein and Lurie, 1990) on return to cool storage.

The heat treatment itself can reduce the microbial load (Neven et al., 2000), decrease titratable acidity while not affecting soluble solids (Porrit and Lidster, 1978; Klein and Lurie, 1992) and cause an increase or decrease in product weight depending on the processed used (Woolf et al., 1995; Riquelme, 1998). Pre-storage heat treatments have been observed to reduce the loss of firmness (Porritt and Lidster, 1978; Klein and Lurie, 1992; Neven et al., 2000) and chlorophyll loss (Klein and Lurie, 1990) during the storage of apples.

2.1.1.1.3. Intermittent Warming During Cool Storage.

Intermittent warming is applied by periodically removing the product from cool temperatures by warming to ambient temperatures for a short period of time (Figure 2.1c) and is primarily used as a method of alleviation of chilling injury (Artés et al., 1998). The warming process generally advances the senescence of the product, ripening the product further than what would have occurred if stored constantly at the lower storage temperature. Duration, temperature and frequency of the warming period can all influence the final product quality. For short storing product (e.g. tomato) oscillations of approximately 6 days at cool

temperatures and 1 day at 20°C are recommended (Artés et al., 1998), whereas long storing products such as citrus are better treated with 3 weeks at cool temperature oscillated with 2 weeks at room temperature (Schirra and Cohen, 1999) to avoid chilling injury.

Intermittent warming during the storage of apples has been observed to reduce the incidence of superficial scald in apples (Watkins et al., 2000a), although not to commercially acceptable levels, and at the expense of advanced ripening and reduced storage life.

Many other fruit cultivars also reduce the incidence of chilling injuries when intermittent warming is applied, including peach (Anderson, 1979; Fernández-Trujillo and Artés, 1997; Perkins-Veazie, 1999), lemon (Artés et al., 1993; Cohen et al., 1990), mango (Nyanjage et al., 1998), mandarin (Schirra and Mulas, 1995), bell pepper (Risse and Chun, 1987), plums (Kotze et al., 1989), oranges (Schirra and Cohen, 1999) and tomatoes (Artés et al., 1998). Additional benefits of intermittent warming treatments include increased titratable acidity (Cohen et al., 1990; Perkins-Veazie et al., 1999) and soluble solids (Nyanjage et al., 1998) and decreased extent of pathogenic decay (Risse and Chun, 1987; Nyanjage et al., 1998).

It has been shown that during intermittent warming treatment of tomato and citrus fruit respiration and ethylene production increases, only to return to its original cool storage levels on subsequent cooling (Cohen et al., 1990; Shira and Mulas, 1995; Artes et al., 1998; Kluge et al., 2003). However, the respiration rate of mandarin was reduced during the cool storage phase of intermittently warmed fruit but was not continued during a subsequent shelf life (Schirra and Mulas, 1995). In contrast, the ethylene production of peaches has been observed to be increased during subsequent cool storage after intermittent warming (Zhou et al., 2001).

Intermittent warming treated fruit have a reduced quality (firmness and chlorophyll degradation) at the completion of the storage period, as a result of faster quality change at the time of exposure to warm temperatures (Fernández-Trujillo and Artés, 1997; Nyanjage et al., 1998). The subsequent rate of quality changes at cool temperatures have not been observed to be influenced by the intermittent warming treatments (Fernández-Trujillo and Artés, 1997).

2.1.1.1.4. Cold Shock Treatment Before Storage

Cold shock treatment has been trialled to prevent chilling injury and subsequently extend shelf life of horticultural products. Exposure to temperatures close to freezing may induce the production of protective metabolites as observed with heat treatments. Goto et al. (1984) provided evidence that a cold shock treatment caused a change in the fatty acid composition of membranes. Cold shock treatment can be applied by dipping product in ice water or salted water for periods of up to 2 hours, or with the use of -1°C air for periods up to 24 hours (Figure 2.1d).

No studies exist investigating the effect of cold shock treatments on apples or pears. This is likely to be due to the fact that these products are optimally stored at close to their freezing point, with only sporadic development of chilling injuries, and hence do not provide much scope for chill shock treatment to take place before freezing. Cold shock treatment of tomatoes has been observed to reduce weight loss during storage and reduce colour development and texture loss (Silva and Vietes, 1998), subsequently increasing shelf life by 10 days without inducing chilling injury (Inaba and Crandall, 1986). Cold shock treatments have also been found to be successful in reducing chilling injury in apricots (Ogata and Sakamoto, 1979) and plums (Goto et al., 1984) and causing degreening of mandarins (Barry and van Wyk, 2006) while being unsuccessful for passionfruit (Silva et al., 1999).

2.1.1.1.5. Intermittent Cooling During Cool Storage

Intermittent cooling unites the idea of the formation of protective metabolites at close to freezing temperatures and the idea of regular exposures to intermittent warming (Figure 2.1e). In an explorative study of the application of intermittent cooling, Artés et al. (1998) stored tomatoes at 12°C and exposed them to 1 day at 2°C every 6 days. Rates of colour change were unaffected by the intermittent cooling treatment and sensory scores favoured the intermittent cooling treated fruit, although incidence of chilling injury was higher.

2.1.1.1.6. Low Temperature Conditioning Before Cool Storage

Low temperature conditioning describes the use of slower cooling to the optimal storage temperature by applying intermediate storage temperatures during cooling (Figure 2.1f). In the apple industry, this technique is referred to as step-wise cooling (Little and Holmes, 2000). Holding cold sensitive products at temperatures slightly above that which induce injury, develops tolerance to those

normally damaging temperatures as cell membranes are given the opportunity to perform compositional changes to enable regular cell function at cooler temperatures (Woolf et al., 2003). Low temperature conditioning has shown to be an effective means of preventing chilling injury in bell peppers (Risse and Chun, 1987; Risse et al., 1987), mango (Chaplin et al., 1986; Graham, 1988), zucchini squash (Kramer and Wang, 1989), grapefruit (Hatton and Cubbedge, 1983), green tomatoes (Lurie and Sabehat, 1997), kiwifruit (Maguire et al., 2005) and avocado (Woolf et al., 2003; Hoffman et al., 2003) often with no other quality differences in comparison to fruit cooled straight to optimal conditions.

2.1.1.1.7. Dual Temperature Storage

Dual temperature storage was developed to alleviate the stress on the product at the point in time in which chilling injury usually occurs by increasing the storage temperature permanently in order to avoid the chilling injuries. Most studies of dual temperature storage are applied to stone fruit. Dual temperature storage reduces chilling injury in plums (Hartmann et al., 1988; Kotze et al., 1989; Taylor and de Kock, 1995), apricots (Taylor and de Kock, 1992) and green tomatoes (Lurie and Sabehat, 1997) and promotes chilling injury in peaches and nectarines (Anderson, 1979). Generally, dual temperature storage results in fruit being in a more advanced state of senescence at the completion of the storage period, in comparison to those fruit stored continuously at the lower temperature (Hartmann et al., 1988; Taylor and de Kock, 1992).

2.1.1.1.8. Oscillating Temperatures Around a Set Point

Regular oscillations of temperature occur in cool storage situations due to refrigeration system operation (Figure 2.1h). The amplitude of fluctuation is largely governed by refrigeration control system set points, whereas the frequency of fluctuation between these set points is a direct reflection of the heat load entering the storage facility and the refrigeration system capacity. The influence of small temperature oscillations of an amplitude of 1-5°C in a cool storage environment was conducted by Ito and Nakamura (1985), which provided some evidence that small oscillations had a small influence on the sensorial measured quality of a number of products stored at an average of 6°C.

2.1.2. Controlled Atmosphere Gas Conditions

The knowledge that change in the components of the gas atmosphere influences the rate of quality changes was established in pioneering storage studies with apples in the 1920's and 1930's (Kidd and West, 1930). In recent times, CA

atmospheres with reduced O_2 and enriched CO_2 are used commercially to significantly extend the storage life of apples. Desirable responses of horticultural products in modified atmospheres include a reduction in oxidative tissue damage and discolouration and the rates of respiration and ethylene production resulting in associated reduced rates of ripening and ethylene mediated phenomenon such as chlorophyll degradation (Beaudry, 1999). Despite the widespread adoption of CA technology in the industry, the mechanisms by which CA influences quality changes are not completely understood (Hertog et al., 2001).

Negatively, CA can induce fermentation, disagreeable flavours and tissue injury, while altering the makeup of microbial fauna (Beaudry, 1999) and reduce aroma biosynthesis (Fellman et al., 1993). Extremely low O_2 levels or excessively high CO_2 levels can induce fermentation, resulting in the generation of off-flavours and tissue damage (Beaudry, 1999). However, occasions do exist where atmospheres close to inducing fermentation provide benefits. Ultra-low O_2 (ULO) storage (0.7% O_2) can significantly reduce the incidence of superficial scald in 'Delicious' apples (Lau, 1997).

The influence of O_2 partial pressures on metabolic activity can be divided into three zones (Beaudry, 1999). Above 5% O_2 the plant is metabolically active and able to provide sufficient energy; between 2-5% O_2 it is suggested that an elastic zone is present in which O_2 uptake declines and marked metabolic adjustments are required to match CO_2 production with O_2 consumption. Below 2% O_2 energy levels begin to decline rapidly and fermentation is induced as possibly the mechanism for the plant material to generate the energy required for survival. CO_2 effects are largely measured above 20% CO_2, although approximately 5% CO_2 can reduce rates of sugar utilisation and alter the activities of a number of enzymes involved in glycolysis. The exact concentrations of O_2 and CO_2 that cause these changes in activity vary for different species and varieties.

Rates of firmness changes in apples are significantly reduced in CA storage conditions. Johnston et al. (2006) reported that the main effect of CA storage was to delay the initiation of rapid softening and slow the overall rate of softening. Siddiqui et al. (1996) reported firmness differences in 'Golden Delicious' apples that had been stored for 4 or 6 months in air, CA (3% CO_2 and 3% O_2) or ULO (3% CO_2 and 1% O_2), with rates of softening being slower with less O_2. Total cell wall content decreased by the same amount on all treatments, but the composition of the cell wall changed differently, with the ULO treatments

containing more pectin and hemicellulose than the other treatments, suggesting that CA may directly influence the cell wall changes that affect firmness.

It has been suggested that the primary action of CA atmospheres is the reduction of ethylene synthesis and sensitivity to ethylene (Gorny and Kader, 1997) and/or reducing respiration (Beaudry, 1999; Hertog et al., 2001). Johnston et al. (2006) showed that CA caused the rate of increase in internal ethylene concentration during climacteric development to be decreased and the maximum internal ethylene concentration to be less than that observed for fruit in air storage.

2.1.2.1. The Influence of Gas Atmosphere Variation on Produce Quality

While the application of constant CA has been shown to have significant quality benefits, with the exception of the requirement to rapidly establish CA after harvest, the influence of changing atmospheric conditions on postharvest product physiology and quality has not been clearly established.

2.1.2.1.1. Delays in Controlled Atmosphere Establishment

A number of studies have investigated the influence of delays in establishing CA on apple physiology during CA and on final product quality at the completion of storage. Almost without exception, all studies have found that delays in excess of approximately 3-6 days in establishing CA results in the apples producing more ethylene while in storage and significantly reducing firmness, titratable acidity and background colour at the completion of storage (Sharples and Munoz, 1974; Lau and Looney, 1982; Liu, 1986; King and Henderson, 1988; Johnston et al., 2006).

2.1.2.1.2. Removing Controlled Atmosphere While Remaining in Cool Storage

When Johnston et al. (2006) shifted apples from CA to RA after 50 or 80 days in CA, rapid softening was either initiated immediately (for 'Royal Gala') or after a small delay of 0-10 days (for 'Cox's Orange Pippin'). Rates of softening in air after CA storage were faster than that of fruit stored constantly in RA for 'Cox's Orange Pippin', while 'Royal Gala' softening was similar to that of fruit constantly in RA. Softening rates at shelf life temperatures were relatively constant, irrespective of previous gas atmosphere treatments.

2.1.3. Ethylene

Ethylene plays an essential role in the complex process of fruit ripening in association with other hormones and developmental factors (Pech et al., 2002).

It is biologically active in trace amounts and its effects are commercially important (Yang and Hoffman, 1984). Delaying senescence by reducing ethylene biosynthesis and action has been a major goal of postharvest physiologists.

Autocatalytic ethylene production in which massive increase in ethylene production is triggered by exposure to ethylene is a characteristic feature of ripening climacteric fruits. Although exogenous ethylene hastens the onset of climacteric ethylene production, the time between an exogenous treatment with ethylene and the onset of climacteric ethylene production may be days or weeks depending on the maturity and sensitivity of tissues. Thus, the role of ethylene in triggering the onset of climacteric ethylene production is complex, and probably indirect (Pech et al., 2002).

In addition to featuring at the onset of ripening of climacteric fruit, ethylene is also associated with physical stress. Actions including, mechanical wounding (e.g. cutting and bruising), radiation, infection, extreme temperatures, drought, flooding and chemical use including herbicides and other pollutants has been found to stimulated ethylene production within 10-30 minutes which later subsides after reaching a peak within several hours (Yang and Hoffman, 1984). The ability for stress to induce ethylene rapidly and for a short time period, demonstrates the responsive ability of the ethylene production system.

2.1.3.1. The Biochemistry of Ethylene in Fruit

2.1.3.1.1. Ethylene Production

Yang and Hoffman (1984) described the conversion of methionine to ethylene via the precursors of S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC), (Figure 2.2). Although this knowledge has been well established the factors that control, regulate and stimulate this biochemical pathway are far from being well understood. This section provides a review of the current knowledge of the ethylene biosynthesis pathway and its rate controlling mechanisms.

Methionine is an essential amino acid that is converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase. The methionine is able to be reconstituted at the cost of energy through the Yang cycle, allowing ethylene to be produced with a limited supply of methionine (Adams and Yang, 1977).

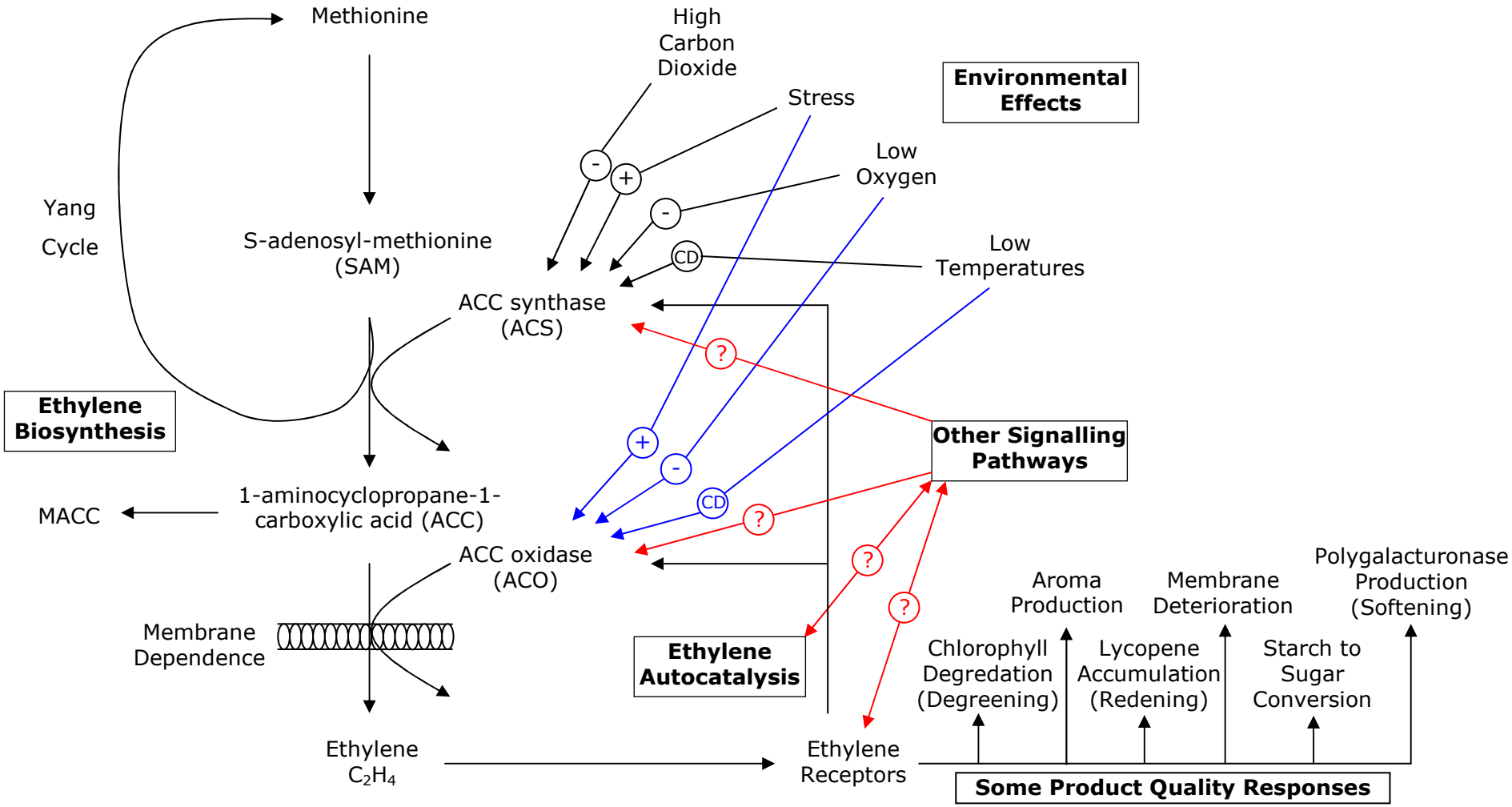

Figure 2.2, Ethylene production and interactions reviewed. + symbols represent an increased abundance and/or activity of enzyme that results in a faster rate of reaction, – symbols the opposite. CD indicates a cultivar dependent response. ? indicates a likely response of an unknown direction.

SAM is a major methyl donor in plants and is used as a substrate for many biochemical pathways. The ability to convert methionine to SAM has been shown in preclimacteric fruit that produce very little to no ethylene (Yang and Hoffman, 1984). Subsequently, this step in the ethylene biosynthesis process is not considered to influence the rate of ethylene production.

The conversion of SAM to ACC is conducted and regulated by the ACC synthase (ACS) enzyme also producing 5'-methylthioadenosine (MTA), which is then converted back to methionine through the Yang cycle. The amount of ACC and ACS has been found to be low in preclimacteric tomatoes (Kende and Boller, 1981), avocado and banana (Hoffman and Yang, 1980). In all cases, initiation of the climacteric ethylene production is partnered by a marked increase in ACC content. These results lead to the belief that the conversion of SAM to ACC (controlled by the abundance and activity of ACS) is a rate limiting step of ethylene production in climacteric fruit.

The conversion of ACC to ethylene is regulated by the ACC oxidase (ACO) enzyme. In preclimacteric fruit, the addition of ACC causes a slight increase in ethylene production, far short of that observed during climacteric ethylene production, indicating that ACO also has a role in controlling the rate of ethylene biosynthesis (Yang and Hoffman, 1984).

The ACO protein is located on the plasma membrane (Ramassamy et al., 1998) and hence ACO activity is in some way dependent on membrane and tissue integrity (Anderson et al., 1979). An increase in ACC and an accompanied low ethylene production has been observed as some fruit become overripe (Hoffman and Yang, 1980) while treatment with processes that deteriorate the membrane structure and disrupt membrane functionality were found to inhibit conversion of ACC to ethylene (Anderson et al., 1979).

2.1.3.1.2. Control of Ethylene Metabolism

The question of what stimuli and at what level those stimuli regulate both rate controlling enzymes ACS and ACO has been the focus of modern ethylene research. An emerging theory is that different isoforms of ACS and ACO exist, each of which are differently regulated (Wang et al., 2002). Developmental, environmental, and chemical factors could control the expression of the same or of different ACS and ACO genes. Pech et al. (2002) studied the ACS gene family in 'Prass Crassane' pear. The expression of ACS1 gene was strongly stimulated during cold storage, whereas the ACS3 gene expression was significant at harvest and absent during chilling treatment conditions. Another two genes, ACS4 and ACS5 were essentially related to the climacteric peak of ethylene

production. When the ripening process was initiated, all 4 genes exhibit high levels of expression.

A theoretical model to explain the induction of autocatalytic ethylene production was put forth by McMurchie et al. (1972). This model describes two ethylene biosynthesis induction mechanisms that operate in climacteric fruit, designated as system I and system II. System I is functional during normal vegetative growth, is ethylene autoinhibitory and is responsible for producing basal ethylene levels that are detected in all tissues including those of non-climacteric fruit. Immature fruit have a nonautocatalytic system I ethylene production capability. When the competency to ripen occurs, an autocatalytic system II ethylene biosynthetic capability is induced. System II usually commences in one region of a fruit, spreading to neighbouring regions as ethylene diffuses freely from cell to cell and integrates the ripening process throughout the fruit (Alexander and Grierson, 2002).

Much debate exists as to what triggers mature fruit to shift from system I to system II ethylene biosynthesis. The potential for cross talk between ethylene, other hormones such as auxin, abscisic acid and cytokinin; and the sugar signalling pathway makes the process of unravelling the role that ethylene plays in ethylene production and fruit ripening complex (Alexander and Grierson, 2002).

In ethylene-suppressed melons, ACS activity is induced at the same time as in control melons, indicating that ACC biosynthesis during the early stages of ripening seems to be an ethylene independent process (Pech et al., 2002). Thus ethylene triggers the onset of ripening and as a consequence of ripening, a massive increase in ethylene production occurs resulting from the development of both ACS and ACO which are limited in preclimacteric fruits, (Pech et al., 2002).

As more components of the ethylene response pathway come to light, it is apparent that there are numerous ways to induce hormone production and perceive the signal, effecting further ethylene production and fruit ripening (Johnson and Ecker, 1998). Further characterisation of members of the ACO and ACS gene families may eventually identify individual members associated with system I and system II ethylene production. Alternatively, the same genes may be regulated by different factors in preclimacteric and climacteric fruit (Leliévre et al., 1997).

2.1.3.2. The Influence of Ethylene on Fruit Ripening

The sharp increase in climacteric ethylene production at the onset of ripening controls the initiation of changes in colour, aromas, texture and flavour of climacteric fruit while other quality changes are ethylene independent. Ethylene operates via a perception and transduction pathway to induce the expression of genes responsible for the biochemical and physiological changes observed during ripening (Pech et al., 2002).

The discrimination between ethylene-dependent and independent pathways during ripening has been facilitated by the availability of transgenic plants and the molecular analysis of naturally-occurring mutant lines. In ethylene-suppressed tomatoes the accumulation of lycopene is strongly impaired and chlorophyll degredation is totally prevented while sugar and acid accumulation is unaffected by ethylene suppression (Murray et al., 1993). Other ripening events like softening and membrane deterioration comprise both ethylene-dependent and independent components (Pech et al., 2002). In all of the transgenic plants obtained so far with a reduced capacity for ethylene synthesis the ripening phenotype is altered and can be at least partially reversed by the application of exogenous ethylene (Leliévre et al., 1997).

Each ethylene sensitive ripening event shows differential sensitivity to ethylene (Pech et al., 2002) in each species and cultivar. In melon the threshold level for degreening of the rind is 1 ppm, while 2.5 ppm is required to trigger some components of the softening process. The saturating level of ethylene dependent ripening related effects is less than 5 ppm, which is by far lower than the internal ethylene concentrations found in the fruit at the climacteric peak (100 ppm) (Pech et al., 2002). Fruit softening is known to be one of the ripening processes that is most sensitive to ethylene with the treatment of immature pear fruits with low levels of ethylene (0.1 ppm) selectively inducing softening but not other aspects of ripening (Leliéve et al., 1997).

2.1.3.3. Temperature Influence on Ethylene Production

The effects of lowering the temperature to cause slower rates of metabolism, applies to the production of ethylene by climacteric fruit. However, exposure to one temperature can influence subsequent ethylene production at other temperatures. In fruits such as some apple cultivars, pears and kiwifruit, low temperatures can promote homogeneous ripening and hasten the induction of a competency to synthesise autocatalytic ethylene (Leliévre et al., 1997).

One of the most studied cultivars in which temperatures influence on ethylene production has been assessed is the ‘Granny Smith’ apple cultivar. Cold treatment in preclimacteric

'Granny Smith' apples stimulates ethylene production on return to ambient temperature by the conversion of ACC to ethylene through the induction of ACO activity and ACO protein accumulation (Jobling et al., 1991; Leliévre et al., 1995). This production of ethylene is in contrast with the extremely slow evolution of ripening of fruit held constantly at 4°C. Knee (1988) and later Larrigaudiere et al. (1997) both showed that the temperature effect in ethylene production is cultivar dependent. In the work of Larrigaudiere et al., (1997) ethylene production for 'Royal Gala' and 'Starkling Delicious' was significantly greater at 20°C than at 1°C, while fruit stored at 1°C for 10 days before transfer to 20°C, increased to a similar ethylene production to fruit constantly stored at 20°C. Meanwhile, Tian et al. (2002) found that 'Braeburn' apples performed similarly to 'Granny Smith' apples and related this to the increased activity of ACS during low temperature storage.

Other fruit provide further examples of the diversity of ethylene responses that are observed as a result of temperature and temperature changes. In winter pears, a cold treatment is required for the induction of autocatalytic ethylene production (Leliévre et al., 1997, Pech et al., 2002) while low temperatures predisposes cucumber tissue to produce ACS when the fruit are transferred from cold to warm which in turn leads to formation of ACC and an acceleration of ethylene production (Wang and Adams, 1982). Zhou et al. (2001) stored peaches at 0°C or 20°C or at 0°C with a single day at 20°C in the middle of a four week period (an intermittent warming treatment). The intermittent warming treatment caused enhanced ethylene production in the fruit when returned to 0°C and the ethylene remained higher than 0°C stored fruit for the remainder of the storage period as a result of induced ACS and ACO production.

2.1.3.4. CA Effects on Ethylene Production

Manipulating the postharvest gas environment (lowering O_2 and increasing CO_2) can suppress ethylene production for many products (Golding et al., 2005). It is generally accepted that the suppression of ethylene production and action is one of the primary mechanisms by which controlled atmospheres extends the storage life of apples (Gorny and Kader, 1996). Low O_2 has also been shown to reduce the expression of ethylene-independent fruit ripening-related genes indicating that not all low oxygen-induced effects are due to the reduction in ethylene biosynthesis (Leliévre et al., 1997).

Nitrogen atmospheres cause a cessation in ethylene production by pears and apples only for a surge in ethylene production to occur upon reexposure of the tissue to air. In air, methionine is efficiently converted to ethylene; whereas in nitrogen, it is metabolised to ACC only (Adams and Yang, 1977).

Gas compositions used in commercial controlled atmosphere storage conditions can have dramatic effects on the biosynthesis of ethylene and its precursors (Leliévre et al., 1997). In 'Cox's Orange Pippin' and 'Granny Smith' apples oxygen levels between 0-2% O_2 reduce the ethylene production rate to close to zero, whereas above 8% O_2, ethylene production remains at its maximal rate (Dadzie et al., 1996). Ethylene biosynthesis of climacteric apple fruit is significantly reduced in an atmosphere of low O_2 due to reduced ACS abundance, subsequent ACC availability (Lau et al., 1984) and reduced activity of ACO (Gorny and Kader, 1996). Ethylene biosynthesis in climacteric fruit placed in an atmosphere of air and 20% CO_2 is reduced, due to reduced ACS and with no effect on either the competency or abundance of ACO (Gorny and Kader, 1996). Subsequently storage in 0.25% O_2 is more effective in reducing ethylene production than in 20% CO_2 due to the dual effect (on ACO and ACS activities) of the low O_2 treatment. In pears, ethylene production is reduced when in atmospheres with 0% CO_2 and less than 6% O_2 (de Wild et al., 1999). In combination with a 5% CO_2 atmosphere, ethylene production is further reduced at all levels of oxygen by a further 30%.

2.2. THE 'CRIPPS PINK' APPLE CULTIVAR

2.2.1. Origins and Appearance

The 'Cripps Pink' apple cultivar is a late maturing 'Lady Williams' by 'Golden Delicious' apple cultivar cross, developed at Stoneville Horticultural Research Station, Western Australia (Corrigan et al., 1997). 'Pink Lady™' is the registered trademark of first quality 'Cripps Pink' apples. The apples are oblong-conical in shape and bi-coloured, with large areas of green-yellow and solid pinkish-red skin (Cripps et al., 1993). The appearance is preferred by many consumers over other commercially available cultivars (Corrigan et al., 1997). 'Cripps Pink' is a late maturing variety, resulting in commercial harvest beginning approximately mid-April in the southern hemisphere and late October in the northern hemisphere, depending on orchard locality and seasonal conditions.

Considered Australia's premium apple, the 'Cripps Pink' cultivar represents 23% of all the apple plantings, and 18% of all apple production in Australia (Anon, 2006). 'Cripps Pink' is also grown commercially in South Africa, New Zealand, South America, the United States, and continental Europe. Consumers prefer fruit with at least 13% sugar, more than 60% blush and not greasy to touch (Melvin-Carter and Little, 1997). The amount and intensity of blush is an attribute that sells 'Pink Lady™' and leads to greater economic returns (Golding et al., 2005; Shafiq and Singh, 2005).

2.2.2. Current 'Cripps Pink' Knowledge

Due to the relative youth of the cultivar, research into the behaviour of the apple to postharvest treatments is both brief and recent. Much of the work has been published during the time of preparation of this thesis, and subsequently did not substantially influence the direction of the work being undertaken.

2.2.2.1. At Harvest Maturity

Although development of blush on 'Pink Lady™' is not an accurate indication of maturity (Melvin-Carter and Little, 1997), Shafiq and Singh (2005) found that delaying harvest can improve development of 'Pink Lady™' blush. A reduction in firmness and titratable acidity as a result of a delayed harvest is consistently reported (Drake et al., 2002; Hurndall, 2003b, Figure 2.3; Shafiq and Singh, 2005). Jobling (2002) reported a 3 kgf difference in firmness as a result of a 15-day difference in harvest. Results of harvest maturity effect on soluble solids have been mixed with Shafiq and Singh (2005) reporting a reduction, while Hurndall (2003b) reported a consistent increase (Figure 2.3). A reduction in background hue angle as a result of a later harvest was reported by Gualanduzzi et al. (2005).

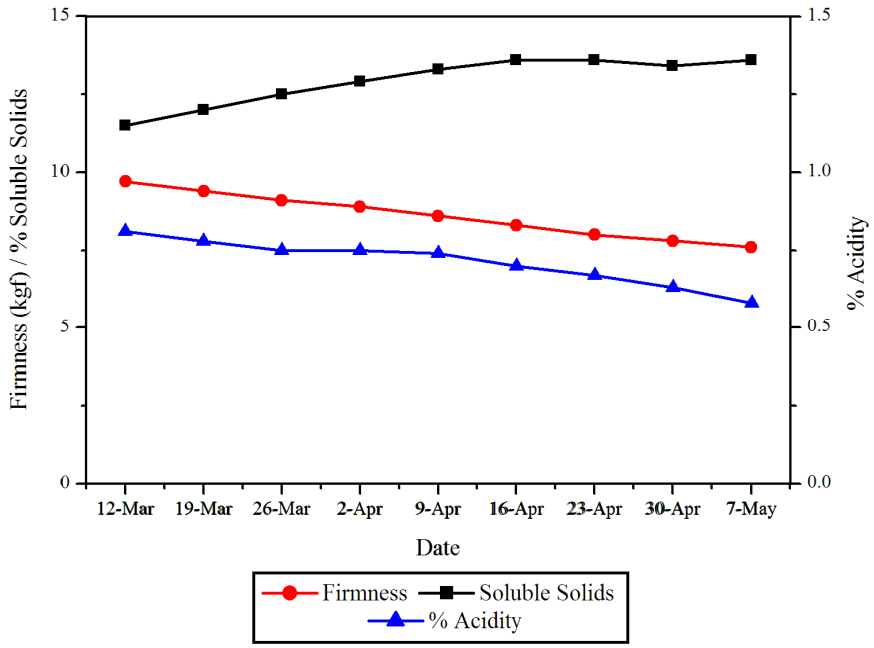

Figure 2.3, Change in quality factors of 'Cripps Pink' apples with harvest date as reported by Hurndall (2003b).

Maguire et al. (2000) studied the water vapour permeance and subsequent weight loss of 'Cripps Pink' and 3 other commercial apple cultivars. In this study they report that the water vapour permeance of 'Cripps Pink' was lower than the other cultivars and remained relatively constant, despite other varieties water vapour permeance increasing with later

harvest date. Both factors contribute to a reduced likelihood of shrivel development during storage of 'Cripps Pink' apples.

2.2.2.2. Cool Storage Performance

Recently reported data on the physiological and quality behaviour of 'Pink Lady™' apples in both air and controlled atmosphere storage conditions by Drake et al. (2002), Crouch (2003), Kupferman (2003), Golding et al. (2005), Gualanduzzi et al. (2005), Saftner et al. (2005), and Shafiq and Singh (2005) provide a valuable benchmark for which to compare the results obtained in this thesis.

Golding et al. (2005), Gualanduzzi et al. (2005) and Saftner et al. (2005) observed firmness to reduce linearly throughout coolstorage period, irrespective of harvest. Shafiq and Singh (2005) showed that 'Pink Lady™' could maintain acceptable fruit quality for a maximum 90 days at 0°C. Reduced rates of firmness change as a result of application of CA have been reported by Hurndall (2003a) and Golding et al. (2005) while Drake et al. (2002) indicated that storage for 180 days in CA conditions resulted in apples with firmnesses not significantly different from that at harvest.

Corrigan et al. (1997) stored 'Pink Lady™' apples at 0°C in air and found that sensory perception of sweetness increased, which was likely due to the shift in the brix to acid ratio (i.e. loss of titratable acidity with no change in brix). Hurndall (2003a) and Saftner et al. (2005) both reported a reduction in soluble solids (°Brix) in air while Drake et al. (2002) found no significant changes in 'Pink Lady™' soluble solids after 180 days of storage at 1°C in air. Both Kupferman (2003) and Hurndall (2003a) found no significant changes in soluble solids during CA storage. Titratable acidity reduces throughout cool storage in air (Drake et al., 2002; Saftner et al., 2005) and CA (Drake et al., 2002; Kupferman, 2003; Hurndall, 2003a). Yellowing of the initially green background colour is a characteristic of 'Pink Lady™' in storage (Gualanduzzi et al., 2005).

2.2.2.3. Ethylene and Storage

'Pink Lady™' is a relatively moderate to high ethylene producer (Melvin-Carter and Little, 1997). Golding et al. (2005) found that internal ethylene concentrations of 'Pink Lady™' apples increased dramatically over the first 50 days of storage in air and stabilised at this concentration over the remainder of storage. Ethylene concentrations in CA stored fruit were approximately 25-30% that of fruit stored in air, but showed the same rapid increase in the first 50 days of storage (Golding et al., 2005).

In recent times there has been a considerable research on the use of ethylene blocking agents aminoethoxyvinylglycine (AVG) and 1-methylcyclopropane (1-MCP) to control ethylene and subsequently ripening during storage, especially for apples (Fan et al., 1999; Watkins et al., 2000b). Crouch (2003) found that 'Pink Lady™' treated with 1-MCP at 20°C within 4 hours after harvest resulted in firmness differences of 2.2 kgf after 6 months storage at -0.5°C in air, and resulted in higher titratable acidity and soluble solids, and reduced superficial scald. Wilkinson et al. (2005) found that treatment of 'Pink Lady™' at 0°C for 1 day, 1 week after harvest aided the maintenance of firmness over a 15 week period while stored in CA conditions. This study also found that 1-MCP treatments had no effect on internal browning development.

Phan-Thien et al. (2004) applied the ACC synthase (and hence ethylene production) inhibitor aminoethoxyvinylglycine (AVG) 3 weeks prior to commercial harvest of 'Pink Lady™' apples, and found that the application delayed ripening by 5 days and the onset of softening by 7 days. In another study, AVG treated fruit were observed to have less ethylene production during storage, irrespective of being stored in air or CA, and resulted in generally firmer fruit at the completion of storage and after a 2 week shelf life (at 20°C) period (Golding et al., 2005). Clearly, ethylene plays a significant role in the ripening and quality changes of apples, including the 'Cripps Pink' cultivar.

2.2.2.4. Storage Disorders

The investigation into the causes of internal browning development has been the focus of a world-wide research effort with 'Pink Lady™' (Jobling et al., 2005; de Castro Hernandez et al., 2005; James et al., 2005). The flesh browning disorder is sporadic in nature and occurs in both CA and in air storage, although it seems that high CO_2 levels can induce higher incidence of the disorder (Jobling et al., 2005). It has been established that internal browning disorders develop during storage and are have a higher incidence in both longer stored (Kupferman, 2003) and later harvested fruit (Drake et al., 2002; Brown et al., 2003; Gualanduzzi et al., 2005; Jobling et al., 2005; James et al., 2005).

Superficial scald is a postharvest physiological disorder of some apple cultivars that manifests itself as browning of the skin with no influence on the flesh, and often develops upon removal from coolstorage temperatures. The disorder is sporadic in nature, affected by season, growing location and harvest and is caused by the oxidation of α-farnesene, a metabolite promoted by ethylene. Early harvested 'Cripps Pink' are more likely to develop superficial scald (Hurndall, 2003b; Gualanduzzi et al., 2005).

2.3. USING MATHEMATICAL RELATIONSHIPS TO DESCRIBE POSTHARVEST FRUIT PHYSIOLOGY AND QUALITY

Models that are able to predict physiology and quality changes as a result of storage conditions are invaluable tools for optimising innovative coolchains that contain heating and cooling processes, and assessing the impact of poor coolchain practices. This section reviews the methods available for the quantification and prediction of the effects of variable storage conditions on fruit quality with a focus on temperature variation.

2.3.1. Empirical Models

Empirical modelling is the practice of fitting an equation to collected data. This fitted equation may include variables known to affect the attribute. Although the curve generated can describe the data collected extremely well, the equations often do not apply to other sets of similarly collected data, severely limiting the application of the models produced. Commonly used empirical models applied to postharvest temperature variable scenarios include the degree-day approach, while others develop simple algebraic equations.

2.3.1.1. Correlations of Quality to Degree-Days

Correlating quality to accumulated degree-days has been borrowed by postharvest science from preharvest studies, where the existence of variable temperature, caused by weather and diurnal patterns need to be negotiated for modelling plant growth. Degree-days are obtained with a simple integration of the temperature versus time history (equation 2.1). By definition, a product experiences one degree-day if it remains at 1°C for one day, where the basis is 0°C. A treatment of 4°C for 6hrs will also result in the product experiencing 1 degree-day. Degree-days can be arbitrarily rescaled (from the basis of 0°C), commonly to the basis of either the freezing temperature of the product or the optimum storage temperature to create units known as accumulated heat units (AHU), (equation 2.1).

$$AHU = \int_0^t (T_P - T_D).dt \qquad [2.1]$$

Accumulated heat unit formula, where T_P = product temperature, (°C); T_D = temperature datum, (°C); t = time (days).

The degree-day approach assumes that the change in rate of a process is linear to change in temperature and constant at each temperature with respect to time and fruit maturity. For a large amount of biological systems this has proven not to be the case.

Calculated AHU values are usually correlated to the quality of the product at an instant in time. Correlating this kind of data with asparagus quality (King et al., 1987; Hurst et al., 1998) and pear firmness, weight loss and colour development (Downs et al., 1989) has been shown to produce positive results, while Klein and Lurie (1992) found that this technique was not effective in predicting quality of heat treated apples.

2.3.1.2. Empirical Fruit Quality Models

Thorne and Segurajauregui Alvarez (1982) modelled firmness and colour of tomatoes in oscillating temperatures by firstly collecting data at constant temperatures, and developing empirical models to predict the changes observed that was a function of both temperature and time. Fluctuating temperature trials were then used to validate the models with some success. Thai and Shelfelt (1990) were to later use the same technique to develop a less successful linear model for peach firmness.

In creating these empirical models the authors assume that the quality changes of their product are both additive and commutative, (Figure 2.4). In making this assumption, the previous time-temperature history of the product is assumed to not affect the rate of quality change. Only the current quality and the temperature at which the product is stored are required to model future product quality. Although this assumption may hold in simple cold storage regimes, those regimes that produce protective mechanisms, such as pre-storage heat treatments (Ferguson et al., 2000) are unlikely to hold to this assumption. Despite being crucial to the model accuracy these assumption have rarely been tested or validated.

A "time shift" method has been used to predict tomato (Thai et al., 1990) and peach (Thai and Shelfelt, 1990; Thai and Shelfelt, 1991) colour change in variable temperature treatments. This method predicts the rate of colour change as dependent on current temperature and colour and assumes that current colour change behaviour is independent to the previous temperatures and colour change history.

Most recently, Johnston et al. (2001) used the same empirical equation to model apple firmness changes for 4 different apple cultivars, with fitted parameters for each cultivar, and temperature modelled with a simplified Arrhenius' equation. This equation using the time shift method was later applied to variable temperature scenarios with some success (Johnston et al., 2005).

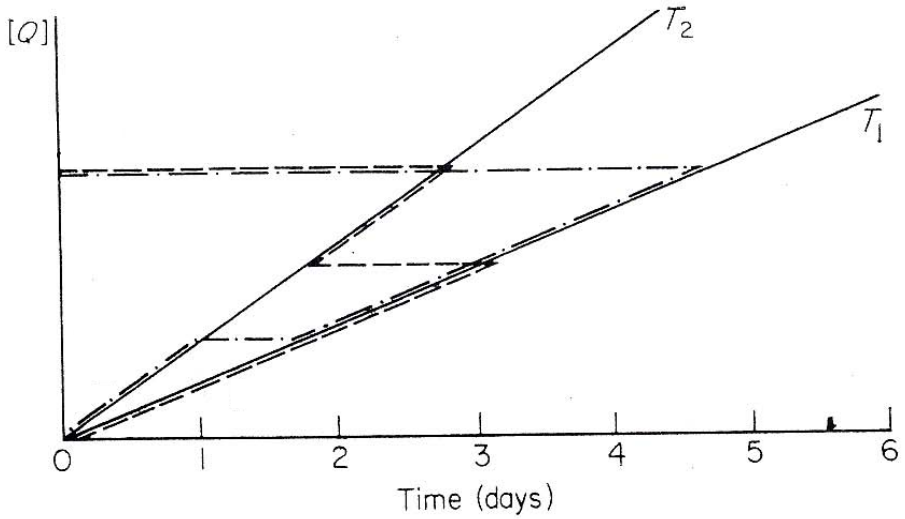

Figure 2.4, A diagrammatic representation of the assumptions of additivity and comutativity. Solid lines T_1 and T_2 represent a (constant) rate of quality change (Q) at temperature T_1 and T_2 respectively. The dashed line represents a treatment of 3 days at T_1 followed by on day at T_2, while the dot-dashed line represents 1 day at T_2 followed by 3 days at T_1.(Source: Thorne and Segurajauregui Alvarez, 1982).

2.3.2. The Kinetic Assumption Approach

The quality changes that occur during fruit ripening and senescence are a result of a multitude of complex biochemical reactions, driven by enzymes. These processes can be described by reaction kinetics and the rate of the reaction by kinetic principles. The models developed are theorised mechanisms and simplified descriptions of these complex metabolic processes. However, this simplification can be justified as many biological pathways are controlled by a single rate controlling reaction. The models created may yield insights about the underlying mechanisms of fruit ripening and senescence (De Smeldt et al., 2002).

The models created have weaknesses in that values representing kinetic rates and temperature dependence are required to be calculated empirically (i.e. fitted to experimental data). Significantly different values can be obtained between batches, growers, locations and seasons, resulting in a lack of confidence of being able to apply these models to predict future events. The section briefly discusses the use of kinetic based models to describe fruit physiology and quality.

2.3.2.1. Kinetics Modelling Formulation

A naturally occurring change in any attribute can usually be approximated by one of the four basic types of kinetic mechanisms (Tijskens and Polderdijk, 1996).

a. *Zero order kinetics.* The rate of quality loss is constant and independent of time and/or product condition. The rate of change in the attribute is subsequently linear with time (equation 2.2).

$$\frac{dQ}{dt} = -k \qquad [2.2]$$

A zero order reaction for the loss of quality, where: Q = quality (unit); k = rate constant (unit.s^{-1})

b. *First order kinetics.* These reactions are commonly encountered in natural processes. The rate of reaction is dependent on the quantity of the current product condition (equation 2.3). As a result a characteristic exponential decay or rise of the attribute is observed.

$$\frac{dQ}{dt} = -kQ \qquad [2.3]$$

A first order reaction for the loss of quality, where: Q = quality (unit); k = rate constant (s^{-1}) and t = time (s).

c. *Michaelis Menten kinetics.* This type of kinetic is based on enzyme reaction theory. When the amount of reactant (S) is small this limits the amount of reaction capable. However when reactants are plentiful, the amount of enzyme available (E) to process the reactant limits the ability for the reaction to proceed.

$$\frac{dQ}{dt} = k_n E\left(\frac{S}{K_m + S}\right) \qquad [2.4]$$

Mechaelis menten kinetics for the gain in quality, where: Q = quality (unit); E = enzyme concentration; S = substrate concentration; t = time (s); k_n **=** rate constant (s^{-1}); K_m = rate constant (unitless).

d. *Logistic kinetics.* This behaviour is regarded as an expression of either autocatylitic processes, diffusion controlled processes, cascades of reactions or complex growth. In logistic kinetics, there is a definite maximum and minimum of the attribute, which are transferred between through time.

$$\frac{dQ}{dt} = -kQ\left(1 - \frac{Q}{Q_{\inf}}\right) \qquad [2.5]$$

Logistic kinetics for the loss of quality, where: Q = quality (unit); k = rate constant (s^{-1}); t = time (s) and Q_{inf} = quality after infinite time (unit).

Using these mechanisms in combination can produce a result that mimics that of biological systems. There are three basic means in which mechanisms can be combined:

a. *Reaction Sequences.* These sequences describe possesses in which one substance is converted to another with any number of intermediates between (equation 2.6).

$$R \xrightarrow{k_1} R_1 \xrightarrow{k_2} P \qquad [2.6]$$

An irreversible reaction sequence, where R = reactants; P = products and k_1, k_2 are rate constants (s^{-1}).

b. *Parallel Reactions.* Reactants can transform into a number of products or the same product by more than one means. Each reaction acts independently from the other (equation 2.7).

$$R \begin{array}{c} \xrightarrow{k_1} \\ \xrightarrow{k_2} \end{array} P \qquad [2.7]$$

A parallel reaction, where R = reactants; P = products and k_1, k_2 are rate constants (s^{-1}).

c. *Reversible Reactions.* All reactions are potentially reversible, but in most cases the energy required to conduct the reverse reaction is so great that the reverse reaction can be ignored (equation 2.8).

$$R \begin{array}{c} \xrightarrow{k_1} \\ \xleftarrow{k_2} \end{array} P \qquad [2.8]$$

A reversible reaction, where R = reactants; P = products and k_1, k_2 are rate constants (s^{-1}).

2.3.2.2. Modelling the Effect of Temperature on the Rate of Change

Temperature affects the rate of reaction in metabolic processes with higher temperatures generally resulting in faster rates of reaction. For the purposes of modelling reactions in temperature variable scenarios, rate values in the kinetic equations are required to be a function of temperature. This relationship is usually defined with the use of Arrhenius' equation (equation 2.9).

$$k = k_{ref} e^{\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad [2.9]$$

The Arrhenius' equation, where k = rate constant at temperature T (s^{-1}); kref = rate at the reference temperature (s^{-1}); E_a = activation energy ($J.mol^{-1}$); R = universal gas constant (8.314 $J.mol^{-1}K^{-1}$); T_{ref} = reference temperature (K); and T = product temperature (K).

The constants (E_a and k_{ref}) required to use the Arrhenius equation are process specific and hence determined empirically from data collected at different temperatures for the system being modelled. Rate constants at a constant temperature and constants required for Arrhenius' equation are usually generated by optimising the values to fit measured data with the use of statistical optimisation computer software.

In applying Arrhenius' equation, Tijskens and Verdenius (2000) showed that the equivalent temperature method (which calculates temperature dependent rates from temperature before integrating) provides more accurate results than the average temperature method (integrating the temperature data and then determining the rate).

Most biological processes have an optimum temperature where the maximum rate of change occurs. In many cases temperatures greater than 30°C in whole fruit can result in reduced rates of change, due to reduced enzyme functionality as a result of higher instability of the enzyme at the higher temperature (Johnston et al., 2001).

In systems that possess an optimum reaction temperature it is often assumed that a reaction occurs that is driven by an enzyme which can exist in two states, of which only one state can cause the reaction to occur. The active state is assumed to obey Arrhenius equation but the existence of the inactive form is favoured at the higher temperatures.

The total amount of active enzyme is quantified with the Boltzmann distribution function, and subsequently the combination of this with the Arrhenius equation, results in a modified Arrhenius equation (equation 2.10) to describe the change in rate with temperature, for an attribute with an optimum (Johnson and Thornley, 1985).

2.3.2.3. Quality Modelling Using the Kinetic Approach

In recent times, modellers of fruit quality and physiology have turned away from pure empirical models, to using the kinetic based approach in order to gain further knowledge, and incorporate the knowledge generated of the possible mechanisms of observed physiological and quality changes. As critical quality characteristics, firmness has been modelled in apples (Tisjkens et al., 1999), peaches (Tisjkens et al., 1998) and tomatoes (Wells and Singh, 1988), while colour changes have been modelled in apples (Johnson

and Thornley, 1985; Dixon and Hewett, 1998), cucumber (Schouten et al., 1997) and tomatoes (Hertog et al., 2004a).

$$k = \frac{k_a e^{\frac{-E_a}{RT}}}{1 + e^{\frac{\Delta S}{R}} e^{\frac{-\Delta H}{RT}}} \qquad [2.10]$$

Modified Arrhenius eqaution for calculation of the rate constant, where k_a = the maximum possible rate constant; E_a = activation energy ($J.mol^{-1}$); R = universal gas constant (8.314 $J.mol^{-1}K^{-1}$); ΔS = increment of entropy ($J.mol^{-1}K^{-1}$); ΔH = increment of enthalpy ($J.mol^{-1}$); T = product temperature (K). (Adapted from Johnson and Thornley, 1985)

2.4. CONCLUSIONS AND OPPORTUNITIES FOR RESEARCH

The 'Cripps Pink' ('Pink Lady™') apple cultivar is a relatively new cultivar that has become the most important cultivar in the Australian apple industry. While in recent times, the depth of knowledge of the quality performance of the cultivar as a result of postharvest treatments, including temperature, and atmosphere adjustment and the application of ethylene blockers (AVG and 1-MCP) has been investigated, establishment of clear behaviour patterns in long-term storage has yet to be clarified. This thesis aims to document the postharvest behaviour of three harvests of 'Cripps Pink' apples in air and one of these harvests in CA, and makes comparisons to other recently published studies for the cultivar. As a result, expected postharvest physiology and quality changes for the 'Cripps Pink' apple cultivar will be clearly established.

The use of applied variable temperature scenarios either prior to or during long-term storage has been used as a means to reduce chilling injuries or apply a disinfestation treatment. These treatments have been shown to influence product quality both beneficially and negatively, depending on the treatment. In the Australian and New Zealand apple industries, it is common practice to sort, grade and pack apples previously stored for a long-term period, prior to shipment to foreign marketplaces. At this time, temperature breaks can occur, of which the influence on the final delivered product quality is currently unknown. Quantification of the effect of such temperature breaks on the 'Cripps Pink' apple cultivar, both at the time of the break and in subsequent optimal storage conditions will be sought in this thesis. Potential influencing factors such as harvest maturity, length of storage prior to break in temperature control, length of break in temperature control and multiple breaks in temperature control are all investigated.

The benefits of storing apples in CA have been well established, as has the need to establish CA rapidly, to obtain the most benefit. However, the influence of commercial CA practices; the breaking of CA in large storerooms in order to remove some of the fruit and the selection of air or CA atmosphere during refrigerated shipping, on product physiology and subsequent quality has not been quantified. The effect of both of these practices on 'Cripps Pink' apples, with and without combination with breaks in temperature control, will be investigated in this thesis. The findings for the influence of atmosphere during shipping will be validated in the commercial shipping environment.

Finally, the knowledge of the ethylene production pathway and the influence of ethylene on produce quality change have also been well established, including for the 'Cripps Pink' cultivar. In recent times, the use of mathematical models based on kinetic principles to model fruit physiology and quality changes during postharvest storage and distribution has been established. However, models that describe ethylene changes during the posthavest period are few and far between. In this thesis an attempt will be made to use the kinetic based modelling approach to predict ethylene production for 'Cripps Pink' apples subjected to variable temperature regimes.

3. Characterisation of the Postharvest Changes of 'Cripps Pink' Apples Stored at Refrigerated Temperatures

3.1. INTRODUCTION

The 'Cripps Pink' cultivar is the result of a cross between the cultivars 'Lady Williams' x 'Golden Delicious' and was developed in Western Australia in the mid 1980s. Since this time the cultivar has proven to be commercially successful, developing into a cultivar that attracts a commercial price premium; and represents an increasing part of the Australian apple industry (Anon., 2006). 'Pink Lady™' is the registered trade mark name for high quality 'Cripps Pink' apples.

Due to the relative youth of the cultivar, little fundamental research has been conducted documenting the postharvest behaviour of 'Cripps Pink' apples either in air or controlled atmosphere storage. The collection of postharvest storage data would provide the industry with guidelines for optimal storage conditions and timing, would allow the prediction of postharvest fruit quality and may form the basis of post-storage quality indices. In addition, comparison of the postharvest behaviour of 'Cripps Pink' to other apple cultivars may provide further insight to 'Cripps Pink' fruit behaviour, and its storage benefits and limitations.

This chapter documents the physiological and quality changes of three harvests of 'Cripps Pink' apples from two different seasons and districts. All three harvests were monitored while stored in air at 0°C, while one harvest was also monitored during controlled atmosphere (CA) storage (2% O_2, 1% CO_2). The focus of this research was to measure the physiological behaviour of 'Cripps Pink' apples (respiration rate and ethylene production) and the changes in fruit quality during storage. This work adds to other recently reported work on the physiological and quality behaviour of 'Pink Lady™' apples in both air and controlled atmosphere storage conditions by Drake et al. (2002), Crouch (2003), Golding et al. (2005), Gualanduzzi et al. (2005), Saftner et al. (2005), and Shafiq and Singh (2005). This work did not extend to the scope of extensive measurement of internal browning disorders that 'Cripps Pink' is known to suffer from after prolonged storage. More detailed information on 'Cripps Pink' internal browning storage disorders is available in the works of Jobling et al. (2005) and James et al. (2005).

3.2. METHODOLOGY

This experiment was conducted over a two year period, the first (2003) being conducted at Massey University, Palmerston North (New Zealand) and the second (2004) at Food Science Australia, Sydney (Australia). Subsequently differences in measurement techniques between years occurred due to equipment availability.

3.2.1. Fruit

In 2003, commercially produced 'Cripps Pink' apples (4000 fruit) were sourced from an orchard in Hawkes Bay, New Zealand. Fruit were harvested on two separate harvest dates (2000 fruit per harvest), 9/04/03 (optimum – OPT) and 22/04/03 (late – LAT). Fruit were transported to Palmerston North on the day after harvest.

In 2004, commercially produced 'Cripps Pink' apples (5000 fruit) were harvested from an orchard in Batlow, NSW, Australia at the optimal harvest date. These fruit were stored for 3 days at 0°C in air at Batlow and then transported unrefrigerated to North Ryde, Sydney (a journey of 1 day).

3.2.2. Storage Conditions

3.2.2.1. Air Storage

The 2003 harvests were both stored in refrigerated air at 0 ± 0.5°C. Throughout a 200 day period, fruit were stored in z-pack corrugated cardboard telescopic cartons (18 kg and 100 fruit per carton, 4 layers of 25 fruit per layer).

The 2004 apples were stored at 0°C in sealed 60L barrels. Non-humidified air was supplied to the barrels at 400 $mL.min^{-1}$ in a flow through system (identical to Figure 3.1, with exception to gas mixing). The composition of the atmosphere within the barrels was confirmed as not substaintially different from air on regular occasions via sampling through a fitted septum in the barrel (sample point B, Figure 3.1) and measuring the concentration of oxygen and carbon dioxide via gas chromatography (section 3.2.3.3).

3.2.2.2. CA Storage

CA storage was applied to the 2004 harvest only. The CA set up used is shown in Figure 3.1. As with the air stored apples the fruit were stored in 60L barrels. Pure nitrogen (N_2), carbon dioxide (CO_2) and non-humidified air (as an oxygen (O_2) source) were mixed to obtain CA conditions of 2% O_2 and 1% CO_2 prior to splitting and supplying to individual barrels through a manifold. Gas

concentrations were regularly checked by sampling triplicates of 1 mL at sample point A (Figure 3.1) and analysed by gas chromatography analysis as stated in 3.2.3.3. Each barrel was supplied with a gas mixture at a rate of approximately 400 $mL.min^{-1}$ in a flow through system. Each barrel was checked for sealing by measuring outlet airflow on a regular basis.

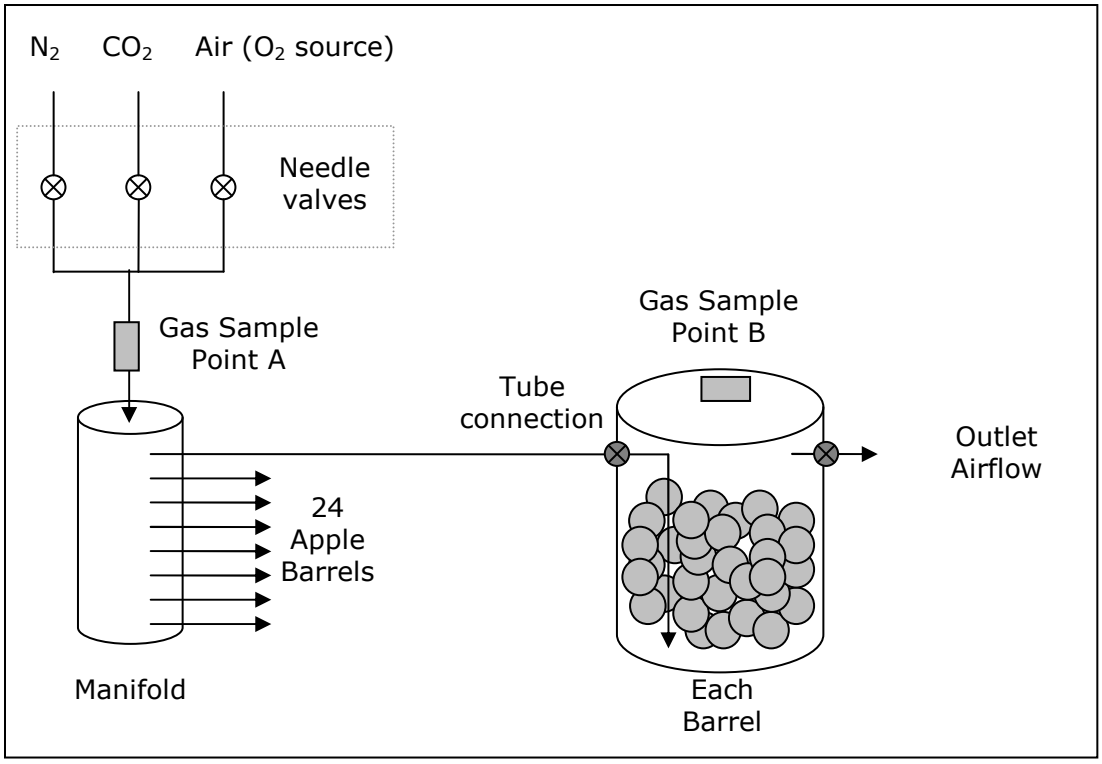

Figure 3.1, Experimental set up used to maintain CA conditions.

3.2.3. Physiological Status

3.2.3.1. Air Storage

Ethylene production and respiration rate of individual fruit were assessed simultaneously at 0°C at regular intervals throughout storage. Fruit were enclosed in an air tight plastic 1 L container and gas samples (2 x 1 mL) were taken at the time of closure and after approximately 1.5 hours. One sample was analysed for CO_2 concentration (section 3.2.3.3) with the other sample analysed for ethylene concentration (section 3.2.3.4). Rates of ethylene production and respiration were calculated as the rate of production of ethylene and CO_2, respectively (equation 3.1) and expressed as recommended by Banks et al. (1995).

$$\text{Production Rate} = \frac{\left(V_C - \frac{m}{\rho}\right)(C_1 - C_0)P}{R.T.m.t} \qquad [3.1]$$

Calculation of production rate (for either CO_2 or ethylene, $mol.kg^{-1}.s^{-1}$), where V_C = container volume (m^3); m = fruit mass (kg); ρ = density ($kg.m^{-3}$); C_0 = initial concentration (%); C_1 = concentration at time t (%); P = atmospheric pressure (Pa); R = universal gas constant (8.314 $Pa.m^3.mol^{-1}K^{-1}$); T = temperature (K); t = time between samples (s).

In 2003, 20 fruit per treatment were measured on each occasion, whereas in 2004, only 10 fruit per treatment were measured on each occasion. The same fruit from each treatment were measured on each occasion.

3.2.3.2. CA Storage

Respiration rate and ethylene production were assessed "in situ", at the treatment temperature and gas conditions. Gas flow was temporarily stopped by fitting sealing plugs into inlet and outlet ports of the barrels. Six samples of initial gas composition were taken with 1 mL syringes through septums fitted for the purpose of gas sampling (sample point B, Figure 3.1). These samples were analysed for CO_2 and ethylene concentration as two sets of triplicates by gas chromatograph analysis. After approximately 4 hours, a second set of 6 samples was collected and analysed. The change in concentration of CO_2 and ethylene were converted to rates of respiration and ethylene production by taking into account the time between samples, volume of the barrel and the weight of apples in the barrel. Apple weight was determined by weighing the entire barrel and subtracting the weight of the barrel.

3.2.3.3. Respiration Rate Determination

In 2003, CO_2 concentration in gas samples were analysed using a miniature infrared CO_2 transducer (Analytical Development, Hoddesdon, UK), with O_2-free N_2 as a carrier gas, and a Hewlett Packard Integrator (model 3396A, USA) with area under the peak used as an indicator of concentration. In 2004, CO_2 concentration was analysed with a Gow-Mac gas chromotograph (series 580, USA), fitted with a CTR1 column (Alltech, Australia), with helium as a carrier gas and a Riken Denshi chart recorder (model SP-G6P, Japan); peak height was used as an indicator of concentration. In both years, the equipment was calibrated with external CO_2 standards (0.274% and 0.194% for each year respectively, as certified as β-standard by B.O.C Gases, New Zealand or Australia). Linearity of output to sample concentration was validated by reducing the concentration of

the standard through mixing with air (by volume) in the sample syringe. Linearity of output to sample volume was validated by measuring output to volume of sample analysed.

3.2.3.4. Ethylene Production Determination

In 2003, ethylene concentration was analysed with a gas chromatograph (Varian 3400, USA) fitted with a flame ionisation detector and a mesh alumina column with nitrogen as a carrier gas and a Hewlett Packard Integrator (model 3396A). In 2004, ethylene concentration was analysed with a Shimadzu gas chromatograph (model GC-17A, Japan), with a GS-Q column (J and W Scientific, USA) and helium carrier gas and a Shimadzu integrator (model C-R7A, Japan). In both years, equipment was calibrated with external ethylene standards (certified as β-standard by B.O.C Gases) with 101 ppm and 11.2 ppm standards used in 2003 and 2004 respectively. Linearity to concentration and sample volume was validated in both years.

3.2.4. Firmness

3.2.4.1. Non-Destructive Firmness Measurements

Non-destructive firmness measurements were conducted on the same fruit measured for respiration rate and ethylene production.

3.2.4.1.1. Compression Firmness

In 2003, non-destructive firmness measurements were conducted at 0°C by compression with a Texture Analyser (TA-XT2, Stable Micro System) with a standard Effegi 11mm round probe. A single compression of 1.5mm at 0.5mm.s^{-1} was conducted in an equatorial position. Measured peak force (kgf) was used as a fruit firmness index. Twenty fruit were averaged at each sampling time.

3.2.4.1.2. Acoustic Firmness (Stiffness)

In 2004, an acoustic firmness measurement of each apple was conducted with an acoustic firmness sensor (Aweta, Netherlands). The average of 3 measurements per fruit was considered to be the apple stiffness ($10^6 Hz^2.kg^{-2/3}$). Thirty fruit were measured at each sampling time.

3.2.4.2. Destructive Firmness Measurement

Destructive (Magness-Taylor) firmness was measured at 20°C after allowing 3 hours for the fruit to acclimatise to that temperature from 0°C (Figure 3.2). This

was achieved by placing the apples directly in front of a household fan in order to significantly decrease the time to acclimatise.

Destructive firmness was measured using an electronic penetrometer (HortPlus, New Zealand) fitted with a standard Effegi 11mm round probe. The average maximum force required to puncture pared tissue to a depth of 8 mm on opposite sides of the fruit equator was recorded. Twenty fruit were measured at each sampling time in 2003 whereas 25 fruit were measured in 2004. Forced measured by the load cell within the penetrometer was calibrated against calibrated scales by applying an force between the two devices.

3.2.5. Background Colour

As the 'Cripps Pink' cultivar is a bi-coloured fruit, at the onset of the experiment, the "greenest patch on the apple shoulder" on each fruit was identified and marked by circling this region with a pen. This location was continuously measured on each measurement occasion (20 fruit per measurement in 2003, 30 fruit per measurement in 2004). Fruit that recorded a hue angle of less than 90 at the onset of the experiment were considered not to have any significant green region and removed from calculation of the treatment average.

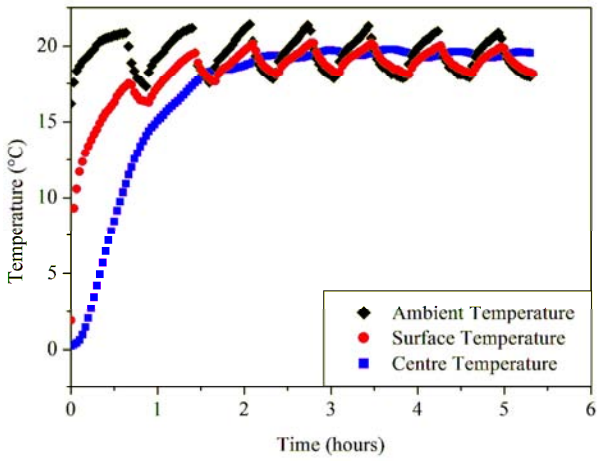

Figure 3.2, Heating curve for size 100 (approx. 180g) apples placed directly in front of a household fan.

In 2003, a 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) was used. In 2004, a Minolta model CR-400 calibrated with a white colour standard (Commission Internationale de l'Éclairage units of Y=92.9, x=0.3134, y=0.3196 using an illuminant C light source) was used.

3.2.6. Other Quality Attributes

3.2.6.1. Weight Loss

Apples used for non-destructive measurements (respiration rate, ethylene production, background colour and non-destructive firmness) were weighed on an individual basis prior to initial storage. On each quality measurement occasion these fruit were reweighed. Weight loss was calculated as a percentage of the initial weight measurement. In 2003, fruit were weighed to 0.001g accuracy (Mettler PG-503S, Toledo, Switzerland), whereas in 2004 a fruit were weighed to 0.01g accuracy (Mettler PR-5002, Toledo, Switzerland).

3.2.6.2. Soluble Solids and Titratable Acidity

Soluble solids and titratble acidity measurements were conducted on the same fruit as used for the destructive firmness tesing. Soluble solids (°Brix) were measured in 2003 only using a hand-held refractometer (Atago N-20, Japan). Freshly released juice from the site of the first penetrometer measurement was used as a sample. Measurements of 20 fruit were averaged for each treatment at each assessment.

Titratable acidity was only measured in 2003. Twenty apple halves from 20 apples were used in groups of 5 apples resulting in 4 measurements on each measurement occasion. Each set of 5 apple halves were homogenised in a household blender, and 1 mL of juice was extracted. Distilled water (50 mL) was added to the juice and titrated to pH 8.2 with 0.1N NaOH with an automatic titrator (Mettler Toledo, USA). The acidity was expressed as moles of malic acid per mL of sample ($mol.mL^{-1}$).

3.2.6.3. Disorders Incidence

Disorders incidence was also conducted on the same fruit used to assess destructive firmness. The incidence of internal storage disorders was determined by slicing each apple in half at an equatorial position and visually assessing (20 fruit in 2003 and 30 fruit in 2004) against a scale developed during the experiment (Figure 3.3). In 2003, samples were assessed at regular time intervals (approximately weekly). In order to remove some of the variation in the data, mean averages of five assessments ranked by time were calculated and presented. In 2004, assessment of internal browning incidence was only conducted at the completion of the experiment.

Figure 3.3, Internal browning assessment scale used to evaluate degree of internal browning in 'Cripp's Pink' apples.

3.3. RESULTS AND DISCUSSION

3.3.1. Physiological Status

3.3.1.1. Air Storage

'Cripps Pink' apples from the three different harvests (from 2 years) stored in air at 0°C all displayed a similar respiration rate pattern while in storage (Figure 3.4a). A significant increase in respiration rate is observed during the 'Cripps Pink' climacteric development (in the first 30-40 days). Respiration rate peaked at 28 $nmol.kg^{-1}s^{-1}$ 20 days after storage. Over the remainder of the storage period (170 days), respiration rate decreased by 25% in an approximate log-linear fashion. A similar trend in respiration rate during storage was reported for parent cultivar 'Golden Delicious' (Saftner et al., 2003), although the increase in respiration rate during coolstorage is not characteristic for all apple cultivars as demonstrated for 'Pacific Rose' and 'Granny Smith' cultivars (Johnston et al., 2002b; Figure 3.5a).

Ethylene production showed a more than 100-fold increase in the first 30 days of storage as a result of climacteric development (Figure 3.4b) and peaked at 0.1 $nmol.kg^{-1}s^{-1}$ for both 2003 harvests. In 2004 ethylene production reached peak rates at approximately 20 days into storage, suggesting that the 2004 harvested fruit were more mature at the time of receival at the laboratory than both of the 2003 harvests, possibly due to the time delay required between harvest and delivery to the laboratory. Rates of ethylene production reduced slightly (approximately 35%) in a log-linear fashion over the remainder of the storage period (160 days). Expectedly, this pattern of ethylene production is identical to that reported for internal ethylene concentrations of 'Pink Lady™' apples stored in air (Golding et al., 2005; Figure 3.6). An increased ethylene production during storage followed by the maintenance of relatively high production rates is similar to that observed for 'Granny Smith' and 'Pacific Rose' (Johnston et al., 2002b),

'Fuji' (Jobling et al., 2003) and for parent cultivar 'Golden Delicious' (Saftner et al., 2003; Figure 3.5b). Ethylene production rates of fruit from the optimal 2003 harvest were observed to reduce slightly from day 50 to day 100 of storage in comparison to both their ethylene peak rate and the ethylene production rate of the other harvests.

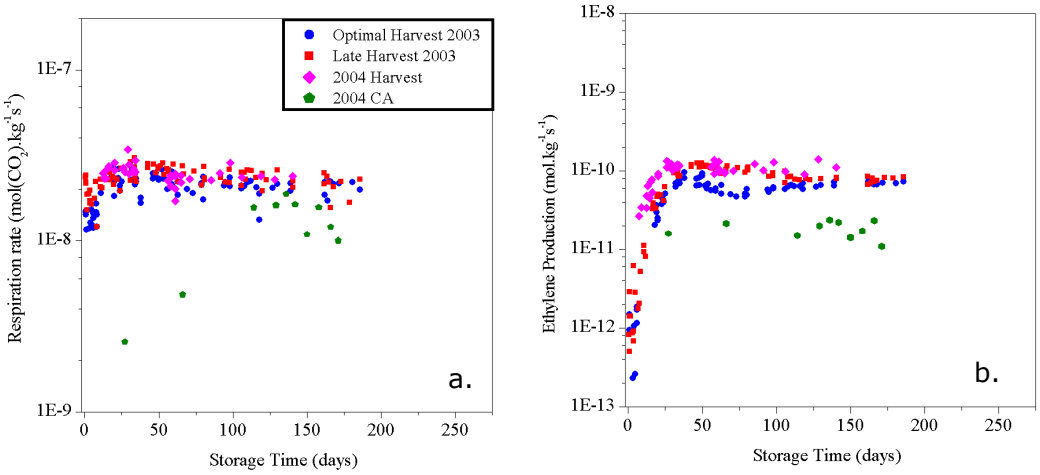

Figure 3.4, Respiration rate (a) and ethylene production (b) of 'Cripps Pink' apples from 3 harvests. Optimal Harvest 2003, Late Harvest 2003 and 2004 Harvest were stored in air at 0°C whereas 2004 CA was stored in CA (2% O_2 and 1% CO_2) at 0°C. Each point represents an average of 20 and 10 fruit for the 2003 and 2004 harvests respectively.

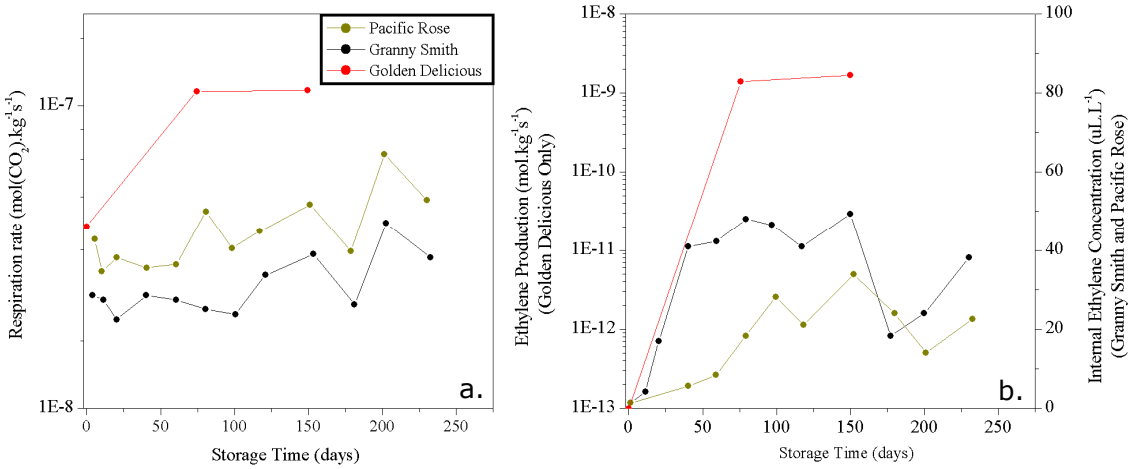

Figure 3.5, Respiration rate (a) and ethylene production (b) of for 'Granny Smith', 'Pacific Rose' and ethylene production for 'Golden Delicious' apples. Data for 'Granny Smith' and 'Pacific Rose' cultivars was extracted and transformed from Johnston et al. (2002b), where fruit were stored at 0.5°C and measurements were conducted at 20°C immediately after removal from cool storage. Data for 'Golden Delicious' was extracted and transformed from Saftner et al. (2003), where fruit were stored at 0°C and measurements were conducted at 20°C.

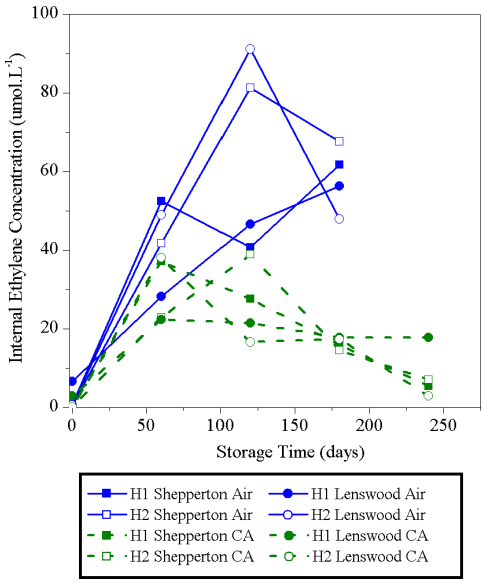

Figure 3.6, Internal ethylene concentration of 'Pink Lady™' apple stored in air and CA (2% O_2 and 1% CO_2) from two harvests from two orchards (Shepperton, VIC; and Lenswood, SA). Adapted from Golding et al. (2005).

3.3.1.2. CA Storage

Application of CA storage at 0°C severely reduced respiration rates on initial storage in comparison to fruit at 0°C in air (Figure 3.4a). This result is comparable to the observed reduction in respiration rate of 'Golden Delicious' apple in CA (Andrich et al., 1998). Additionally, the time required to develop peak rates of respiration was significantly lengthened (to approximately 100 days) while the rate of respiration at the peak was approximately half that of peak respiration rates in air.

'Cripps Pink' apples in CA produced ethylene at a constant rate throughout a 200 day period (Figure 3.4b). However, the initial frequency of measurement was low, and hence the previously documented climacteric ethylene development of 'Cripps Pink' apples in CA (Golding et al., 2005; Figure 3.6) may have been missed. The ethylene production rate of 20 pmol.kg^{-1}s^{-1} was one fifth of that of fruit stored in air, a similar difference to that found for internal ethylene concentrations (Golding et al., 2005). Johnston et al. (2006) reported an approximate 50% reduction in internal ethylene concentration for 'Cox's Orange Pippin' and 'Royal Gala' cultivars as a result of application of CA while de Wild et al. (2003) reported a 75% reduction in ethylene production in 'Conference' pears. It would seem that reducing O_2 from 21% (air) to approximately 2% results in at least a 50% reduction in ethylene production in pome fruit.

3.3.2. Firmness

3.3.2.1. Air Storage

The initial Magness-Taylor firmness of the 2004 harvested fruit being less firm than either of the 2003 harvests ($P<0.0001$). Firmness was observed to change relatively linearly throughout the storage period, irrespective of harvest (Figure 3.7a) agreeing with previously published results by Golding et al. (2005), Gualanduzzi et al. (2005) and Saftner et al. (2005) (Figure 3.7b). Johnston (2001) suggests that all apple firmness changes (independent of cultivar) can be described by a triphasic curve (with each cultivar having its own curve). However, data collected in this trial and that of others (Golding et al., 2005; Gualanduzzi et al., 2005) suggests that a triphasic curve may provide little benefit over a simple linear relationship for the 'Cripps Pink' cultivar.

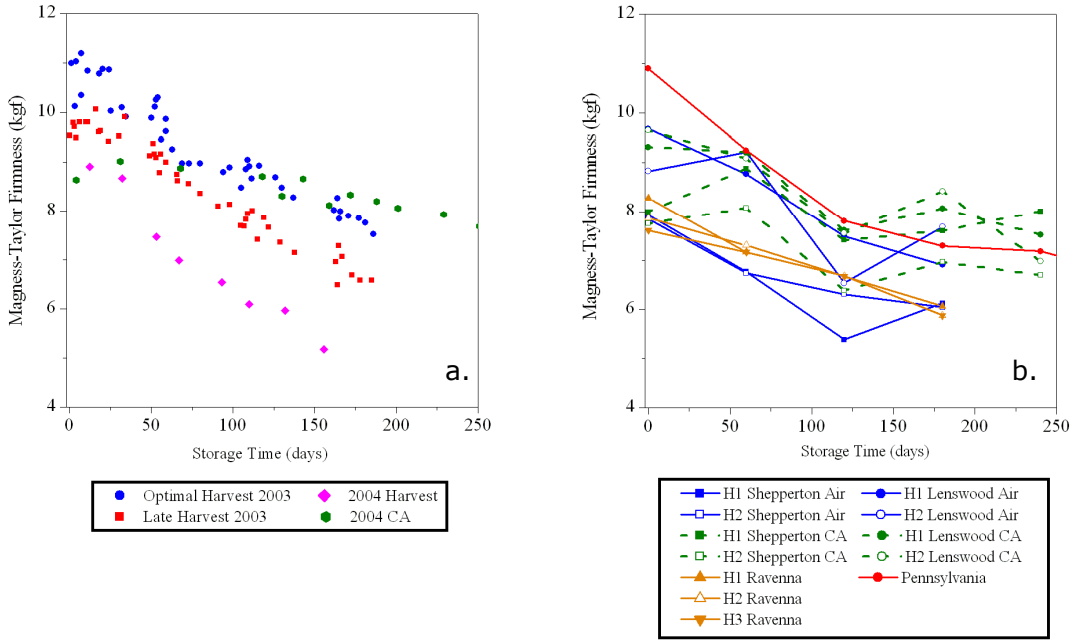

Figure 3.7, Measured (a) and previously reported (b) firmness changes of 'Cripps Pink' ('Pink Lady™') apples stored in air and CA (2% O_2 and 1% CO_2). Previously reported data is adapted from Golding et al. (2005) (Shepparton and Lenswood), Gualanduzzi et al. (2005) (Ravenna) and Saftner et al. (2005) (Pennsylvania). Each data point in (a) represents an average of 20 (2003) or 30 (2004) fruit.

The non-destructive measurements of firmness using the non-destructive compression showed significant scatter in 2003 (Figure 3.8a). Subsequently in 2004, the AWETA acoustic firmness sensor was adopted as a non-destructive firmness measurement technique (Figure 3.8b). This technique was found to produce a more consistent measurement. Stiffness was found to decrease in a linear manner throughout the storage period for 2004 (Figure 3.8b). In either

case, the use of non-destructive firmness provided no additional information on the firmness changes of 'Cripps Pink' apples through storage than the destructive penetrometer technique.

'Cripps Pink' apples lost approximately 3 kgf over a 180-day period (Figure 3.7). Gaulanduzzi et al. (2005) reported that the firmness of 'Pink Lady™' became unacceptable once below 6 kgf and hence suggests that 'Cripps Pink' have potentially a 150-180 storage life at 0°C in air. In comparison, firmness loss for 'Granny Smith' and 'Pacific Rose' cultivars over the same duration of time (180 days) is 1.5 kgf whereas rapid softening cultivars 'Royal Gala' and 'Cox's Orange Pippin' lose 3 kgf of firmness (Johnston, 2001). However, significantly, the 'Cripps Pink' apple cultivar is harvested at an initial firmness of 11-9 kgf, whereas all cultivars studied in the work of Johnston (2001) were harvested at approximately 7 kgf. Hence, although 'Cripps Pink' loses firmness at a rate similar to that of rapid softening cultivars, long-term air storage of 'Cripps Pink' is still commercially feasible, due to the hardness of the fruit at harvest.

In this trial an increased rate of softening was not induced upon an increase in the rate of ethylene production, a contradiction of evidence that ethylene plays a major role in softening of apple cultivars. Johnston (2001) reported that the development of the climacteric and subsequent increase in internal ethylene caused rapid ripening and hence a triphasic softening curve in 'Royal Gala' and 'Cox's Orange Pippin' cultivars. Furthermore, the use of 1-MCP (an ethylene production inhibitor) has comprehensively showed that reducing ethylene production in apples stored in air results in reduced firmness losses during storage (Fan et al., 1999; Watkins et al., 2000b; Rapusinghe et al., 2000), including the 'Cripps Pink' cultivar (Crouch, 2003). It is entirely possible that changes other than increased ethylene concentration may initiate rapid softening of triphasic softening fruit. Kim et al. (1999) found that kiwifruit softening was initiated without changes in ethylene production, ACC concentration or ACO acitivity. These authors suggest that fruit produce more ethylene receptors or receptors become more sensitive to ethylene as fruit mature, initiating the rapid softening phase.

Other studies have previously demonstrated the possibility that ethylene may not be explicitly linked to apple softening. In the study of Johnston (2001), 'Pacific Rose' did not show a rapid softening phase at shelf life temperatures despite high internal ethylene concentrations. Additionally, Gussman et al. (1993) reported

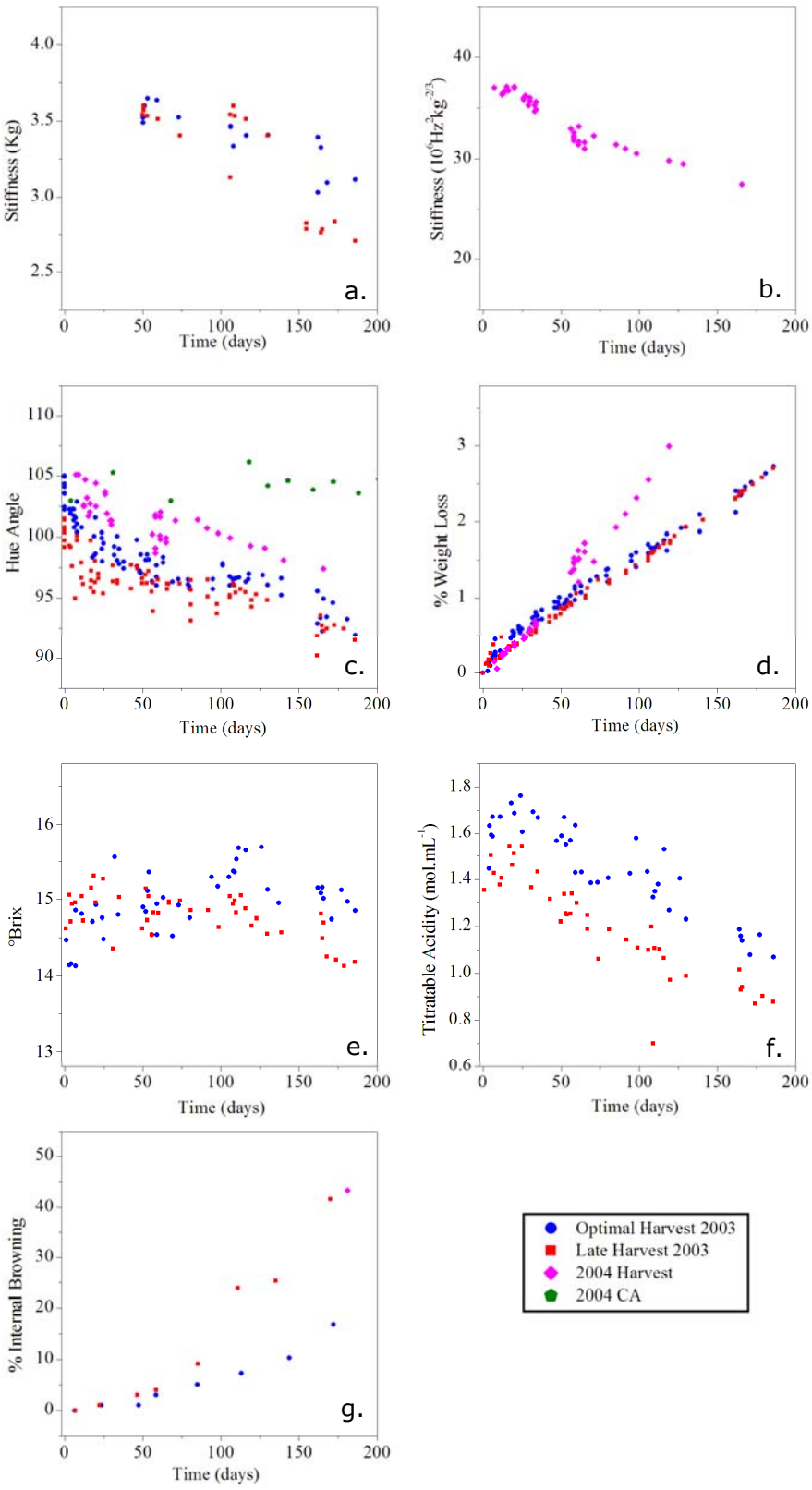

Figure 3.8, 'Cripps Pink' quality changes while stored at 0°C. Optimal Harvest 2003, Late Harvest 2003 and 2004 Harvest were stored in air at 0°C whereas 2004 CA was stored in CA (2% O_2 and 1% CO_2) at 0°C. Optimal Harvest 2003 was harvested 9/04/2003 and Late Harvest 2003 harvested 22/04/03 from Hawkes Bay (New Zealand). 2004 apples were harvested on 30/4/2004 from Batlow, NSW (Australia). Each data point represents an average of 20 (2003) or 30 (2004) fruit.

large differences and no links between ethylene production and rates of softening between five apple cultivars. These works indicate that sensitivity to ethylene rather than ethylene production (or internal ethylene concentration) may be the key in regulation of apple softening. Other fruit properties such as density may play a role in the relationship of ethylene and softening changes. Fruit with larger cells and more intercellular spaces are generally considered to have weaker tissue than fruits with smaller cells and less intercellular spaces (Harker et al., 1997). In either case, comparing the results obtained in this trial to the results published for other cultivars (e.g. Johnston, 2001), it would seem that the softening of the 'Cripps Pink' apple cultivar seems to be relatively insensitive to ethylene, and hence may explain the lack of evidence of the typical triphasic softening profile.

Work with ethylene inhibitors on the 'Cripps Pink' cultivar provides some insight that ethylene still plays some role in softening of the 'Cripps Pink' apple cultivar. Crouch (2003) found that ethylene inhibitor 1-MCP was beneficial in preserving fruit firmness of 'Pink Lady™' apples stored in air. Similarly, Golding et al. (2005) showed that the application of ethylene inhibitor aminoethoxyvinylglycine (AVG) prior to harvest reduced the rate of firmness loss of 'Pink Lady™' during storage in air and Phan-Thien et al. (2004) found that pre-harvest application of AVG resulted in delayed 'Pink Lady™' softening prior to harvest.

3.3.2.2. CA Storage

Rates of firmness change in CA conditions were observed to be significantly slower than that observed in air (Figure 3.7a). Over a 220-day period a firmness reduction of only 1 kgf was observed. Similar slow rates of firmness change as a result of application of CA have been reported (Golding et al., 2005) while Drake et al. (2002) indicated that storage for 180 days in CA conditions resulted in apples with a firmness that was not significantly different from that at harvest.

3.3.3. Background Colour

3.3.3.1. Air Storage

The 2004 harvest had the highest background colour hue angle at the beginning of storage, indicating a less mature fruit than those harvested in 2003 ($P < 0.0001$). This indicated that there were differences in the initial maturity, between harvests. However the effect was opposite to that observed for the climacteric physiology development and flesh firmness and hence demonstrates the difficulties that exist in accurately determining harvest maturity. The differences may be largely due to seasonal and geographical effects. As

expected, fruit from the later harvest of 2003 had a lower initial hue angle than those of the optimal harvest in that year, agreeing with results observed by Gualanduzzi et al. (2005).

Differences in the rate of change of hue angle were observed between years (Figure 3.8c) ($p = 0.001$). In 2003, the change in hue angle during storage was found to follow a biphasic pattern with a rapid decrease in the first 30 days of storage followed by a phase of slower change for the remainder of the storage period. This pattern was not observed in 2004, where the change in the hue angle was observed to change linearly over the duration of the storage period at a rate of approximately 0.04 °hue.day^{-1} (R^2=0.84, $P<0.001$). After approximately 165 days all harvests had changed hue angle by approximately the same value in comparison to their harvest background colour, with hue angle differences remaining apparent as a result of the differences at the time of harvest.

The rapid change in hue angle observed while the fruit were preclimacteric with a relatively low ethylene production does not support the link that has been made between ethylene production during climacteric development and an increased rate of breakdown of chlorophyll and subsequent yellowing, observed in 'Granny Smith' (Johnston, 2001) and 'Golden Delicious' cultivars (Saftner et al., 2003). The results of this trial agree with that of Crouch (2003) who reported that application of 1-MCP to 'Pink Lady™' apples had little effect on the rate of background colour change, suggesting that ethylene may not play a large role in influencing the degreening of 'Cripps Pink' apples and hence provides further evidence of the lack of sensitivity of the 'Cripps Pink' cultivar to ethylene.

3.3.3.2. CA Storage

Application of CA was proven to be highly effective in eliminating background colour changes in 'Cripps Pink' apples over a 200-day period (Figure 3.8c). Brackmann et al. (1994) found that the application of 3% O_2 and 1% CO_2 reduced all background colour changes for 'Cripps Pink' parent cultivar Golden Delicious over an eight month period.

3.3.4. Other Quality Parameters

3.3.4.1. Weight loss

Weight loss in 'Cripps Pink' apples was found to be highly linear (Figure 3.8d). Rates of weight loss for the two harvests of 2003 were found to be similar. This

is in agreement with Maguire et al. (2000) who found that the water vapour permeance of 'Cripps Pink' apples changed little with harvest time. In this study, rates of weight loss differed dramatically between each year. This difference is likely to be caused by the differences in storage methods applied in each respective year. In 2003, where fruit were stored as they would be commercially, in z-pack style cartons, the rate of weight loss was approximately 0.013 $\%.day^{-1}$. However in 2004, fruit were stored in 60 L sealed containers supplied with nonhumidified air, and lost weight at 0.025 $\%.day^{-1}$.

3.3.4.2. Soluble Solids and Titratable Acidity

No significant changes in 'Cripps Pink' total soluble solids (°Brix) were observed as a result of harvest date or time in storage (Figure 3.8e), agreeing with the findings of Drake et al. (2002) and Kupferman (2003) and contradicting the constant reduction in soluble solids reported by Saftner et al. (2005). However, acidity at the optimal harvest was greater than the later harvest (Figure 3.8f), with this difference being maintained throughout the 190 days of storage. Acidity was stable in the first 25 days of storage (for either harvest), and later reduced at a linear and similar rate for both harvests (-0.0035 $mol.mL^{-1}day^{-1}$, $R^2 = 0.82$) suggesting a strong link between ethylene production and changes in fruit acidity. Saftner et al. (2005) also reported a reduction in acidity throughout storage in air at 0°C. The stability of soluble solids (°Brix) coupled with a decrease in titratable acidity during maturation of 'Cripps Pink' agrees with the previous findings by Corrigan et al. (1997) and Drake et al. (2002) for this cultivar.

3.3.4.3. Disorders Incidence

Some 'Cripps Pink' apples develop internal browning during cool storage. In this trial, internal browning of 'Cripps Pink' was more prevalent in fruit stored for a longer time (Figure 3.8g) agreeing with the findings of Kupferman (2003). Additionally, a higher incidence of internal browning was found for the later harvest of 2003 than in the earlier harvested fruit. This observed higher incidence of internal browning for 'Pink Lady™' apples from a later harvest supports results from more detailed studies (Brown et al., 2003; James et al., 2005; Gualanduzzi et al., 2005).

Superficial scald is a postharvest physiological disorder of some apple cultivars that manifests itself as browning of the skin with no influence on the flesh, and often develops upon removal from coolstorage temperatures. The disorder is sporadic in nature, affected by season, growing location and harvest and is

caused by the oxidation of α-farnesene, a metabolite promoted by ethylene. (Watkins et al., 2000a). Some of the optimal 2003 harvest fruit developed superficial scald after 160 days in storage and the incidence increased to 20% of the fruit after 180 days in storage. In contrast, only 2 apples (0.2%) of the late harvest fruit were found to develop superficial scald by the completion of the 200 day storage period (data not shown). Similarly, Gualanduzzi et al. (2005) found that only the first of three harvests of 'Pink Lady™' were susceptible to superficial scald. The higher incidence of superficial scald in earlier harvested apples is consistent with those results found by Bramlage and Watkins (1994) for 'Granny Smith', 'Delicious' and 'Cortland' cultivars and Blanpied et al. (1991) for the 'Starkrimson Delicious' cultivar. Almost no superficial scald was observed in the 2004 experimental work. Commercial incidence of scald is often controlled with the application of the antioxidant, diphenylamine (DPA) after harvest (which was not used in this trial).

3.4. FURTHER DISCUSSION AND CONCLUSIONS

3.4.1. Correlations Between Quality Parameters

A Pearson's correlation analysis of the collected quality data was conducted in order to further investigate relationships between the quality factors measured. Firstly, data from all three harvests stored in air at 0°C were pooled and analysed, and then each harvest was analysed separately. The correlations between the quality measurements are displayed graphically in Figure 3.9.

The strongest correlation observed for the pooled data set was for the destructive firmness and titratable acidity (Figure 3.9g) while destructive firmness and weight loss and destructive firmness and hue angle also showed a strong (> 0.800) correlation (Table 3.1). Notably, the destructive firmness and hue angle correlation for air stored apples does not apple to those fruit stored in CA (Figure 3.9i) indicating the different responses of quality parameters to application of CA. Titratable acidity and hue were both highly correlated to weight loss for each harvest, but not as a pooled data set (Figure 3.9e and j). Titratable acidity and hue were highly correlated for the optimal 2003 harvest, but not for the other harvests or the pooled data set. Notably, °Brix was not highly correlated to any other quality parameter nor did it substantially change with storage time, and subsequently should not be considered as a judgement criteria for assessing the quality of 'Cripps Pink' apples.

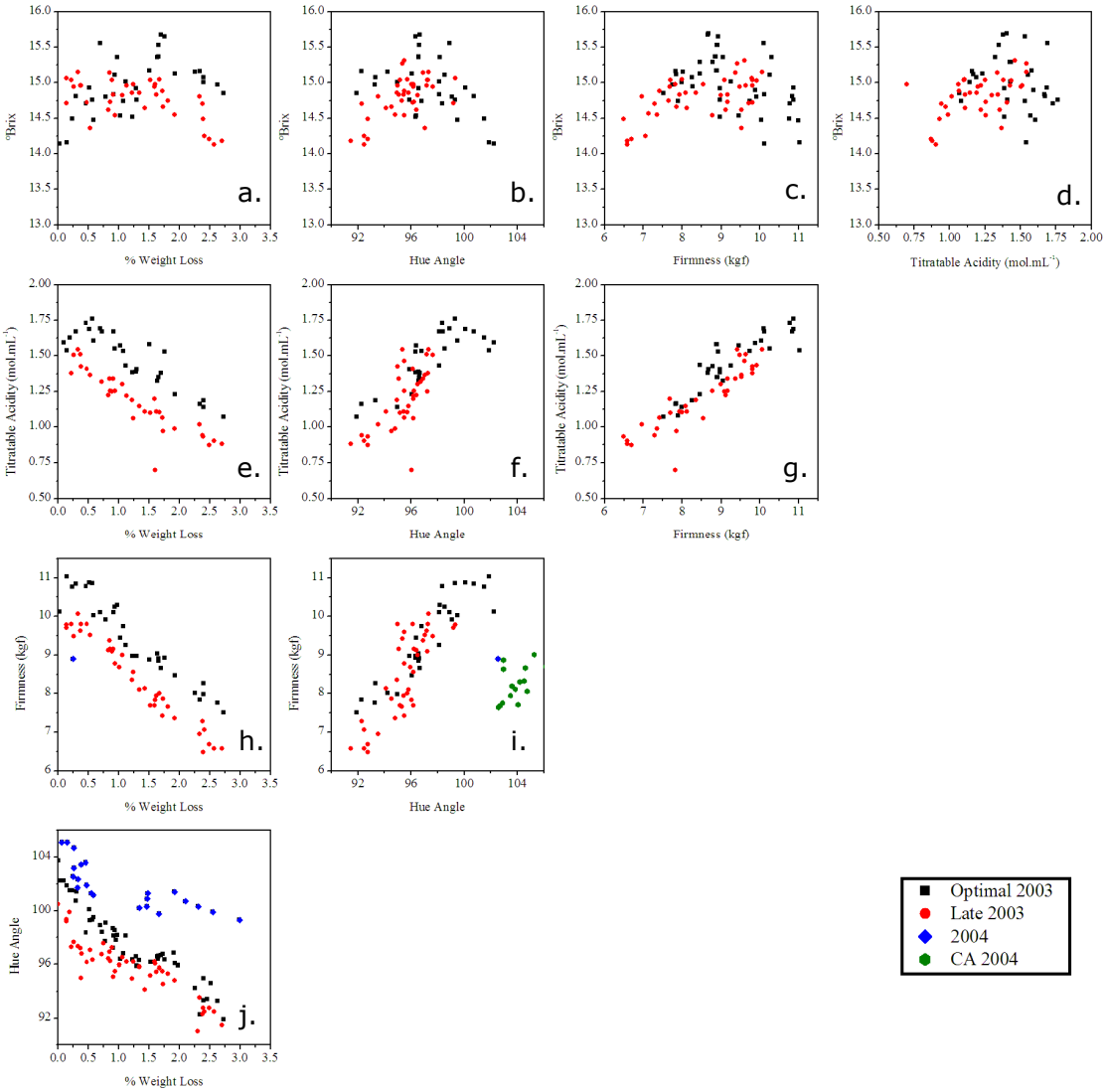

Figure 3.9, Correlations of quality parameters collected from three harvests of Cripps Pink apples. Data presented are identical to that in Figure 3.7a and Figure 3.8.

Table 3.1, Highly Correlated Quality Characteristics as Determined by the Pearsons Correlation. Note: Highly Correlated was defined as (>0.800). Those quality characteristics which were not highly correlated for any harvest were omitted from the table.

			Pearsons Correlation			
	Parameter Pair	Figure	Grouped	OPT03	LAT03	2004
Highly Correlated	Firm – TA	7g	**0.902**	0.903	0.900	-
	Firm – WL	7h	**0.869**	0.952	0.980	-
	Firm – Hue	7i	**0.829**	0.913	0.812	-
Both Harvests Highly Correlated	TA – WL	7e	0.753	**0.884**	**0.899**	-
	Hue – WL	7j	0.680	**0.941**	**0.887**	**0.806**
One Harvest Highly Correlated	TA - Hue	7f	0.781	**0.808**	0.680	-

Note: Firm = destructive firmness (kgf), TA = titratable acidity (mol.mL^{-1}), WL = weight loss (%), Hue = background hue angle (°Hue).

3.4.2. Sensitivity of quality parameters to ethylene in apple cultivars

In this study, the 'Cripps Pink' cultivar has been observed to respond to the increased ethylene production at the climacteric by increasing respiration rate and reducing titratable acidity. However the responses of increased rate of firmness loss and hue angle change observed in other cultivars (Johnston, 2001) were not highly evident for 'Cripps Pink' at 0°C. The reduced rate of softening and colour change of 'Cripps Pink' provides a commercially valuable cultivar with a long storage life.

The results from this work and those conducted with other cultivars (e.g. Gussman et al. 1993; Johnston, 2001) suggests that responses of apples to ethylene are cultivar specific. Ripening processes that are generally accepted as dependent on ethylene, include firmness, respiration rate, hue and titratable acidity. Multiple ethylene receptors have been identified in tomato, although their individual roles are not yet known (Klee et al., 1999). It is possible that each ethylene receptor controls a different ripening pathway. Similarly, it is possible that signal transduction pathways of the ripening responses are different. In either case, differences in response to ethylene between cultivars can be attributed to differences in reception and signal transduction of ethylene rather than the presence of ethylene itself. Detailed investigations into the roles of different ethylene receptors and/or ethylene response signal transduction pathways, possibly aided by the comparison of the different responses observed in different cultivars, will allow the isolation of critical regulatory factors and aid in future successful breeding and/or genetic manipulation of new commercial cultivars with enhanced storability.

3.4.3. Final Conclusions

The 'Cripps Pink' apples in this study showed a typical climacteric ripening pattern, resulting in a dramatically increased ethylene production and increased respiration rate 30 days after storage at 0°C. During storage, the rates of weight loss and firmness loss were relatively linear, whereas hue angle change was harvest dependent and °Brix did not change significantly. The loss of titratable acidity began at the time of climacteric development and was subsequently linear.

Delays in harvesting 'Cripps Pink' apples are likely to cause a decrease in the hue angle, firmness and titratable acidity at the time of harvest and this difference is maintained during storage. Fruit harvested later are also more likely to develop

internal browning and less likely to develop superficial scald. Otherwise, postharvest physiology and quality of 'Cripps Pink' apples is little affected by harvest maturity.

Climacteric ethylene production was found to induce increased respiration rate and the rate of titratable acidity loss. However, the rates of firmness loss and hue angle were not stimulated by the climacteric ethylene production in 'Cripps Pink' apples. Comparing the ethylene reception and response signal transduction pathways of 'Cripps Pink' apples to that of other apple cultivars which have firmness and/or hue angle sensitivity to ethylene may elucidate key regulatory factors that will aid future breeding of commercially successful apple cultivars.

4. The Effect of Breaks in Temperature Control on 'Cripps Pink' Apple Physiology and Quality

4.1. INTRODUCTION

The effects of temperature and gas atmosphere on fresh produce physiology and quality have been extensively studied, to the point where optimal (constant) storage conditions for most products are widely available in the free press (e.g UC Davis website, http://postharvest.ucdavis.edu; USDA Handbook 66, http://www.ba.ars.usda.gov/hb66/). However, in the industrial environment optimal storage conditions are not always maintained throughout the coolchain due to logistic constraints, equipment breakdown, or management decisions. One common scenario in New Zealand and Australia, is the storage of fruit in bins after harvesting. At a later time the fruit are removed from the store, graded and packed (often in a non-refrigerated grading line) before shipping in a refrigerated environment to off-shore markets. How these types of breaks in storage conditions affect product physiology and quality on return of the product to refrigerated storage conditions has rarely been investigated.

The use of controlled applied heat treatments, either prior to storage (pre-storage heat treatment) or during storage (intermittent warming) has been extensively investigated (Klein and Lurie, 1992; Watkins et al., 2000a). While these treatments are primarily applied as a means to reduce chilling injury during coolstorage for most fruit, for apples these treatments are usually applied to reduce the rate of softening during periods of coolstorage (with exception of the study of Watkins et al., 2000a) and hence extend the length of possible storage. This body of work does provide some clues as to the possible influence of unintentional breaks in temperature control. Unintentional breaks differ from the application of intentional treatments as they are sporadic and occasional, and the position of the fruit in relation to the external environment will significantly influence the exposure to the external temperature (i.e. fruit next to the exterior of the package will change temperature rapidly, while product located within a pallet stack may not be significantly influenced). These positional effects of fruit to the environment as determined by packaging systems were however not investigated by experimental protocols in this thesis.

Currently it is generally accepted by industry and researchers that the rate of quality loss of a product is dependent only on the current product quality and storage conditions (Tijskens and Verdenius, 2000). It is frequently assumed (in

state of the art fresh produce modelling) that current fruit physiology and quality is representative of previous storage conditions and that future quality losses and physiology are independent of these previous storage conditions (Uchino et al., 2004).

This investigation aimed to analyse the effect of breaks in temperature control during coolstorage (in air) to confirm or challenge the validity of the assumption that only the current fruit maturity and environmental conditions effect product physiology and rates of quality change. For industry bodies the results could assist in informing decision making, such as the selection of operating conditions or corrective action when there has been a deviation from optimal conditions. If sufficient evidence is found that significant deterioration of product is caused by small temperature breaks this will justify technological solutions to be sought to eliminate or minimise the risk of breaks occurring (e.g. better control systems).

This chapter describes the physiological and quality responses of 'Cripps Pink'' apples observed during the laboratory simulation of alternative coolchain scenarios (without the use of CA technology) in which breaks in temperature control occur. Data was collected over two seasons (2003 and 2004), from harvests from two counties (Hawke's Bay, New Zealand and Batlow, NSW, Australia), with investigations taking place into the influence of harvest maturity, timing of breaks in temperatures control in the coolchain and the implications of multiple breaks in the coolchain. In total, the physiology and quality of 'Cripps Pink' apples were monitored during 23 different simulated coolchain scenarios.

4.2. METHODOLOGY

4.2.1. Fruit, Physiological and Quality Measurements

'Cripps Pink' apples were obtained on two years (2003 and 2004) as explained in section 3.2.1. The methods used for assessing physiological status (respiration rate and ethylene production) and quality (colour, non-destructive firmness, penetrometer firmness, brix, titratable acidity and disorders) were those used in for each year, respectively (sections 3.2.4 to 3.2.6). All non-destructive measurements (respiration rate, ethylene production, hue angle and non-destructive firmness) were taken at the current treatment temperature, whereas all destructive measurements were conducted at 20°C, after allowing 3 hours of acclimatisation to that temperature if required (Figure 3.2). For assessment of physiological status, the time between sampling of headspace gas was reduced to 30 minutes when at 20°C.

4.2.2. Temperature Treatments

The experiment was designed to assess a range of factors that may affect the response of the product to temperature changes. In 2003, the experiment was designed to simulate possible commercial practices, whereby after a period of coolstorage (0, 2, 4 or 6 months), fruit can be exposed to a break in temperature control due to loading, re-grading and packing. This simulated break was 3 days at 20°C (B = break) with each treatment that experienced a break having an equivalent control treatment of 3 days at 0°C (C = control). The apples were then subjected to a simulated temperature regime as if they were transported to a distant marketplace in the coolchain (3 weeks at 0°C) and then sold at retail (2 weeks at 20°C) (Figure 4.1). Treatments were replicated by applying to both optimal and late harvest apples from the same source. Each treatment was represented by 20 fruit, on which all non-destructive measurements were made.

In 2004, the shelf life period was removed from the experiment in order to focus on the subsequent long-term effects on fruit physiology and quality change at coolroom temperatures if the fruit were exposed to an earlier break in temperature control. Additionally, the influence of length of break in temperature control, and the implications of multiple breaks in temperature control were also investigated (Figure 4.2). Physiological status of 10 fruit and quality of 30 fruit were measured (non-destructively) for each treatment.

In all cases, temperature changes were achieved by physically moving fruit from a room at one prescribed temperature to another. Rapid temperature change of fruit was aided by placing the fruit in front of fans for a period of at least 3 hours (Figure 3.2).

4.2.3. Data Transformation

4.2.3.1. Physiological data

In order to remove the influence of natural variability, the quality attributes collected in a non-destructive manner were compared to measured attributes prior to exposure to 20°C on an individual apple basis. Respiration rate and ethylene production data were transformed to relative values based on those measured prior to temperature exposure (equation 4.1). At each time point and for each individual fruit the respiration rate/ethylene production was divided by that measured immediately prior to the first temperature exposure (for those treatments with an exposure) or that measured at the equivalent time (for the

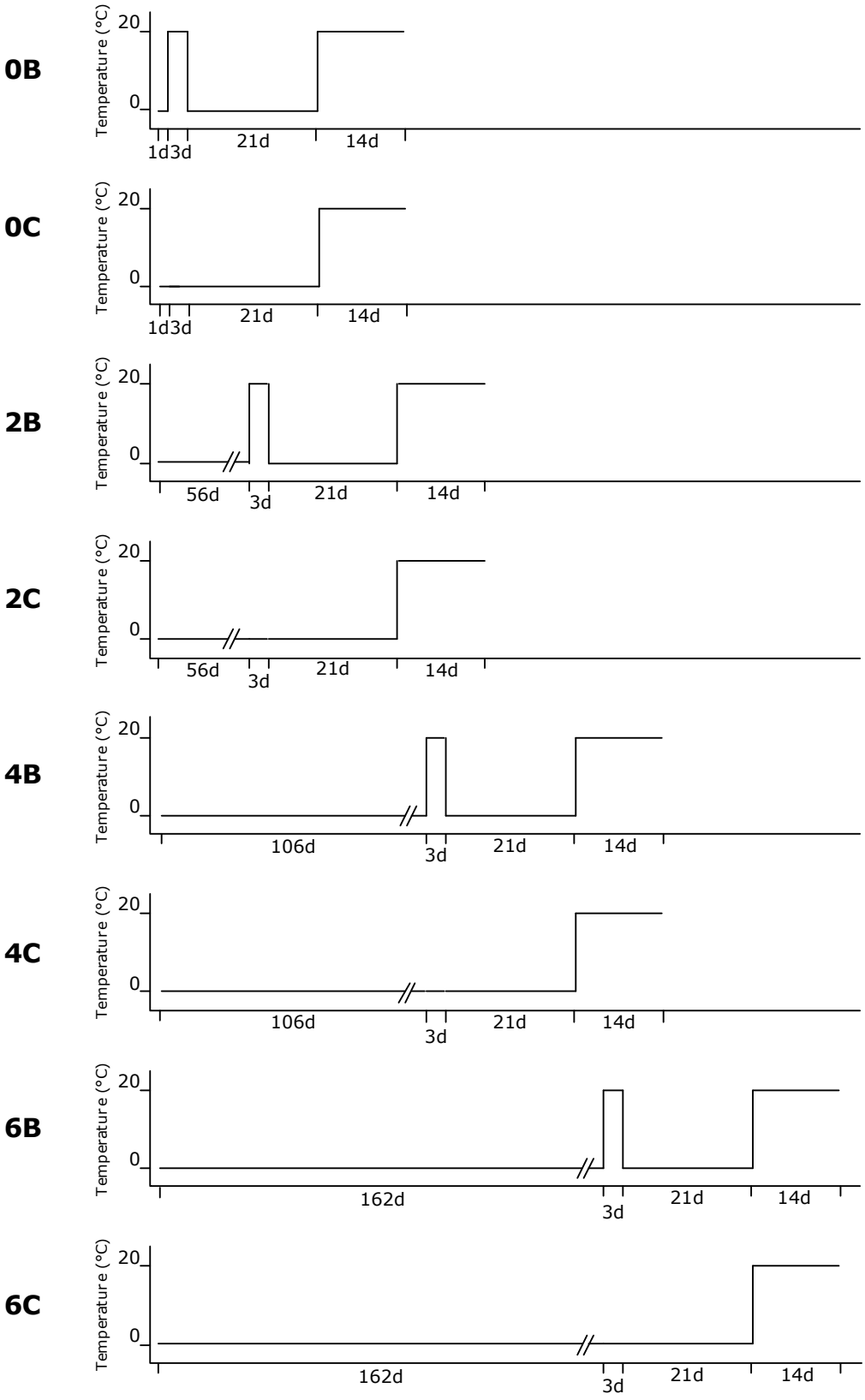

Figure 4.1, Description of treatments used for apples from both (optimal and late) 2003 harvests from Hawkes Bay, New Zealand. Note: d = days.

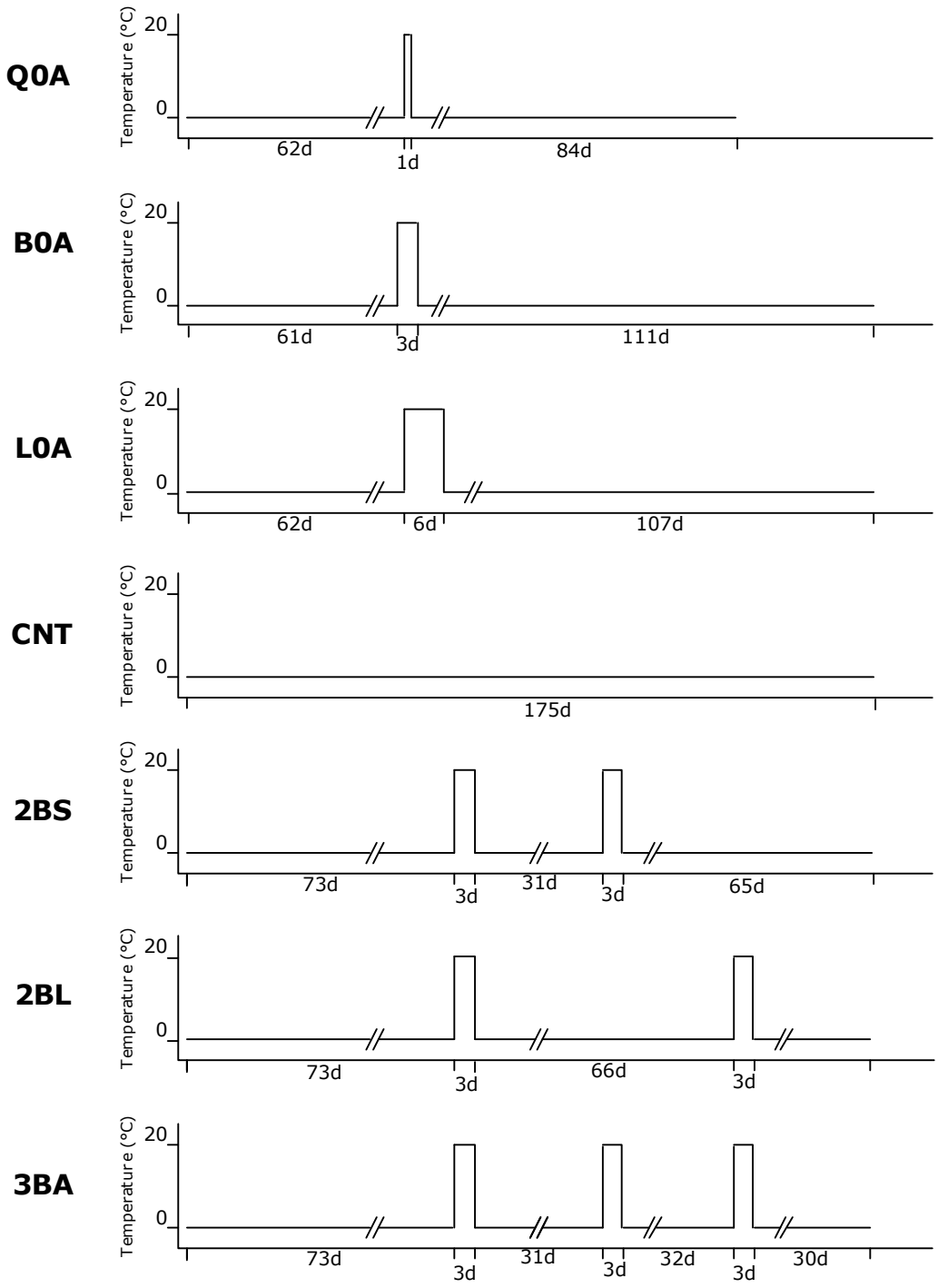

Figure 4.2, Description of treatments used for apples from the 2004 harvest from Batlow, NSW, Australia. Note: d = days.

control treatments). A relative value equal to one indicates no change in physiological status of that fruit, where those values below or above one would indicate a slower or more rapid metabolism, respectively.

In preclimacteric fruit (treatments 0C and 0B) respiration rate and ethylene production could not be converted to relative rates, as the rates at harvest were

not at a point of steady state due to the development of the climacteric (section 3.3.1.1) and hence are presented as their unconverted values.

$$\text{Relative}_{RR} = \frac{\text{Current}_{RR}}{\text{Prior to Initial 20°C Abuse}_{RR}} \qquad [4.1]$$

Equation used to transform physiological data (respiration rate (RR), and ethylene production) from measured values to relative values. Note: RR = respiration rate. Relative ethylene production can be calculated by replacing subscript RR with EP.

4.2.3.2. Non-Destructive Quality Data

Hue angle and stiffness are reported as changes from the time when first exposed to a break in temperature control (equation 4.2). Values for hue angle and stiffness at the time of initial exposure to the temperature break were extrapolated by straight line from previous data points collected prior to temperature exposure. As hue angle and stiffness both reduce during storage of 'Cripps Pink' apples, the parameter change values are expected to get increasingly more negative with time.

$$\text{Change}_{Hue} = \text{Current}_{Hue} - \text{Prior to Initial 20°C Abuse}_{Hue} \qquad [4.2]$$

Equation used to transform non-destructive quality (hue angle and stiffness) measurements to change in quality. Note: Hue = hue angle. Stiffness (ST) can be calculated by replacing subscript Hue with ST.

The incidence of physiological disorders was consolidated by summing the total incidence of each storage disorder (internal browning and superficial scald) in each treatment subsequent to the initial exposure to 20°C (or at the equivalent time for control treatments) and reporting this as a percentage of the total fruit measured.

4.2.3.3. Data Analysis

One-way analysis of variance (Minitab v13.31, Minitab Inc, USA) was conducted at points of interest identified from the graphs constructed. When the variance of data was not homogeneous as assessed by Levene's test ($P<0.05$, Minitab v13.31, Minitab Inc, USA) values were then transformed using log with base of 10 in order to regain homogeneity. Significant differences between treatments and departure from initial physiological status (log relative respiration rate/ethylene production = 0) were determined with the use of Fishers' LSD method ($P<0.05$, Minitab v13.31, Minitab Inc, USA).

Rates of loss of hue angle and stiffness were calculated for individual apples between defined time periods using linear regression (Excel v9, Microsoft Corp, USA). Comparison of rates of loss of stiffness and hue angle between treatments was also conducted using one-way analysis of variance (Minitab v13.31, Minitab Inc, USA) and Fishers' LSD ($P<0.05$, Minitab v13.31, Minitab Inc, USA).

Significant differences in incidence of storage disorders were determined with the use of the chi-squared statistic (Minitab v13.31, USA).

4.3. RESULTS AND DISCUSSION

In 2003, the experiment was replicated by using two harvests from the same source. There were no substantial differences observed between the optimal and late harvest data, except the physiology of fruit exposed to a break in temperature control 0 months after coolstorage (treatment 0B), so only the optimal harvest data is presented and discussed.

4.3.1. Physiological Changes

4.3.1.1. Effect of Length of Storage Duration Prior to a Temperature Break

The physiological status of 'Cripps Pink' apples from the same harvest and stored constantly at 0°C (treatments 0C, 2C, 4C and 6C) provided a baseline against which the response of the apples during and after the temperature exposure (treatments 0B, 2B, 4B and 6B) could be compared (Figure 4.1).

Physiological status at the time of the exposure to break in temperature control was found to have a significant effect on the response of 'Cripps Pink' apple physiology. Those fruit that had yet to demonstrate a rapid increase in ethylene production to a rate of approximately 0.1 $nmol.kg^{-1}s^{-1}$ (Figure 3.4b) were considered to be preclimacteric, while fruit that had reached this rate of ethylene production were considered postclimacteric. Those apples exposed rapidly after harvest (treatment 0B) and still in a preclimacteric state displayed a different behaviour to fruit that had been stored for a period of time (treatment 2B, 4B and 6B) and hence developed their climacteric status while in storage prior to the break in temperature control. This section is therefore presented in two parts, firstly the fruit that were preclimacteric when exposed to a break in temperature control, followed by the fruit that were postclimacteric at the time of exposure to a break in temperature control.

4.3.1.1.1. Influence of a break in temperature control on the physiology of preclimacteric apples

Fruit exposed to 20°C after 0 months storage (treatment 0B) showed an approximate 8-fold increase in the rate of respiration (Figure 4.3a), similar to response of postclimacteric fruit (Figure 4.4a). When returned to cool storage after exposure to 20°C for 3 days, rates of respiration of fruit (treatment 0B) were similar to the levels of control apples (treatment 0C). The rate of respiration during the shelf life period was not substantially influenced by the previous exposure to 20°C.

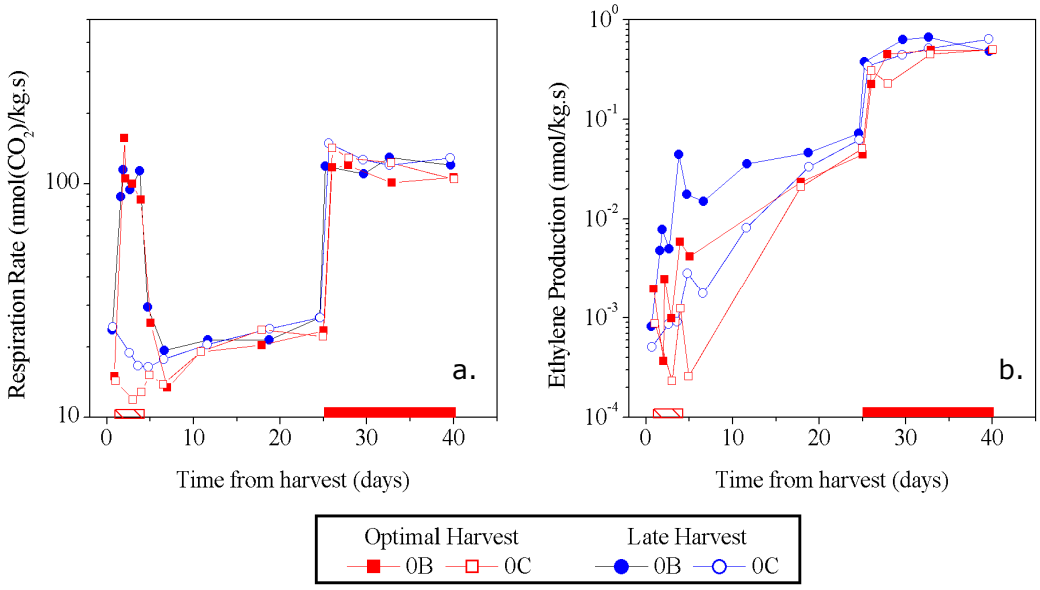

Figure 4.3, The effect of a temperature break (B, 3 days at 20°C) in comparison to control fruit (C, 3 days at 0°C) on 'Cripps Pink' apple (a) respiration rate and (b) ethylene production, after 0 months storage at 0°C for both optimal and late harvest fruit from the 2003 harvest. After the exposure of 3 days at 20°C fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Empty bars represent time of exposure to 20°C for temperature break treatments whereas solid bars represent time at 20°C for all treatments (shelf life).

Ethylene production of preclimacteric apples was observed to increase slightly during the initial exposure to 3 days at 20°C (Figure 4.3b) but not nearly as much as postclimacteric fruit (Figure 4.4b). The increase in ethylene production was maintained on immediate return to 0°C. In the subsequent 21 days at 0°C differences generated between treatment 0B and 0C dissipated with ethylene production of control fruit increasing to a level equal to that of the fruit exposed to the break. It would appear that a period of storage at 20°C for preclimacteric 'Cripps Pink' apples results in advancing the start of the climacteric peak. During the shelf life period ethylene production was not substantially influenced by previous temperature exposure.

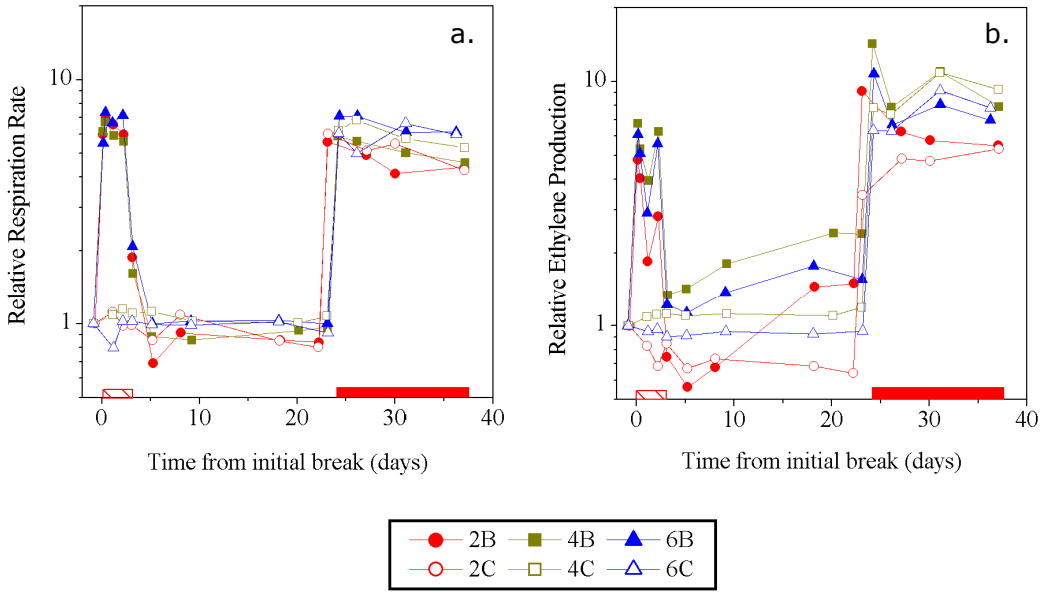

Figure 4.4, The effect of a temperature break (B, 3 days at 20°C) in comparison to control fruit (C, 3 days at 0°C) on 'Cripps Pink' apple relative (a) respiration rate and (b) ethylene production, after 2, 4 and 6 months storage at 0°C. Data presented is for the optimal harvest only. After exposure of 3 days at 20°C fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Empty bars represent time of exposure to 20°C for temperature break treatments whereas solid bars represent time at 20°C for all treatments.

Similar to this work, delays before cooling 'Royal Gala' and 'Cox's Orange Pippin' apple increased internal ethylene concentration at 3 days into a delay (at 20°C) (Johnston, 2001). Upon cool storage (at 3°C), 'Cox's Orange Pippin' apples that had a cooling delay of 3 or 5 days had an internal ethylene production twice that of apples delayed for 1 or 0 days. In 'Royal Gala' apples, when cooling was delayed for 7 days, the apples had a higher ethylene production than the other treatments for the subsequent 100 days in storage (at 0.5°C). These studies provide evidence that delay in cooling of apples after harvest advances the development of the climacteric peak. If the apple cultivar is relatively sensitive to ethylene, this reduction of preclimacteric storage time may result in a reduced storability of the fruit (Johnston, 2001).

4.3.1.1.2. Influence of a break in temperature control on the physiology of postclimacteric apples

Apples stored for 2, 4, and 6 months before exposure to 20°C for 3 days all showed similar patterns of respiration and ethylene production upon exposure to 20°C and during subsequent cool storage and shelf life periods (Figure 4.4). The rate of respiration increased approximately 6-8 times over that at 0°C upon initial

exposure to 20°C. Respiration of the exposed apples then reduced to similar levels as non-exposed apples when they were returned to 0°C. During the simulated shelf life period (2 weeks at 20°C), the respiration rates of all treatments was again 6-8 times greater than at 0°C (Figure 4.4a).

Ethylene production of 'Cripps Pink' apples stored for 2, 4, or 6 months prior to exposure to 3 days at 20°C was initially 5-7 times higher on the day of exposure, reducing to 2 to 4 times higher on the following day and increasing to 4 to 6 times higher on the third day at 20°C (Figure 4.4b). This trend was irrespective of harvest maturity (data not shown) or previous time in storage. A burst of ethylene synthesis upon transfer of produce from chilled temperatures to warmer temperatures is often seen in apples. Jobling and McGlasson (1995) observed a burst of ethylene production in 'Lady Williams' (a parent cultivar of 'Cripps Pink') and 'Fuji' apples after up to 32 days at 0°C which correlated well to accumulated ACC concentrations and ACO activity. Similarly, in cucumber fruits ACC accumulates at chilled temperatures and is rapidly metabolised to ethylene on transfer to 25°C (Wang and Adams, 1982). The rapid burst of ethylene production on exposure to 20°C following storage at 0°C is likely to be a result of accumulated precursors at 0°C which are utilised with increased enzyme activity upon transfer of fruit to the warmer temperature.

On return to cool storage (at 5 days), fruit stored for 2 months prior to exposure (treatment 2B) initially produced ethylene at a rate less than before the break, as did the equivalent control treatment (2C). This result is caused by the reduction in ethylene production immediately after the climacteric observed for the optimal harvest in 2003 (Figure 3.3b). Apples from the optimal harvest and stored for 4 months prior to exposure produced significantly greater ethylene in comparison to the control treatment on return to coolstorage (Table 4.1; Figure 4.4b). Apples from the late harvest and stored for either 2 or 4 months prior to the break both produced ethylene at rates significantly less than prior to exposure (data not shown), while apples from either harvest and stored for 6 months produced ethylene at similar rates than prior to the break. Subsequently, no clear influence of either length of storage (providing apples are postclimacteric) or harvest maturity on the ethylene production on return to coolstorage was established.

Irrespective of harvest maturity (data not shown) or time in storage prior to exposure, all treatments exposed for 3 days at 20°C eventually showed increased

Table 4.1, Comparison of 'Cripps Pink' apple relative ethylene production for treatments exposed to 3 days at 20°C (B) after variable times in storage at 0°C to apples not exposed to a break in temperature control (C). 2, 4, and 6 refer to 2, 4 and 6 months storage at 0°C prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Data points are extracted from those presented in Figure 4.4a.

	Log Relative Ethylene Production							
Time after initial exposure to 20°C	5 days (0°C)	n	18 days (0°C)	n	24 days (20°C)	n	36 days (20°C)	n
2B	-0.313 ^{d*}	14	0.140 ^{c*}	16	0.941 ^{bc}	14	0.703 ^{c}	15
2C	-0.185 ^{c*}	18	-0.200 ^{e*}	17	0.547 ^{e}	12	0.709 ^{c}	14
4B	0.133 ^{a*}	19	0.359 ^{a*}	19	1.131 ^{a}	19	0.899 ^{ab}	16
4C	0.039 ^{b}	16	0.031 ^{d}	15	0.881 ^{cd}	16	0.944 ^{a}	16
6B	0.034 ^{b}	20	0.229 ^{b*}	20	1.019 ^{b}	20	0.814 ^{b}	20
6C	-0.041 ^{b}	19	-0.038 ^{d}	19	0.794 ^{d}	19	0.879 ^{ab}	18
$LSD_{0.05}$	0.085		0.085		0.091		0.104	

Different letters in columns indicate significant differences between treatments within the column ($P<0.05$). Asterisk (*) indicates significant differences from the physiological status immediately prior to breaks in temperature control (0) for fruit at 0°C.

ethylene production at a level approximately twice that of the pre-break ethylene production (at 18 days), and significantly different from those treatments not exposed to a break in temperature control. It would seem that exposure of postclimacteric 'Cripps Pink' apples stored at 0°C to short periods at 20°C results in a shift in the ethylene homeostasis on return to coolstorage. While at 20°C, the precursors involved in ethylene production (ACC, SAM) and/or enzymes (ACO, ACS) may alter markedly in concentration and/or activity. Each precursor/enzyme may have a different temperature sensitivity. These changes result in a shift in balance on return to coolstorage, which ultimately causes a change in the rate of ethylene production.

Upon initiation of the shelf life period at 20°C (at 24 days after the break), fruit that had previously been exposed to 20°C continued to produce ethylene at a significantly higher rate over those not previously exposed. This difference was observed to decrease during the shelf life period and resulted in both break and control fruit treatments producing ethylene at the same rate after 2 weeks at 20°C (at 36 days, Table 4.1). This result suggests that the exposure to 20°C causes the shift in ethylene production homeostasis (and not the return to 0°C from 20°C), as the control treatment is able to produce ethylene at the same rate as the treatment previously exposed after a period of time at 20°C.

4.3.1.2. Effect of Length of a Single Break During Storage

The effect of length of time at 20°C on the physiological response of 'Cripps Pink' apples on return to coolstorage was investigated in 2004. The respiration rate of 'Cripps Pink' apples on return to 0°C was observed to be not affected by length of exposure to 20°C (Figure 4.5a). Respiration rates of all treatments were not substantially different to those prior to the temperature break (relative respiration rate = 1) or to fruit not exposed (treatment CNT).

The length of time of exposure to 20°C significantly influenced the pattern of ethylene production of 'Cripps Pink' apples on return to coolstorage (Figure 4.5b). Fruit that were exposed to 1 day at 20°C (treatment Q0A) initially (at 5 days after initial exposure) produced about 50% less ethylene than before the temperature break, whereas fruit exposed to 20°C for 6 days (treatment L0A) returned to ethylene production at the same level as prior to the break (Table 4.2). This result suggests that only a short time at 20°C can trigger the induced increase in ethylene production, although time is required for the increase in ethylene production to occur.

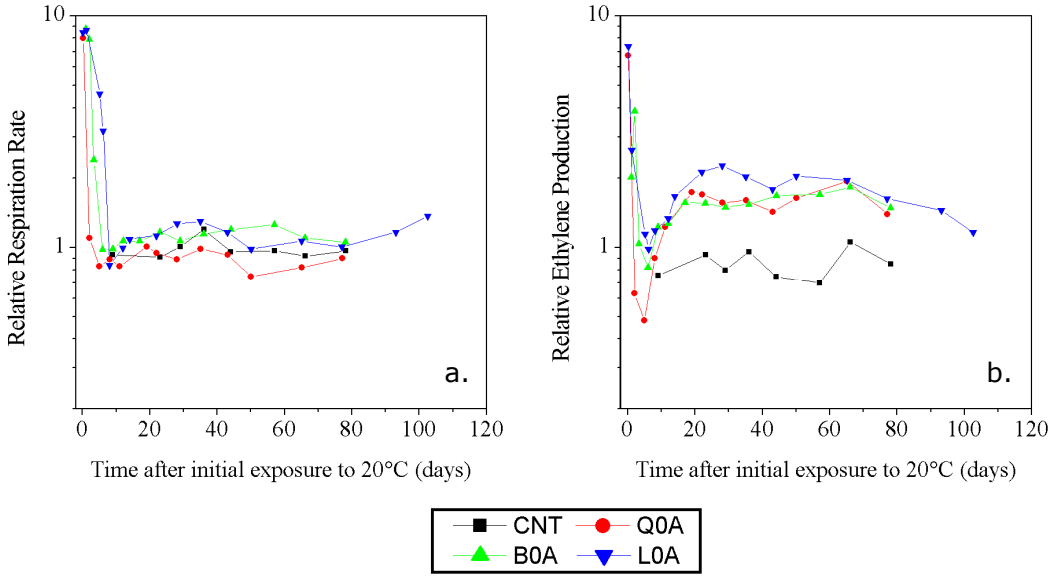

Figure 4.5, Influence of the length of exposure to 20°C on the physiological response of 'Cripps Pink' apples after 2 months at 0°C. Treatments CNT, Q0A, B0A and L0A represent exposure time of 0, 1, 3, and 6 days of exposure to 20°C respectively.

Table 4.2, Comparison of 'Cripps Pink' apple relative ethylene production for treatments exposed to varying times at 20°C after 60 days of storage at 0°C. Treatments CNT, Q0A, B0A and L0A represent exposure time of 0, 1, 3, and 6 days of exposure to 20°C respectively. Data points are extracted from those presented in Figure 4.5b.

	Log Relative Ethylene Production					
Time after initial exposure to 20°C	5 days	n	25 days	n	65 days	n
CNT	-0.123 ^{ab}	10	-0.041 ^{c}	9	-0.167 ^{b*}	10
Q0A	-0.355 ^{b*}	10	0.208 ^{ab*}	10	0.204 ^{a*}	9
B0A	-0.126 ^{ab}	10	0.174 ^{b*}	10	0.182 ^{a*}	10
L0A	0.044 ^{a}	10	0.307 ^{a*}	10	0.288 ^{a*}	10
$LSD_{0.05}$	0.308		0.116		0.113	

Different letters in columns indicate significant differences between treatments within the column ($P<0.05$). Asterisk (*) indicates significant differences from initial physiological status (0).

Apples exposed to 20°C (for any length of time) and stored at 0°C for another 25 days produced 50 to 100% greater ethylene than prior to the exposure. This altered rate of ethylene production, induced by the previous exposure to 20°C was maintained for the remainder of the experiment (80 days at 0°C). Apples that were not exposed to any break in temperature control (treatment CNT) maintained a relatively constant ethylene production throughout the 80 day period of the experiment.

4.3.1.3. Effect of Multiple Temperature Breaks During Storage

In 2004, the physiology of 'Cripps Pink' apples was monitored before, during and after exposure to a primary, secondary and (in one treatment) a tertiary exposure to 20°C for 3 days, separated by periods of storage at 0°C. On each exposure to 20°C, respiration rates of 'Cripps Pink' apples increased by 7-9 fold (Figure 4.6a). These results are not unlike that found for tomatoes stored at 9°C and warmed to 20°C at weekly intervals, which were observed to alter respiration rate and ethylene production by similar levels on each warming occasion (Artés et al., 1998). On return to cool storage at 0°C the rate of respiration was not significantly different to those prior to the initial exposure.

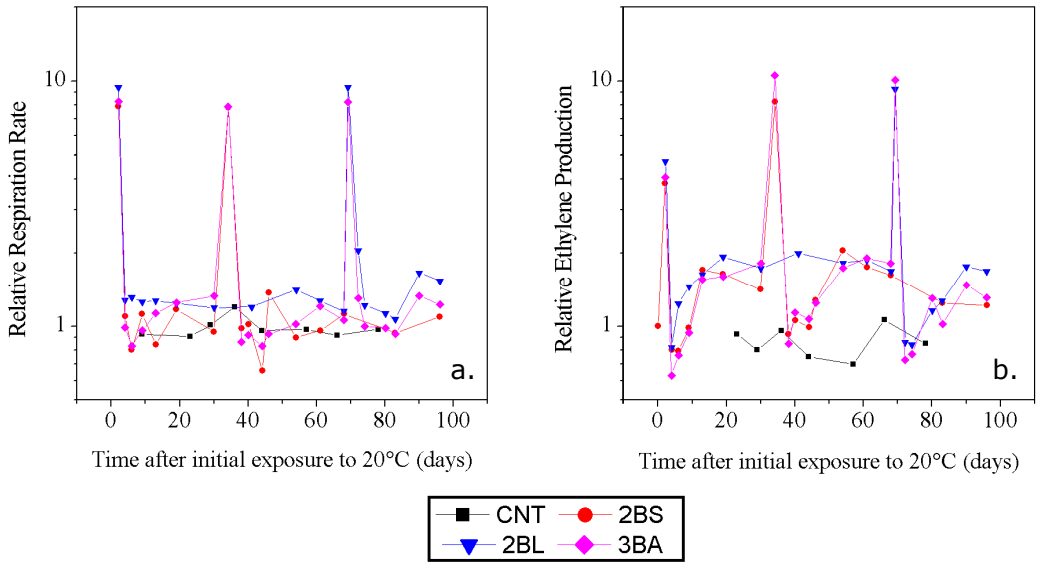

Figure 4.6, Effect of multiple temperature breaks of 20°C for 3 days during storage at 0°C on (a) respiration rate and (b) ethylene production. Note: CNT = no exposure; 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days). Data for CNT is identical to that presented in Figure 4.5. Apples were stored at 0°C for 73 days prior to initial exposure.

Primary exposures of the treatments resulted in the development of increased ethylene production on return to 0°C, as previously discussed. Secondary exposures or a tertiary exposure to 20°C for 3 days mimicked the response observed for the primary exposure (Figure 4.6b). On each exposure the ethylene production of the 'Cripps Pink' apples increased 5-6 times when moved to 20°C. Absolute ethylene production was greater during the second and third fruit exposures, possibly due to a higher rate at 0°C induced by the previous temperature exposure. This result strengthens the finding of 2003 in which fruit previously exposed to 20°C produced more ethylene than control treatments on the initiation of the shelf life period (Table 4.1).

On initial return to 0°C after the second (or third) exposure (e.g. at 40 days for treatments 2BS and 3BA), fruit produced ethylene at rates similar to that of fruit not exposed to any period at 20°C (Table 4.3). This result is in spite of the fact that the fruit were producing approximately double this amount of ethylene prior to a secondary exposure as a result of the primary exposure.

As seen in the case of a single exposure to 20°C, apples exposed for a second occasion produced ethylene at rates similar to that of apples not exposed for approximately 5 days after return to 0°C (Figure 4.7). After 5 days, ethylene production once again increased until reaching a production rate of approximately double that of non-exposed apples 18 days after exposure. The observed pattern of a delayed induced increase in ethylene production as a result of exposure of postclimacteric 'Cripps Pink' apples to 3 days at 20°C remains consistent even when a third exposure to 20°C is applied (Figure 4.7a) and this change is independent of the time at 0°C between exposures (Figure 4.7b). These results suggest that any time period spent at 20°C triggers an alteration to the apples homeostasis ethylene production. A new point of homeostasis results, producing a sustained period of ethylene production approximately double that prior to exposure to 20°C.

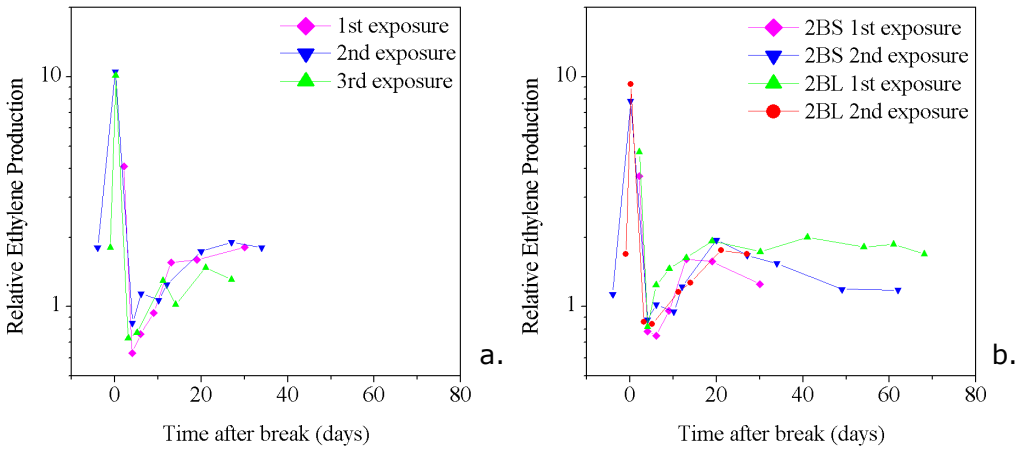

Figure 4.7, Comparison of the 'Cripps Pink' apple relative ethylene production response of (a) the same fruit to 3 breaks in temperature control (treatment 3BA) and (b) fruit to 2 breaks in temperature control with different times between exposure (treatments 2BS and 2BL).

Table 4.3, Comparison of 'Cripps Pink' apple relative ethylene production at 0°C for treatments exposed to 3 days at 20°C on multiple occasions. Note: CNT = no exposure; 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days). Data points are extracted from those presented in Figure 4.6b.

	Log Relative Ethylene Production									
Time after initial exposure to 20°C	20 days	n	40 days	N	60 days	n	75 days	n	95 days	n
CNT	-0.04 [a]	9	-0.03 [a]	9	-0.17 [a]*	10	-0.10 [a]	10	-	-
2BS	0.20 [b]*	9	-0.02 [a]	10	0.23 [b]*	9	0.08 [b]	9	0.06	10
2BL	0.27 [b]*	10	0.28 [b]*	10	0.25 [b]*	10	-0.13 [a]	9	0.20	9
3BA	0.19 [b]*	10	0.03 [a]	9	0.25 [b]*	10	-0.10 [a]	9	0.08	10
$LSD_{0.05}$	0.10		0.14		0.13		0.17		NS	

Different letters in columns indicate significant differences between treatments within the column ($P<0.05$)
* indicates significant differences from initial physiological status (0).
NS indicates no significant differences

4.3.2. Firmness Changes

4.3.2.1. Effect of Length of Storage Duration Prior to a Temperature Break

4.3.2.1.1. Destructive Firmness Changes

There were no consistently significant differences in firmness between 'Cripps Pink' apples exposed to 3 days at 20°C and their equivalent control fruit (Figure 4.8a). The degree of scatter in the data indicates that the amount of natural variability in the population is a larger effect on observed differences in fruit firmness than that of exposure of fruit to 20°C for 3 days.

4.3.2.1.2. Compression Firmness Changes

Exposure of 'Cripps Pink' apples to 20°C resulted in an increase in the rate of non-destructive firmness change as evidenced by the steeper slope during the shelf life period (Figure 4.8b). However, change in firmness of fruit exposed to 20°C for 3 days earlier in storage (all B treatments), were similar to their control equivalents (all C treatments) throughout both the subsequent coolstorage and shelf life periods.

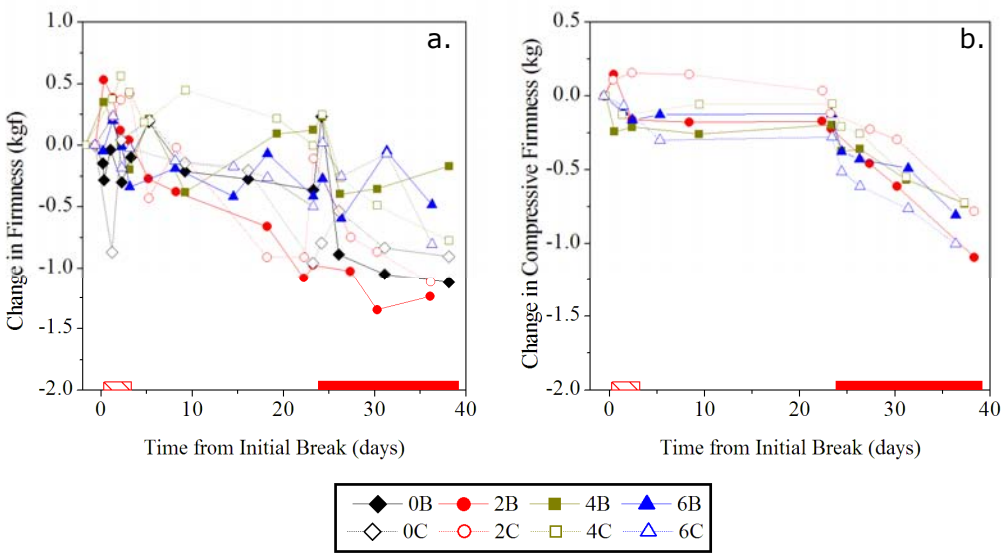

Figure 4.8, The effect temperature breaks (B - 3 days exposure to 20°C) in comparison to control fruit (C – 3 days at 0°C) on 'Cripps Pink' apple firmness after 0, 2, 4, and 6 months storage at 0°C for optimal harvest fruit. Figure 4.8a presents data collected by penetrometer where Figure 4.8b presents data collected by compression. 0, 2, 4, and 6 refer to 0, 2, 4 and 6 months storage at 0°C in air prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Empty bars represent time of exposure to 20°C for temperature break treatments whereas solid bars represent time at 20°C for all treatments.

4.3.2.2. Effect of Length of a Single Break During Storage

4.3.2.2.1. Acoustic Firmness (Stiffness) Changes

Exposure to 20°C resulted in a rapid reduction in the change in stiffness as measured by the acoustic firmness sensor (Figure 4.9a). Substantial differences in change in stiffness between treatments occurred as a result of different exposure times to 20°C (Figure 4.9a) with those fruit exposed to 20°C for a longer time showing a greater change in stiffness. Despite the effect on change in stiffness at 20°C, on return to coolstorage the subsequent rates of stiffness loss were not significantly different between treatments (P>0.05).

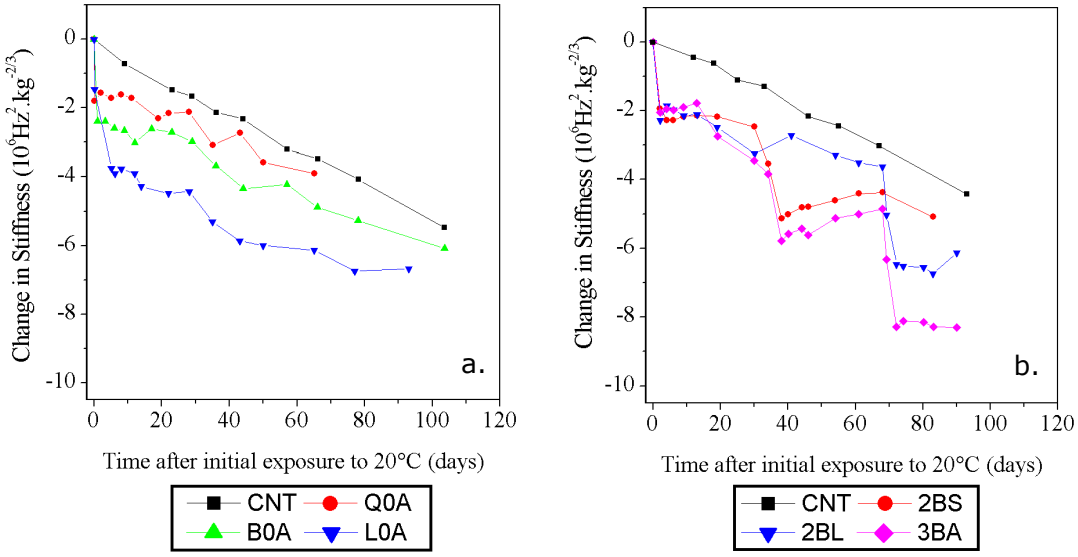

Figure 4.9, Effect of (a) length of exposure time after 2 months storage at 0°C and (b) multiple breaks of 20°C for 3 days during storage at 0°C on change in 'Cripps Pink' apple stiffness as measured by an acoustic firmness sensor. Treatments CNT, Q0A, B0A and L0A represent exposure time of 0, 1, 3, and 6 days of exposure to 20°C. 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days). Data for CNT is identical in both figures, although based on different reference times.

4.3.2.3. Effect of Multiple Temperature Breaks During Storage

4.3.2.3.1. Acoustic Firmness Changes

Exposures to 20°C for 3 days resulted in substantial reductions in stiffness with exposures later in storage being observed to cause a larger change in stiffness (Figure 4.9b). Whether this increase in change in stiffness on secondary exposures is influenced by previous exposure or greater fruit maturity is not known. The rate of change of stiffness on subsequent return to coolstorage (at 0°C) was significantly affected by multiple exposures to 20°C for 3 days (Figure 4.9b, Table 4.4). Rates of stiffness loss were not significantly influenced by a single exposure. However, after a secondary

exposure to 20°C, rates of stiffness loss were significantly less. Why this result was observed is difficult to explain.

In recent times, speculation of the value of the acoustic firmness method (stiffness) as an accurate means to assess firmness of fruit have been published (Hertog et al., 2004b). Many authors have demonstrated the influence of mass loss on stiffness measurement of apples (Roth et al., 2004; East, unpublished data). However, in this case, fruit exposed to two or more periods of 3 days at 20°C continue to lose weight at a rate similar to both non-exposed apples and that of themselves prior to exposure (Figure 4.14b), providing no explanation to the change in rate of stiffness loss.

Table 4.4, Rate of change of stiffness per day between times of exposure of 3 days at 20°C. Treatments 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days) and CNT = no exposure. The data is collected from the linear regression of the individual apples that make up the average behaviour presented in Figure 4.9b.

	Period of Time after Initial Temperature Break						
Treatment	Days 4 - 34	n	Days 37 - 69	n	Days 72 – 100	n	$LSD_{0.05}$
C0A	-0.059 ^{b}	18	-0.045 ^{b}	20	-0.053 ^{b}	28	NS
2BS	-0.017 $^{a\ B}$	22	0.025 $^{a\ A}$	26	-		0.022
2BL	-0.038 $^{ab\ B}$	18	-0.033 $^{b\ B}$	24	0.003 $^{a\ A}$	25	0.030
3BA	-0.050 $^{b\ B}$	19	0.026 $^{a\ A}$	25	0.002 $^{a\ A}$	24	0.031
$LSD_{0.05}$	0.028		0.020		0.031		

Different lowercase letters in columns indicate significant differences between treatments within the column (P<0.05). Different uppercase letters in rows indicate significant differences between time periods for the same treatment (P<0.05). NS indicates no significant differences

4.3.3. Colour Changes

4.3.3.1. Effect of Length of Storage Duration Prior to a Temperature Break

The effect of exposure of 'Cripps Pink' apples to 3 days at 20°C on hue angle change was dependent on length of storage prior to exposure. For those apples exposed to 20°C after 0 months at 0°C, the rate of hue angle change was not affected by the 3 day exposure to 20°C (Table 4.5). Significantly greater rates of hue angle reduction were observed for both exposed and control fruit stored for 0 months in comparison to fruit stored for 2 or more months (Figure 4.10).

The change in hue angle was similar for apples exposed 3 days at 20°C after 2, 4, or 6 months (Figure 4.10). Apples exposed to 3 days at 20°C had a substantially greater change in hue angle on return to cool storage than control treatments. On the initiation of the shelf life period (at 4 days), differences in the change in hue angle between break

and control fruit continued to be evident. However these differences diminished during the shelf life period (Figure 4.10).

Table 4.5, Comparison of the change in 'Cripps Pink' hue angle for optimal harvest fruit exposed to 3 days at 20°C (B) after variable times in storage at 0°C and fruit not exposed to a break in temperature control (C). 0, 2, 4, and 6 refer to 0, 2, 4 and 6 months storage at 0°C in air prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. All values of stiffness are averages of 20 fruit. Data points are extracted from those presented in Figure 4.10.

	Change in hue angle					
Time after initial exposure to 20°C	8 days (0°C)	N	24 days (20°C)	n	36 days (20°C)	n
0B	-3.87 [a]	13	-4.29 [a]	13	-10.95 [a]	13
0C	-2.83 [ab]	13	-4.01 [a]	13	-12.57 [a]	13
2B	-1.69 [c]	16	-1.06 [b]	16	-5.71 [b]	16
2C	0.64 [d]	14	1.49 [c]	14	-3.7 [c]	14
4B	-1.96 [bc]	15	-1.53 [b]	15	-3.88 [c]	15
4C	-0.36 [d]	12	1.06 [c]	12	-4.44 [c]	12
6B	-2.43 [bc]	12	-1.96 [b]	13	-3.61 [c]	13
6C	1.83 [e]	13	1.21 [c]	13	-3.64 [c]	13
$LSD_{0.05}$	1.09		1.35		1.93	

Different letters in columns indicate significant differences between treatments within the column (P<0.05)

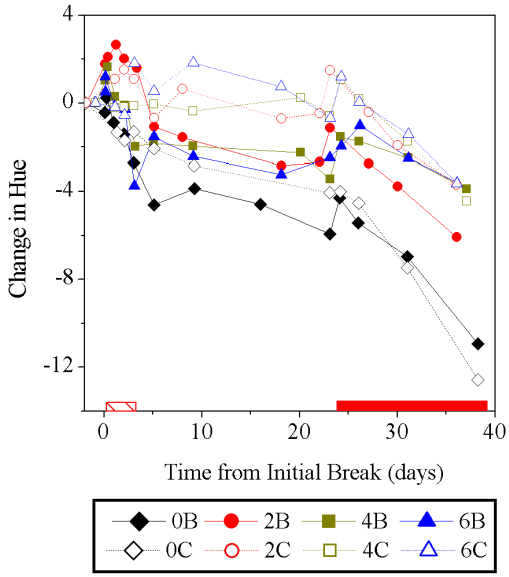

Figure 4.10, The effect temperature breaks (B - 3 days exposure to 20°C) in comparison to control fruit (C – 3 days at 0°C) on 'Cripps Pink' backgound hue change after 0, 2, 4, and 6 months storage at 0°C for optimal harvest fruit. 0, 2, 4, and 6 refer to 0, 2, 4 and 6 months storage at 0°C in air prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Hatched bars represent time of exposure to 20°C for temperature break treatments (B) whereas solid bars represent time at 20°C for all treatments.

4.3.3.2. Effect of Length of a Single Break During Storage

Exposing 'Cripps Pink' apples to 20°C after 2 months storage for any length of time caused a step reduction in hue angle (Figure 4.11a). Apples exposed for longer periods of time had a greater reduction in hue angle. On return to coolstorage at 0°C, rates of hue angle reduction were approximately 0.05 °Hue.day^{-1}, and were not significantly different between treatments.

4.3.3.3. Effect of Multiple Temperature Breaks During Storage

Secondary and tertiary exposures to 20°C for 3 days had similar effects to a primary exposure on change in hue angle (Figure 4.11b). Rates of hue angle change on return to coolstorage were similarly not influenced by previous temperature exposure. These results indicate that the temperature effect on hue angle change may be additive as evidenced by the similar values observed for treatments exposed to a total of 6 days at 20°C (treatments 2BS, 2BL and L0A) at the completion of the experiment (comparing Figure 4.11a and Figure 4.11b).

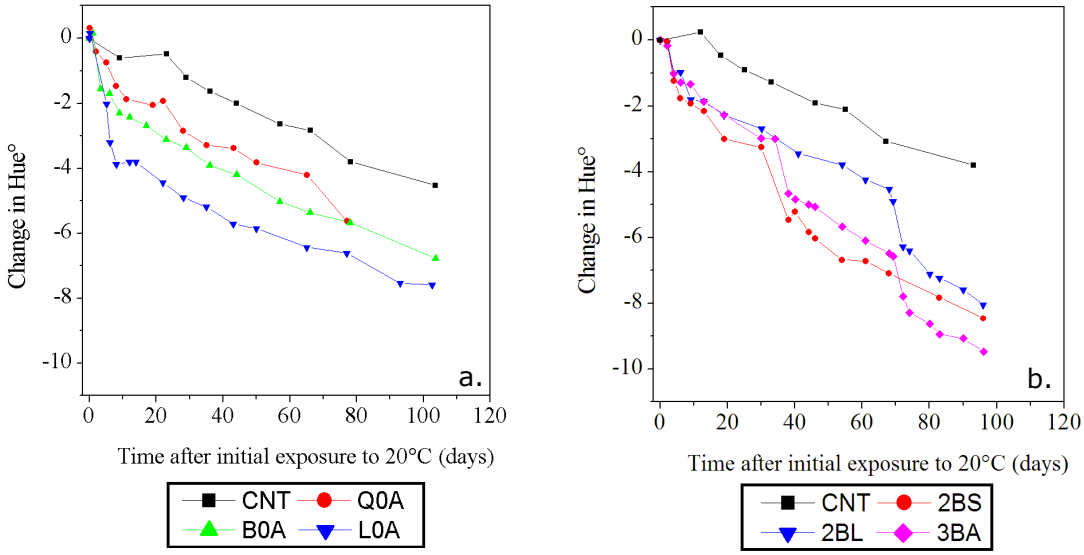

Figure 4.11, Effect of (a) length of exposure time after 2 months storage at 0°C and (b) multiple breaks of 20°C for 3 days during storage at 0°C on change in Cripps Pink apple background hue angle. Treatments CNT, Q0A, B0A and L0A represent exposure time of 0, 1, 3, and 6 days of exposure to 20°C. 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days). Data for CNT is identical in both figures, although based on different reference times.

4.3.4. Other Quality Changes

4.3.4.1. Titratable Acidity

Titratable acidity of the apples was measured in the 2003 season. The transfer of fruit from 0°C to the shelf life period at 20°C was observed to increase the rate of loss of

titratable acidity (Figure 4.12). However, exposure to 3 days at 20°C was not observed to cause substantial differences in 'Cripps Pink' apple titratable acidity (Figure 4.12).

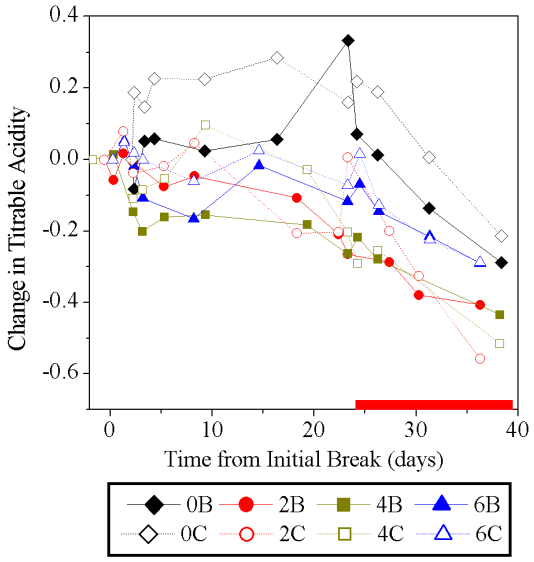

Figure 4.12, The effect of temperature breaks (B - 3 days exposure to 20°C) in comparison to control fruit (C – 3 days at 0°C) on change of 'Cripps Pink' apple titratable acidity after 0, 2, 4, and 6 months storage at 0°C for optimal harvest fruit. 0, 2, 4, and 6 refer to 0, 2, 4 and 6 months storage at 0°C in air prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Empty bars represent time of exposure to 20°C for temperature break treatments whereas solid bars represent time at 20°C for all treatments (shelf life)

4.3.4.2. Weight Loss

The rate of weight loss for 'Cripps Pink' apples increased on exposure to 20°C, and was independent of storage duration before exposure (Figure 4.13), length of exposure (Figure 4.14a) or previous exposures (Figure 4.14b). Rates of weight loss for all treatments were observed to be similar, with a 3 day exposure to 20°C, resulted in an approximately 0.75% loss of fresh weight. The differences created by losing weight during exposure to 20°C were maintained throughout subsequent coolstorage. As a result the influence of temperature on weight loss can be considered additive as clearly demonstrated by the similar weight loss at the completion of storage of the two treatments exposed to two periods of 3 days at 20°C (2BS and 2BL) at different time periods (Figure 4.14b).

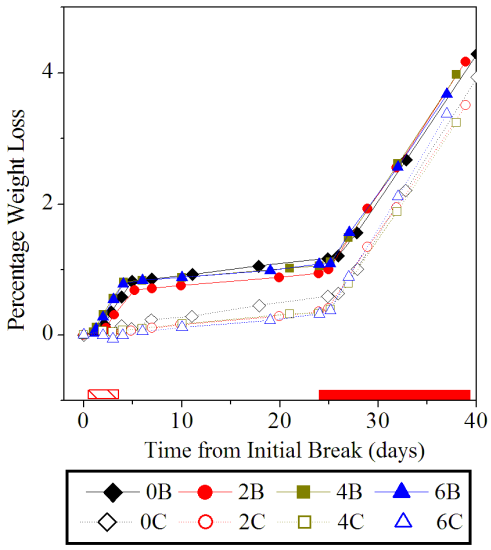

Figure 4.13, The effect temperature breaks (B - 3 days exposure to 20°C) in comparison to control fruit (C – 3 days at 0°C) on 'Cripps Pink' weight loss after 0, 2, 4, and 6 months storage at 0°C for optimal harvest fruit. 0, 2, 4, and 6 refer to 0, 2, 4 and 6 months storage at 0°C in air prior to exposure respectively. After exposure fruit were returned to 0°C for 21 days followed by 14 days at 20°C. Empty bars represent time of exposure to 20°C for temperature break treatments whereas solid bars represent time at 20°C for all treatments (shelf life).

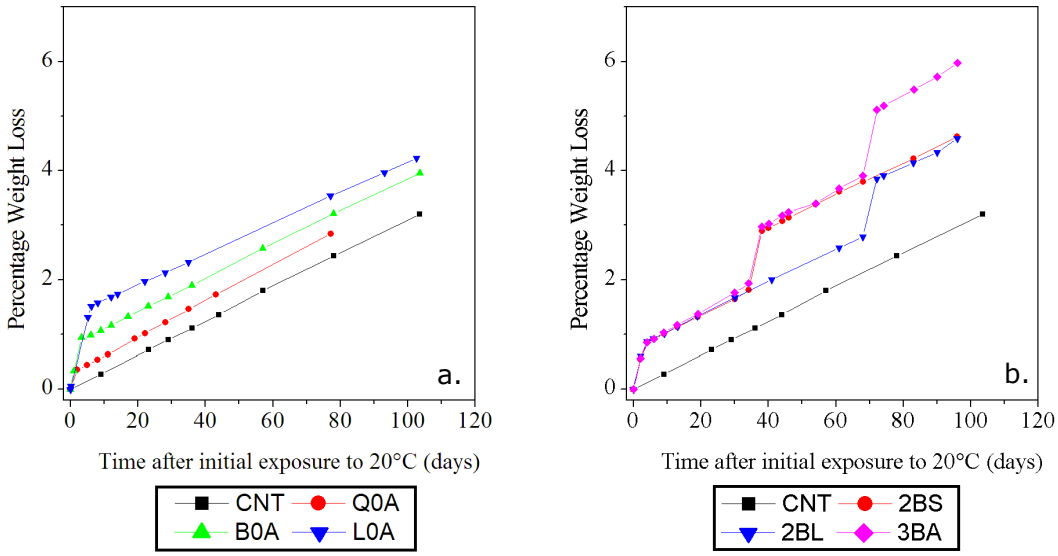

Figure 4.14, Effect of (a) length of exposure time after 2 months storage at 0°C and (b) multiple breaks of 20°C for 3 days during storage on change in 'Cripps Pink' apple weight loss. Treatments CNT, Q0A, B0A and L0A represent exposure time of 0, 1, 3, and 6 days of exposure to 20°C. 2BS = 2 exposures (0 and 34 days); 2BL = 2 exposures (0 and 69 days) and 3BA = 3 exposures (0, 34 and 69 days). Data for CNT is identical in both figures, although based on different reference times.

4.3.4.3. Disorder Incidence

An assessment of the internal and external storage disorders was conducted on the 'Cripps Pink' apples that were destructively assessed for quality attributes throughout the

2003 season. The incidence of 'Cripps Pink' storage disorders was largely influenced by storage duration, with those fruit stored for the longest period of time having the highest incidence of internal browning and superficial scald and hence total storage disorders. A single break of 3 days at 20°C did not influence of incidence of internal browning (Figure 4.15, Figure 4.16). However, significant differences ($P < 0.05$) in incidence of internal browning was observed in 2004 with those treatments with multiple exposures to 20°C (2BS, 2BL and 3BA) or a single long exposure (L0A) having signficantly less incidence of severe internal browning than those the treatments not exposed (CNT) or exposed for 3 days to 20°C (B0A), (Figure 4.16). This result should be treated with caution, as the numbers of fruit used in this assessment are small (30 fruit per treatment). This result of reduced internal browning disorders as a result of breaks in temperature mimics findings for the efficacy of intermittent warming treatments in reducing disorders in tomatoes (Artés et al., 1998), peaches (Fernández-Trujillo and Artés, 1997) and other fruit crops (section 2.1.1.1.3).

A break in temperature control (3 days at 20°C) significantly increased incidence of superficial scald for optimally harvested fruit stored for 6 months prior to temperature exposure (Figure 4.15a). This result is in contrast to previous findings where intermittent warming treatments successfully reduced superficial scald in the 'Granny Smith', 'Cortland', 'Law Rome' and 'Delicious' and 'Pacific Rose' cultivars (Watkins et al., 1995; Alwan and Watkins, 1999; Watkins et al., 2000a). Notably however, in the previously published studies, the lowest rates of superficial scald were obtained for fruit removed from cool temperatures on a weekly basis. In the 2004 harvest, little superficial scald was observed (section 3.3.4.3) and hence subsequent analysis of the treatment effect on incidence of superficial scald was not possible.

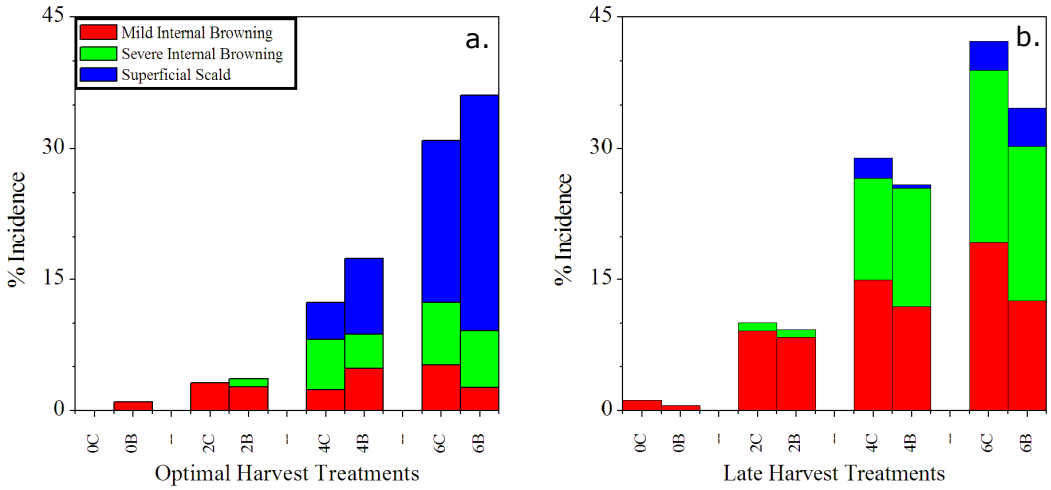

Figure 4.15, Percentage incidence of disorders for both 2003 harvests averaged over the entire length of time subsequent to the initial temperature break.

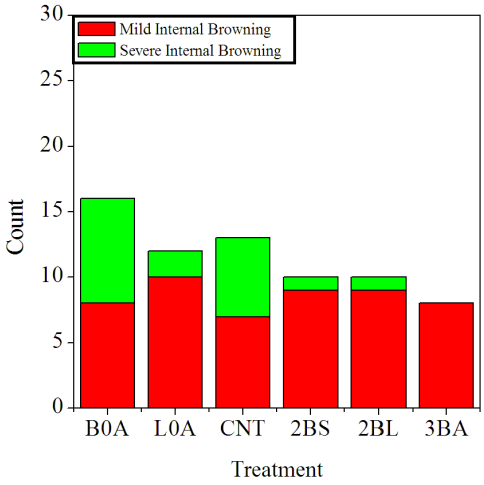

Figure 4.16, Incidence of disorders in 2004 treatments assessed after 175 days. Treatment QOA was completed after 147 days (as opposed to 175 days) and hence not comparable to the other treatments due to the increase in rates of internal browning with storage time (Figure 3.9g).

4.4. FURTHER DISCUSSION AND CONCLUSIONS

This investigation aimed to assess the effects of exposing 'Cripps Pink' apples stored at 0°C in air to short time periods at 20°C on the subsequent fruit physiology and quality changes on exposure to 20°C and on subsequent return to cool storage at 0°C. In investigating the response, the effect of harvest maturity, time in storage prior to exposure, length of exposure to 20°C and multiple exposures to 20°C were studied.

In the first section the effect of temperature breaks on fruit physiology will be discussed followed by a section discussing the effects of breaks in temperature control on fruit quality.

4.4.1. Effect of Breaks in Temperature Control on Postclimacteric 'Cripps Pink' Apple Physiology.

Without exception, exposing postclimacteric 'Cripps Pink' apples to 20°C resulted in a significant change in the ethylene production of the fruit on return to cool storage and initially on return to warm periods (Figure 4.4b, 4.5b and 4.6b). In general, on return to cool storage, ethylene production returned close to that prior to the initial temperature exposure, and remained at this level for approximately 5 days and then increased to approximately 1.5-2 times the initial ethylene production rate over the following 10 days and maintained this increased level of ethylene production for the remainder of the storage period. This ethylene production response of 'Cripps Pink' apples on return to coolstorage (at 0°C) subsequent to exposure to 20°C will be referred to in this document as the "induced increase in ethylene production".

Coupled with this observed induced increase in ethylene production was a lack of response in 'Cripps Pink' respiration rate on return to storage at 0°C after a short time period at 20°C. The respiration rate consistently returned to the values similar to that prior to the break and showed no subsequent change during storage at 0°C. This result demonstrates independence of the two physiological indictors (respiration rate and ethylene production), and hence suggests that both indicators should be measured in order to determine fruit physiological status.

An induced increase in ethylene production has been observed in other scenarios where fruit are considered to be stressed, such as bruising (Lougheed and Franklin, 1974). However, in this investigation, the increased ethylene production as a response to a break in temperature control of postclimacteric 'Cripps Pink' apples is both delayed (by approximately 5 days at 0°C on return to coolstorage) and sustained (for a period of up to 80 days after the initial break in temperature control). Both of these traits suggest that the response is not a result of stress but rather a shift in metabolic control and altered homeostasis of ethylene production.

No clear influences of harvest maturity (data not shown), time in storage prior to exposure to 20°C (assuming that fruit are postclimacteric) or previous exposures to 20°C (Figure 4.7) on the magnitude or rate of the response of the induced increase in ethylene production were evident. However, the duration of exposure to 20°C influenced the initial ethylene production of 'Cripps Pink' apples on return to cool storage (Figure 4.5b), with those fruit exposed for 1 day (treatment Q0A) producing half as much ethylene as to that prior to exposure, whereas those fruit exposed for 6 days (treatment L0A) returned to ethylene production levels similar to that prior to temperature exposure. Additionally, there is some evidence that a larger exposure time may induce a greater increase in ethylene production (Figure 4.5b).

Induced increases in ethylene production on return to cool storage have been observed in other investigations that contained variable storage temperature regimes for other apple cultivars and fruit. Johnston (2001) reported that returning 'Royal Gala' or 'Cox's Orange Pippin' apples to the optimal cool room temperature after exposure to 12°C for 7 days (after 50 days at the optimal storage temperature) resulted in fruit possessing a higher internal ethylene concentration that remained greater than the control fruit for up to 25 days in cool storage. Similarly, Alwan and Watkins (1999) studied the effect of intermittent warming on 'Cortland', 'Delicious' and 'Law Rome' apple cultivars and reported an increased ethylene production in intermittently warmed fruit, although these

observations were inconsistent. In other fruit, Zhou et al. (2001) induced an immediate increase in peach ethylene production on return to storage at 0°C after 1 day at 20°C and Cabrera and Salviet (1990) quadrupled cucumber ethylene production rates at 2.5°C by exposing fruit to 12.5°C for 18 hours every 3 days. The combination of these studies suggests that an increase in ethylene production at coolstorage temperatures may be a widespread response of climacteric fruit exposed to fluctuating temperature regimes.

The major pathway of ethylene biosynthesis in higher plants is methionine → S-adenosylmethionine (SAM) → ACC → ethylene (Yang and Hoffman, 1984). The steps in the pathway are catalysed by SAM synthase, ACC synthase (ACS) and ACC oxidase (ACO), respectively. Both ACS and ACO play a role in regulating ethylene biosynthesis (Alexander and Grierson, 2002). These enzymes are encoded by multigene families, with the expression of each gene in the family differently regulated by various developmental, environmental and hormonal signals (Jiang and Fu, 2000). Turnover of both ACS and ACO is rapid, allowing for rapid control of both enzymes and subsequent ethylene production (Fluhr and Mattoo, 1996).

It is possible that the processes of both exposing 'Cripps Pink' apples to a break in temperature control and/or returning the apples to cool storage, may turn on or off some genes in the ACO and ACS gene families, changing the balance of ACO and ACS and hence ethylene production. This hypothesis is supported by the responses observed for intermittently warmed peaches. When Zhou et al. (2001) exposed peaches to 20°C for 1 day and induced an increase in ethylene production (immediately) on return to storage at 0°C, with the increase in ethylene production being linked to increased activities of both ACS and ACO.

Alternatively, the change in ethylene production homeostasis may be related to compositional changes in the plasma membrane influencing the activity of ACO. Previously, acclimation to low temperatures (low temperature conditioning, section 2.1.1.1.6) by exposing fruit to low non-chilling temperatures prior to refrigeration treatments have been shown to induce compositional changes in the membrane lipids (Goto et al., 1984) that result in reduced sensitivity of the produce to refrigerated temperatures. The influence of temperature on membrane lipid composition has been confirmed in 'Cox's Orange Pippin' apples (Bartley, 1986). It is a common belief that changes in membranes lipids, result in altered membrane fluidity and affect functionality of the associated proteins (Marangoni et al., 1996). Ramassamy et al. (1998) demonstrated that the ACO protein is located on the external face of the plasma membrane of 'Golden Delicious' apples. It is possible that fluctuations in temperature

may also induce membrane lipid compositional changes, and hence functionality. If this is the case, then changes in ethylene production rate are not unlikely due to the location of ACO on the plasma membrane. If this mechanism is proven to have a role in altering the homeostasis of ethylene production, then it is likely that the rate of change of temperature (from cool store to ambient and reverse) may also play a role in the observed effects on ethylene production.

More investigation is required to determine whether the shift in homeostasis of ethylene production of 'Cripps Pink' apples subjected to a break in temperature control is caused by a change in balance of ethylene producing enzymes (ACO and ACS), changes in membrane functionality, or a combination of both effects.

4.4.2. Effect of Breaks in Temperature Control on 'Cripps Pink' Apple Quality.

Exposing preclimacteric 'Cripps Pink' apples to 3 days at 20°C resulted in increased weight loss, but no other significant quality effects over control fruit. The response of preclimacteric apples to a break in the cool chain is cultivar dependent. DeLong et al. (2004) found that a 7 day delay to cooling of 'Honeycrisp' apples had no effect on fruit firmness, soluble solids and titratable acidity after storage for 4 or 6 months in refrigerated air. Johnston (2001) reported that delays in cooling 'Royal Gala' and 'Cox's Orange Pippin' apples for 2 to 4 days before storage resulted in fruit being softer (when compared to those not delayed) upon storage. Brookfield (1996) reported significant losses of firmness and hue angle at the end of 12 weeks cool storage in 'Royal Gala' and 'Pacific Rose' cultivars; a loss in firmness only for 'Braeburn' apples and no significant effect for 'Fuji' apples, as a result of a delayed time to cool before storage.

At 20°C, the loss of the quality of postclimacteric 'Cripps Pink' apples as measured by stiffness (Figure 4.9), hue angle (Figure 4.11), titratable acidity (Figure 4.12) and weight loss (Figure 4.13) was more rapid than at the cool storage temperature of 0°C as most clearly illustrated by the steepening curves during the simulated 2 week shelf life period. This more rapid rate of loss of hue angle, stiffness and weight loss at 20°C generally resulted in significant differences between exposed and control apples on return to cool storage.

The observed reduction in product quality on return to cool storage after a short time exposure to warmer temperatures agrees with research for other apple cultivars and fruit. In Alwan and Watkins' (1999) study of intermittent warming of 'Cortland', 'Delicious' and 'Law Rome' cultivars, firmness losses were generally larger for fruit exposed to 20°C for 1 day every 1 or 2 weeks in comparison to fruit stored constantly at

0.5°C. Similarly, Johnston (2001) reported that an intermittent warming treatment (2 days at 10°C or 2 days at 20°C after 10 days at cool storage temperatures) for the 'Royal Gala' and 'Cox's Orange Pippin' apple cultivars resulted in a lower firmness of the warmed fruit on return to cool storage, in comparison to fruit not warmed. Similar to the results for apples, greater reductions in hue angle and firmness were reported in tomatoes exposed to intermittent warming treatments (Artes et al., 1998).

Brookfield et al. (1998) found that apple cultivar had a significant effect on response to exposure to 4°C or 10°C during coolstorage at lower temperatures. 'Royal Gala' apples were observed to be affected significantly with temperature abuse treatments being less firm, more yellow, and more greasy at the completion of the simulated coolchain, whereas 'Braeburn' apples only showed minor effects on loss of firmness and development of yellow, and 'Pacific Rose' apples showed no effect at all.

In this research differences in quality were established as a result of a previous exposure to 20°C when fruit were returned to cool storage, however these differences were not necessarily translated to significant differences in quality after a period of subsequent shelf life temperatures (Figure 4.10). Similar outcomes (i.e. a significantly softer product while in storage as a result of intermittent warming which is not maintained in a subsequent shelf-life period), have been demonstrated with intermittently warmed tomatoes (Artés et al, 1998) and peaches (Zhou et al., 2001).

In this work, rates of change of stiffness of 'Cripps Pink' apples at 0°C were found to be dependent on previous time temperature history. A single temperature exposure was observed to not influence the rate of stiffness change (Figure 4.9). However, for fruit exposed to two or three exposures to 20°C, rates of stiffness change in subsequent cool storage were significantly less than control fruit (Table 4.4). In addition, exposures to 20°C later in storage were observed to cause a larger effect of change in stiffness while at 20°C (Figure 4.9b). While initially, these results look unusual, confidence in the accuracy of the result is provided by the continued and constant loss of stiffness observed in the control treatments. These results are in contrast to those of Johnston (2001) who found that the softening rate of 'Royal Gala' and 'Cox's Orange Pippin' at any temperature between 0-20°C was not influenced by exposure to another temperature, when firmness was assessed with a penetrometer. Recent research presents scepticism about the acoustic firmness technique, especially with respect to the influence of water loss (Roth et al., 2005) and the interpretation of the results with respect to consumer preferences (Johnson and Dover, 2005). Although these results may be true for stiffness (acoustic firmness) there is no means of ascertaining whether this trend would be

observed for penetrometer firmness, nor how these results would transfer to consumer acceptability.

With the exception of weight loss, it would seem that a break in temperature control of up to 3 days has little effect on the quality of 'Cripps Pink' apples presented to the consumers. While short times at higher temperatures can cause statistically significant quality changes at the time of higher temperature, exposure does not accelerate quality losses on return to cool storage and the differences caused are reduced during the shelf life period.

4.4.3. Linking 'Cripps Pink' Apple Physiology to Quality

While exposing postclimacteric fruit to 20°C was observed to cause quality differences, exposing preclimacteric 'Cripps Pink' apples to 20°C for 3 days after 0 months on storage (treatment 0B) had no significant effect on changes in hue angle (Figure 4.10). It is thought that chlorophyll loss is stimulated by the presence of ethylene (Saltviet, 1999) causing yellowing of the skin (a reduction in hue angle). Significantly, preclimacteric fruit (treatment 0B) also showed a small increase in ethylene production during the 3 days at 20°C (Figure 4.3b) suggesting that both high temperature and high ethylene production are required to cause more rapid hue angle change. This conclusion is further supported by research with ethylene-suppressed mutant tomatoes cultivars, in which chlorophyll degredation is totally prevented (Murray et al., 1993).

It was found that during the first 25 days at 0°C, following an exposure to 20°C, fruit go through the process of developing the induced increase in ethylene production after which time, ethylene production stabilises at up to twice the initial ethylene production. When, Saftner et al. (2003) treated postclimacteric 'Golden Delicious' apples (a parent cultivar of Pink Lady™) with 1-MCP, a significant reduction in ethylene production was induced by the 1-MCP treatment. The reduced ethylene production was coupled with reduced respiration rates and reduced rates of colour loss and volatile production but did not affect firmness changes. If ethylene has a stimulating effect on chlorophyll degradation and subsequent skin yellowing, we would expect the rate of change of hue angle to increase as the induced increase in ethylene production develops. However, this response to the increased ethylene production was not observed for 'Cripps Pink' in this experiment.

Similarly, endogenous ethylene levels of apple fruit and the amount of ethylene accumulated in storage atmospheres influence the content of α-farnescene in apple skin and hence the development of superficial scald (Rapusinghe et al., 2000). In 2003, the

only year when incidence of superficial scald was sufficient to conduct a robust statistical analysis, the influence of a break in temperature control (and hence increased ethylene stimulation of α-farnescene production) was observed to increase subsequent superficial scald on one occasion.

The study of the influence of ethylene on ripening has recently been facilitated by the availability of transgenic plants and naturally occurring mutant lines of other fruit crops. These lines of fruit generally have no ability to produce auto-catalytic ethylene and hence the influence of ethylene concentration can be clearly studied with the application of exogenous ethylene. In transgenic melons, the rate of ripening parameters such as degreening of the rind, flesh softening, and the production of aromas are strongly reduced by the absence of ethylene (Flores et al., 2001). These various melon ripening events were shown to have different sensitivities with 1 ppm required for degreening of the rind, while 2.5 ppm was required to initiate some components of the softening process. The saturating level (i.e. the concentration of ethylene that when exceeded does not further increase the rates of quality loss) of the ethylene dependent ripening events was less than 5 ppm for all ripening factors, which is by far lower than the internal ethylene concentrations found in the non-transgenic fruit at the natural climacteric peak (100 ppm) (Pech et al., 2002). Hence it is speculated that the climacteric ethylene production of 'Cripps Pink' apples at 0°C may already be at saturation levels for chlorophyll degredation, while being close to the limit for α-farnescene production. If this is the case, the subsequent increase in ethylene concentration as a result of breaks in temperature control would not induce any increases in rate of chlorophyll degredation while still increasing the incidence of superficial scald.

Further evidence of the existence of saturation ethylene concentrations in postclimacteric apples is provided by Tan and Bangerth (2000) who applied ethylene to 'Golden Delicious' apples (a parent cultivar of 'Cripps Pink') at four different stages of maturity. Fruit were exposed to 100 ppm of exogenous ethylene for 25 days at 25°C, with the first three harvest dates (of preclimacteric apple) being stimulated to produce more ethylene (through auto-catalysis) whereas the late harvest (already climacteric) showed no stimulation. These results suggest that increases in internal ethylene concentration (either by self-production or exogenous supply) in preclimacteric apples stimulate further ethylene production and more rapid quality degeneration. However, subsequent increases of ethylene (either by self-production or exogenous supply) to postclimacteric fruit may not result in quality effects, as postclimacteric apples may already produce ethylene at a level that saturate stimulation of the some quality responses.

4.4.4. Other Potential Consequences of the Observed Results

4.4.4.1. Potential for Increased Volatile Production

A quality attribute of apples that was not measured in this study and is known to be influenced by ethylene is volatile production (Mattheis et al., 2005; Lurie et al., 2002). Song and Bangerth (1996) showed that rates of ethylene production were closely correlated to rates of aroma production for 'Cripps Pink' parent cultivar 'Golden Delicious' apples. Subsequently it is possible that the induced increase in ethylene production may prove to be beneficial in the production of volatile compounds during storage.

4.4.4.2. Consequences on Laboratory Methods

This experiment has demonstrated that even a small period (1 day) at non-refrigerated temperatures can result in altering the physiology of a fruit on return to coolstorage, although quality indices may not be immediately substantially different. This result suggests that in all postharvest experiments breaks in temperature control should be avoided, whether due to measurement protocol or other circumstances as these breaks may influence the results of the experiment itself. However, this provides the researcher with a difficult quandary, if one was to design an experiment in which fruit stored at different temperatures are to be compared. Basic scientific technique suggests that we should measure all treatments at the same temperature, as calibration errors will no doubt occur if treatments are measured at different temperatures. However, moving fruit to a single temperature may influence the future response of that fruit to their treatment temperature.

4.4.5. Final Conclusions

Breaks in temperature control during storage of postclimacteric 'Cripps Pink' apples at 0°C in air, cause the fruit to produce approximately double the ethylene upon return to subsequent coolstorage. Meanwhile, some quality characteristics (weight loss, background hue angle and titratable acidity) are observed to change significantly during the time of exposure. Despite strong links between ethylene and rates of change of apple quality characteristics (firmness, and background hue angle), this induced increase in ethylene production was not observed to increase rates of quality change. It is suggested that the lack of response of quality characteristics to the increase in ethylene is because ethylene levels in non-exposed fruit already exceed saturated stimulation levels. The observed results may prove to have an influence on fruit volatile production, and also present a case for maintaining constant temperatures during the measurement of postharvest studies.

5. The Effect of Breaks in Controlled Atmospheres on 'Cripps Pink' Apple Physiology and Quality

5.1. INTRODUCTION

Apple quality attributes, such as firmness and colour, change during refrigerated storage as part of the normal metabolism of the product (Figure 3.7 and Figure 3.8). In the commercial environment it is common practice to apply controlled atmospheres (CA) to apples that are to be stored for extended periods of time. Rates of firmness change (and other quality attributes) have been slowed by storing apples in low oxygen (O_2) for cultivars such as 'Delicious', 'Jonagold', 'Golden Delicious', 'Granny Smith', 'Fuji' (Drake, 1993); 'Royal Gala', 'Cox's Orange Pippin' (Johnston, 2001); 'Braeburn' (Hertog et al., 2001) and 'Cripps Pink' (Drake et al., 2002).

The mechanisms of action of decreased O_2 and elevated CO_2 on quality changes, whether ethylene dependent and independent, are still unclear (Watkins, 2002). It is possible that the changes in O_2 and CO_2 could directly affect the quality change reactions (Siddiqui et al., 1996) or the quality change reactions might not be stimulated as a result of suppression of ethylene production (Saltviet, 1999). Alternatively, they could be affected by the reduced pool of energy available as a result of reduced rates of respiration (Hertog et al., 2001).

To transport and ship product to foreign marketplaces, CA conditions need to be broken to allow removal of the product from the storage facility in a safe manner. In large CA storage facilities, it is possible that only a portion of the fruit in the store could be removed at any one time, exposing fruit not removed to a short period of time without CA while being maintained under refrigeration. When fruit are removed from CA and possibly regraded and packed, they may also be removed from refrigeration, thus exposing the fruit to breaks in temperature control and CA atmosphere control. The effect of short-term (1-7 day) breaks in CA conditions, with or without breaks in temperature control on product physiology and quality during storage are not well understood for any fruit.

During shipping, exporters can choose to transport refrigerated product in either refrigerated air (RA) or refrigerated CA conditions (at an additional cost). Currently it is assumed that CA shipping will result in higher quality product delivery, as the fundamentals of the technology are no different to that proven for onshore storage. However, some debate exists as to whether shipping in CA results in significant benefits in final quality as the total time to deliver product to the consumer is relatively short (up

to 6 weeks) in comparison to the previous time in storage (up to 6 months). Previous work by Mare et al. (2005) has shown that shipping plums in CA, as opposed to air, lengthened shelf life by 2 to 3 weeks. To the best of the author's knowledge, the potential quality benefits of investing in shipping apples in CA (as opposed to air) have not previously been published for apples.

In the shipping environment temperature control is generally less consistent than it is during onshore storage. This is largely due to the relatively high surface area to volume ratio of shipping containers in comparison to refrigerated coolstores (Tanner and Amos, 2003). Consequently the effects of small temperature differences (a comparison between 0°C or 3°C) during the shipping period (either in air or CA) were also investigated in this work.

This investigation aims to quantify the physiological and quality changes in 'Cripps Pink' apples that occur during, and subsequent to, breaks in optimal storage conditions that are representative of breaks that can occur in commercial practice. The focus of this research was not only to assess the influence of exposure to non-optimal storage conditions during the time of the exposure, but also to assess if exposure to breaks in optimal storage conditions resulted in altering the physiology and rate of quality deterioration on return to optimal storage conditions.

5.2. METHODOLOGY

5.2.1. Fruit

'Cripps Pink' apples were obtained from Batlow, NSW, Australia in 2004 (section 3.2.1). On arrival at Food Science Australia, Sydney, apples were randomised into plastic netting bags of 25 fruit each. Six bags were then selected randomly and placed into each barrel and sealed. Bags from treatments with identical storage histories were resorted between barrels when breaks in CA occurred. This resorting was done to remove the influence of any differences between barrels prior to examining the effects in new storage environments.

5.2.2. Storage Conditions

5.2.2.1. Storage Technique

While at refrigerated temperatures (0°C and 3°C) all fruit were stored in sealable 60L plastic barrels that had a capacity of 150 apples (6 bags of 25 fruit) per barrel. Unhumidified gas was supplied via a flowthrough system at 400 $mL.min^{-1}$ (Figure 3.1). Controlled atmosphere (CA) gas mixtures were created prior to splitting the flow and

supplying gas to individual barrels. A CA mixture of 2% O_2, 1% CO_2 and 97% nitrogen (N_2) was created by mixing air (as the O_2 source), and CO_2 with pure N_2. This mixture was chosen as it had previously been shown to provide significant quality benefits for 'Cripp's Pink' apples (Golding et al., 2005). Concentrations of CO_2 and O_2 were checked on a weekly basis by gas chromatograph analysis via sampling of the gas in the barrels (sample point B, Figure 3.1). Gas chromotography was calibrated against 3% and 1% CO_2 and 20%, 10%, 6.75% and 1% O_2 β-standards (BOC Gases, Australia). Treatments that were stored in air (RA) were provided with a flow rate of non-humidified air at 400 $mL.min^{-1}$. The flow rate of gas at the outlet of the barrel was checked on regular occasions to ensure that the barrel remained under positive pressure and hence the atmosphere within was equal to that being supplied.

Treatments at 3°C were created by insulating the 60L plastic barrels and placing two in-house manufactured and tested, electronically regulated 30W fan heaters (Food Science Australia, Sydney) inside the barrels with the fruit. This technique allowed the practical supply of identical gas conditions, independent of treatment temperature (0°C or 3°C). Maintainance and distribution of temperature within the barrel was checked prior to the experiment with another set of apples. Temperature variation within the barrels did not exceed 0.5°C.

During times in which apples were exposed to 20°C, apples were removed from the barrels and shifted to a room maintained at 20 ± 1°C. Atmosphere changes at 0°C and 3°C during the experiment were achieved by changing the gas supply line from CA to RA or vice versa.

5.2.2.2. Treatments Combinations

The experiment was designed to assess a range of CA and temperature condition scenarios that apples may potentially be subjected to in a commercial coolchain after a period of storage in CA (Figure 5.1). Each treatment was designed to simulate a single scenario after a period of CA storage where:

- MB1 – a single 3 day break in CA conditions but no exposure to temperature fluctuations.
- MB2 – exposure to a 3 day break in CA conditions on 2 occasions but no exposure to temperature fluctuations.
- CNT – control - no exposure to gas or temperature fluctuations (shipped in CA).
- TAS – no exposure to temperature fluctuations but shipped in air.

- Four treatments were exposed to a break in CA and temperature control (3 days at 20°C in air) to simulate load-out and were then simulated as being shipped at:
    - 0GS – 0°C in CA
    - 3GS – 3°C in CA
    - 0AS – 0°C in air
    - 3AS – 3°C in air.

5.2.3. Physiology and Quality Assessment

5.2.3.1. Respiration Rate and Ethylene Production

Respiration rate and ethylene production were assessed "in situ", at the treatment temperature and gas conditions. Gas flow was temporarily stopped by fitting sealing plugs to inlet and outlet ports of the barrels. Six samples of initial gas composition were taken with 1 mL syringes through septums fitted for the purpose (sample port B, Figure 3.1). These samples were analysed for carbon dioxide and ethylene concentration as two sets of triplicates by gas chromatograph analysis (sections 3.2.3.3 and 3.2.3.4). After approximately 4 hours, a second set of 6 samples was collected and analysed. The change in concentration of CO_2 and ethylene were converted to rates of respiration and ethylene production by taking into account the time between samples, volume of the barrel and the weight of apples in the barrel (equation 3.1).

5.2.3.2. Firmness and Colour

As the effect of a break in CA was being evaluated in this study, and apples were removed from the CA conditions in order to measure the quality attributes, each apple was only able to be measured on one occasion. This constraint to the experiment, removed the possibility of conducting multiple non-destructive measurements on individual fruit as conducted in the temperature break experiment (section 3.2.4.1.2). Subsequently, all quality measurements were conducted at 20°C, after allowing 3 hours of acclimatisation to that temperature if required (Figure 3.2). Methods used for assessment of quality (colour and penetrometer firmness) were identical to those used in Chapter 3 (sections 3.2.5 and 3.2.4.2). In the case of the assessment of hue angle, the greenest region of each apple was measured. Apples with a hue angle less than 80 were removed from the calculation of the average as they were considered as not having any significant green region. Similarly, fruit with storage rots, severe internal browning or CO_2 pitting were removed from the calculation of the average fruit firmness (in some cases up to 40% of the fruit in a sample).

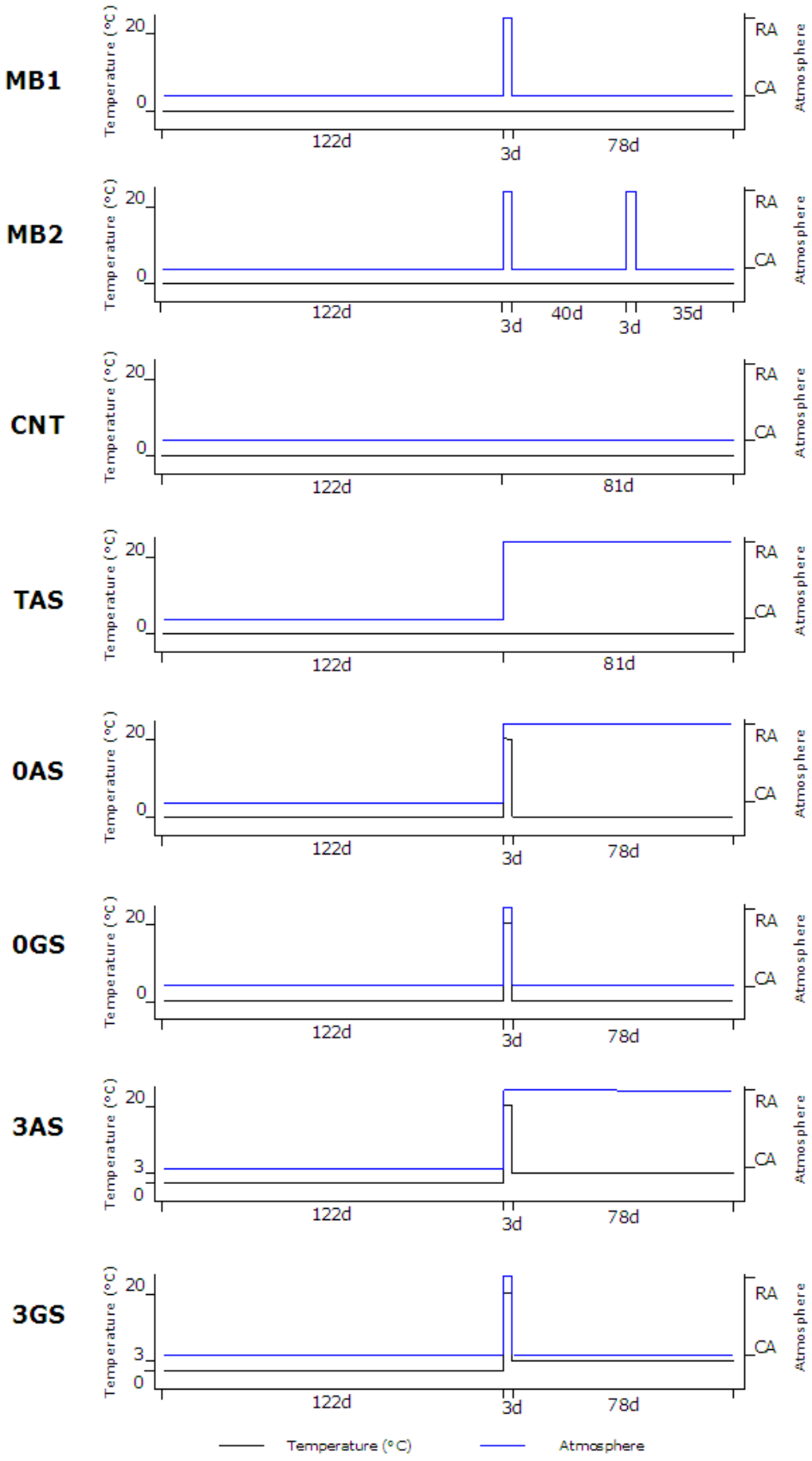

Figure 5.1, Time profiles of laboratory simulations investigating the effect of breaks in controlled atmosphere and temperature on 'Cripps Pink' apple fruit physiology and quality. Note: d = days; CA = controlled atmosphere (2% O_2, 1% CO_2) and RA = air).

5.2.4. Data Analysis

For each quality assessment time a one-way analysis of variance (Minitab v13) was conducted. The significance difference between the treatments was determined with the use of Fishers LSD. When significant differences were found at a measurement time, the LSD was plotted as a bar above the associated data points. Rates of quality loss were assessed per treatment with linear regression.

Comparison of distributions of data were conducted with the Kolmogorov-Smirnov test (Kirkman, 2005). This test is able to determine if the nature of the distribution of two populations is significantly different regardless of the nature of the distribution of each of the populations.

5.3. RESULTS AND DISCUSSION

5.3.1. Breaking CA at Refrigerated Temperatures

5.3.1.1. Permanent Removal from CA

A change in atmosphere from CA to air results in an increase in the rate of apple metabolism. Apples stored in air at 0°C after a period of CA storage (treatment TAS, Figure 5.2) had an average respiration rate and ethylene production over the last 81 days of 22 nmol(CO_2).kg^{-1}s^{-1} and 43 pmol.kg^{-1}s^{-1}, respectively, in comparison to the respiration rate and ethylene production of 14 nmol(CO_2).kg^{-1}s^{-1} and 17 pmol.kg^{-1}s^{-1} (treatment CNT, Figure 5.2), respectively, for fruit stored constantly in CA. These differences represent a 58% and 145% increase in the rate of respiration and ethylene production, respectively, as a result of being removed from CA to RA storage at 0°C.

While, removal of 'Cripps Pink' apples from CA to air at 0°C shifts the respiration rate to that of fruit constantly stored in air (Figure 5.2a), previous storage in CA influenced the subsequent production of ethylene in air storage (Figure 5.2b). Postclimacteric 'Cripps Pink' apples constantly stored in air respired at an average rate of 24 nmol(CO_2).kg^{-1}s^{-1} and produced ethylene at 111 pmol.kg^{-1}s^{-1} (Figure 3.4; treatment AIR, Figure 5.2). The behaviour of 'Royal Gala' at refrigerated temperature in air is dependent on previous time in storage in CA, with those fruit stored in CA for 80 days (and postclimacteric), taking another 150 days until they produced ethylene at the same rate as those constantly stored in air (Johnston, 2001). Similarly, Jobling et al. (2003) reported that CA storage limited the ability of 'Fuji' apples to produce ethylene on return to air at 20°C. Subsequently, these results suggest that application of CA for long periods (in excess of 50 days), not only suppresses ethylene production during the time of CA storage, but also reduces ethylene production (by approximately 50%) in subsequent air storage. In

contrast to 'Cripps Pink' and 'Royal Gala', 'Cox's Orange Pippin' immediately changes from ethylene production rates under CA conditions to ethylene production rates of fruit constantly stored in air, once removed from CA to air (Johnston et al., 2006), suggesting that the effect of CA on subsequent physiology in refrigerated air storage is cultivar specific.

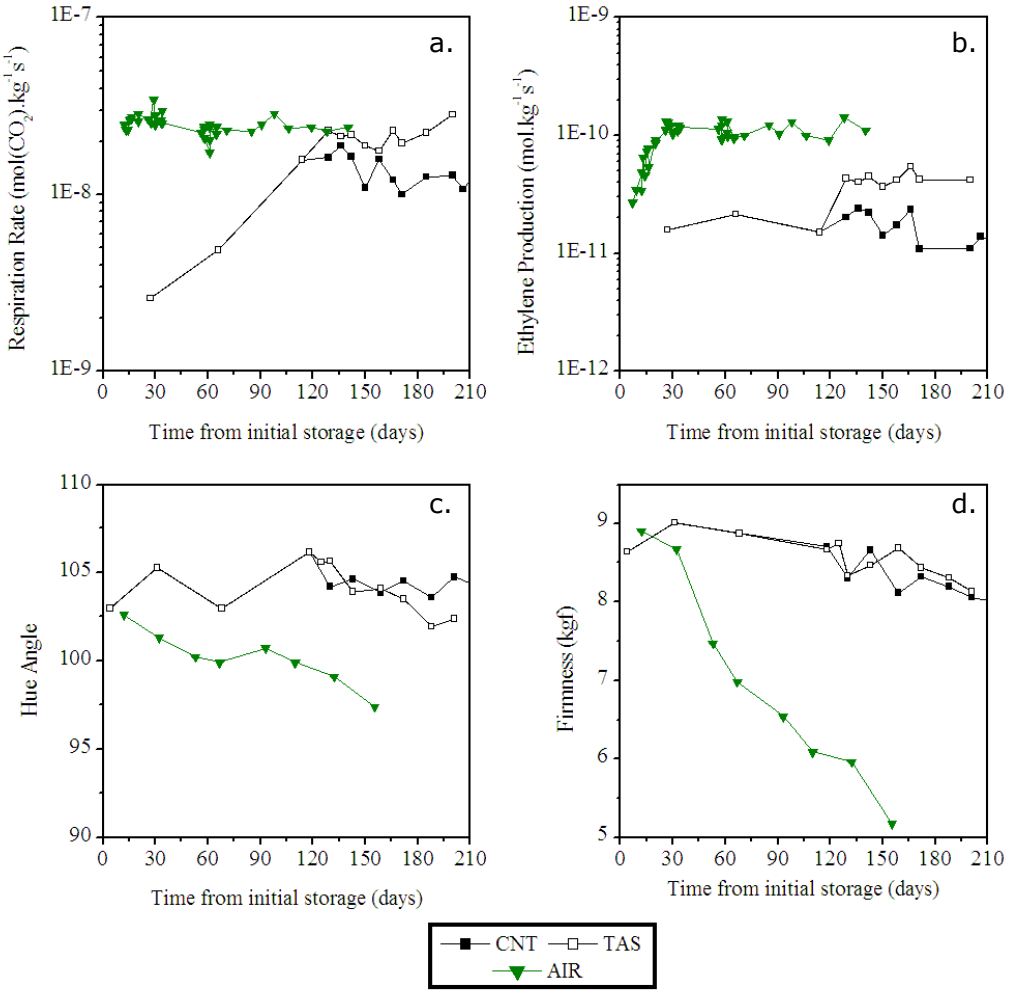

Figure 5.2, The effect of atmosphere and change in atmosphere on refrigerated 'Cripps Pink' (a) respiration rate, (b) ethylene production, (c) background hue angle and (d) firmness. Treatment AIR, was stored constantly in air at 0°C (data retrieved from section 3.3.1.1) whereas the other treatments were stored at 0°C in CA (2% O_2 and 1% CO_2) for 122 days. Treatments TAS and CNT were measured as one treatment for the first 122 days as there were no differences in storage during this time. After this time, treatment CNT remained in CA whereas TAS was transferred to air at 0°C.

On transfer to air the change in background colour of 'Cripps Pink' accelerated to rates similar to that of fruit constantly stored in air (Figure 5.2c), whereas change in firmness showed no reaction to the change in atmosphere for the following 80 days. In contrast to this result, when Johnston et al. (2006) shifted 'Royal Gala' from CA atmospheres to

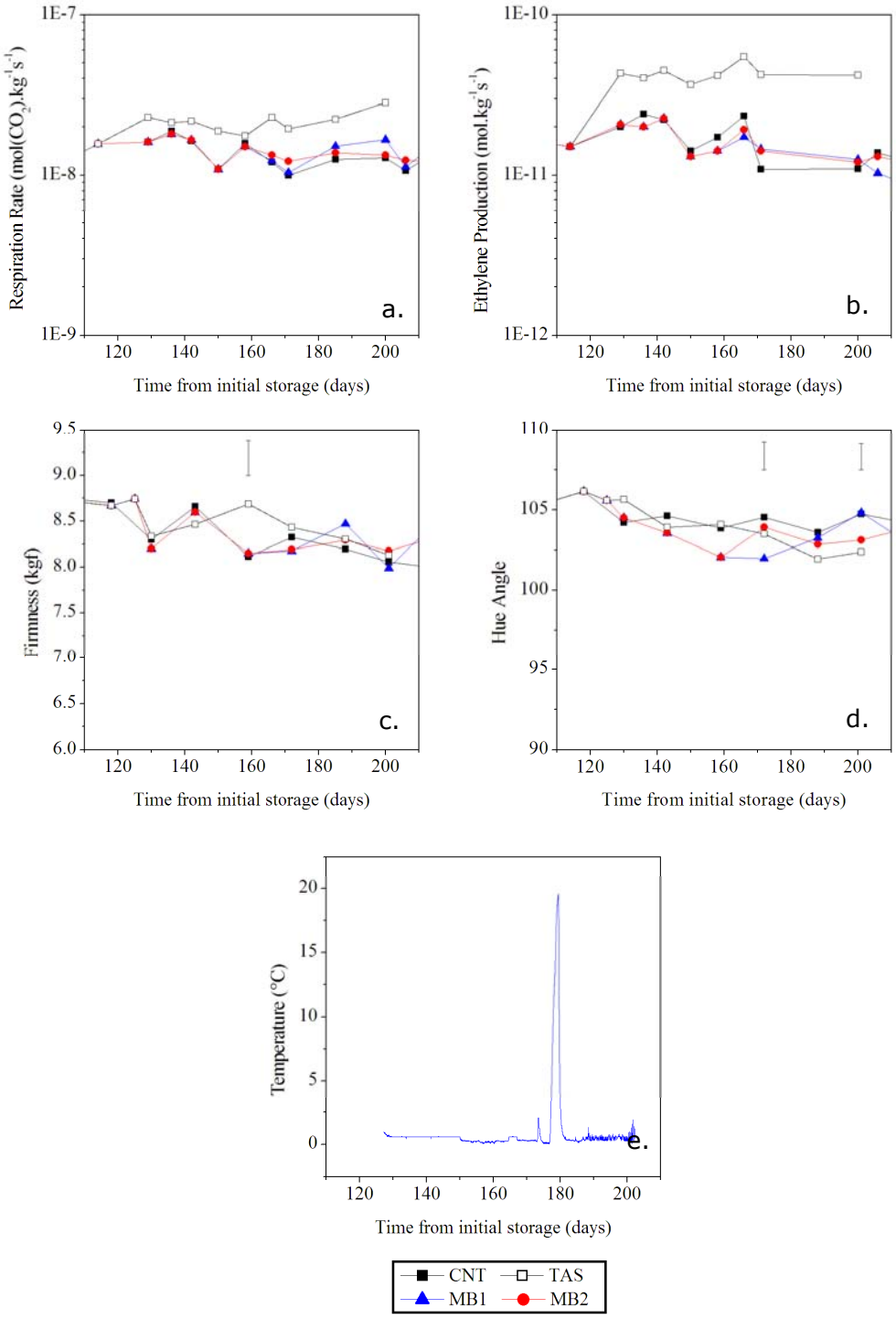

Figure 5.3, The effect of a breaks in CA of 3 days on refrigerated 'Cripps Pink' (a) respiration rate; (b) ethylene production; (c) firmness and (d) background hue angle. All fruit were stored at 0°C in CA (2% O_2 and 1% CO_2) for 122 days. After this time treatments had the same temperature (e) and were different in atmosphere with CNT = 81 days in CA; TAS = 81 days in air; MB1 = 3 days in air + 78 days in CA and MB2 = 3 days in air + 40 days in CA + 3 days in air + 35 days in CA. At times where quality characteristics were significantly different (P>0.05), LSD bars are shown. Data presented for treatments TAS and CNT are identical to that in Figure 5.2. Note the unplanned temperature variation that occurred at day 178 for 3 days in all treatments of the experiment (e).

air at refrigerated temperatures, rates of firmness change immediately shifted from that of CA stored fruit to that of air stored fruit, while 'Cox's Orange Pippin' softened at a faster rate than constantly air stored fruit after a 10 day delay in establishment of the rapid softening period (Johnston et al., 2006).

5.3.1.2. Breaking CA During Storage to Allow other Apples to be Removed

A 3-day break in CA while 'Cripps Pink' apples remained refrigerated did not significantly alter the physiological state or quality of the fruit (MB1; Figure 5.3). One day (29 hours) after apples were removed from CA, the physiological status did not differ significantly from that of apples continuously stored in CA (CNT; at day 167, Figure 5.3a and b). On return to CA the fruit continued to behave similarly to fruit that had not been exposed to a break in CA.

The difference in results observed between apples exposed for 3 days to air (treatments MB1 and MB2) and those moved permanently from CA storage to RA storage (treatment TAS) may be due to constraints caused by using the static method for measuring respiration rate. In measuring the evolution of CO_2 from the fruit over a short period of time, it is assumed that the fruit is at homeostasis (physiological equilibrium) and that all CO_2 emitted is produced as a result of respiration within the fruit. However, in this experiment, respiration was measured after a relatively short time (29 hours) after fruit were removed from an atmosphere of 2% O_2 and 1% CO_2 and placed in air. Hence, some of the CO_2 emitted may have been CO_2 that was adsorbed during exposure to the higher CO_2 atmosphere of the previous CA environment, and not a result of respiration and, secondly, the fruit physiology would most likely have been shifting from one point of homeostasis to another as a result of the changes in internal gas concentrations.

5.3.2. Breaks in CA in Combination with Breaks in Temperature Control at Time of Loadout.

5.3.2.1. Respiration Rate and Ethylene Production

Gas conditions used during the simulated shipping period were observed to substantially influence the respiration rate of the fruit (Figure 5.4a). Fruit shipped in air treatments were observed to consistently respire at approximately 20.0 $nmol(CO_2).kg^{-1}s^{-1}$, 38% higher than those in CA (14.5 $nmol(CO_2).kg^{-1}s^{-1}$), with the treatment in air at 3°C (3AS) respiring at the highest rate of 25.0 $nmol(CO_2).kg^{-1}s^{-1}$. As observed for fruit stored in air (Figure 4.4a) the rates of respiration at coolstorage temperatures were not influenced by any previous exposure to 3 days at 20°C (compare treatments TAS and 0AS, Figure 5.4). Notably, the respiration rate of fruit stored at 3°C in CA did

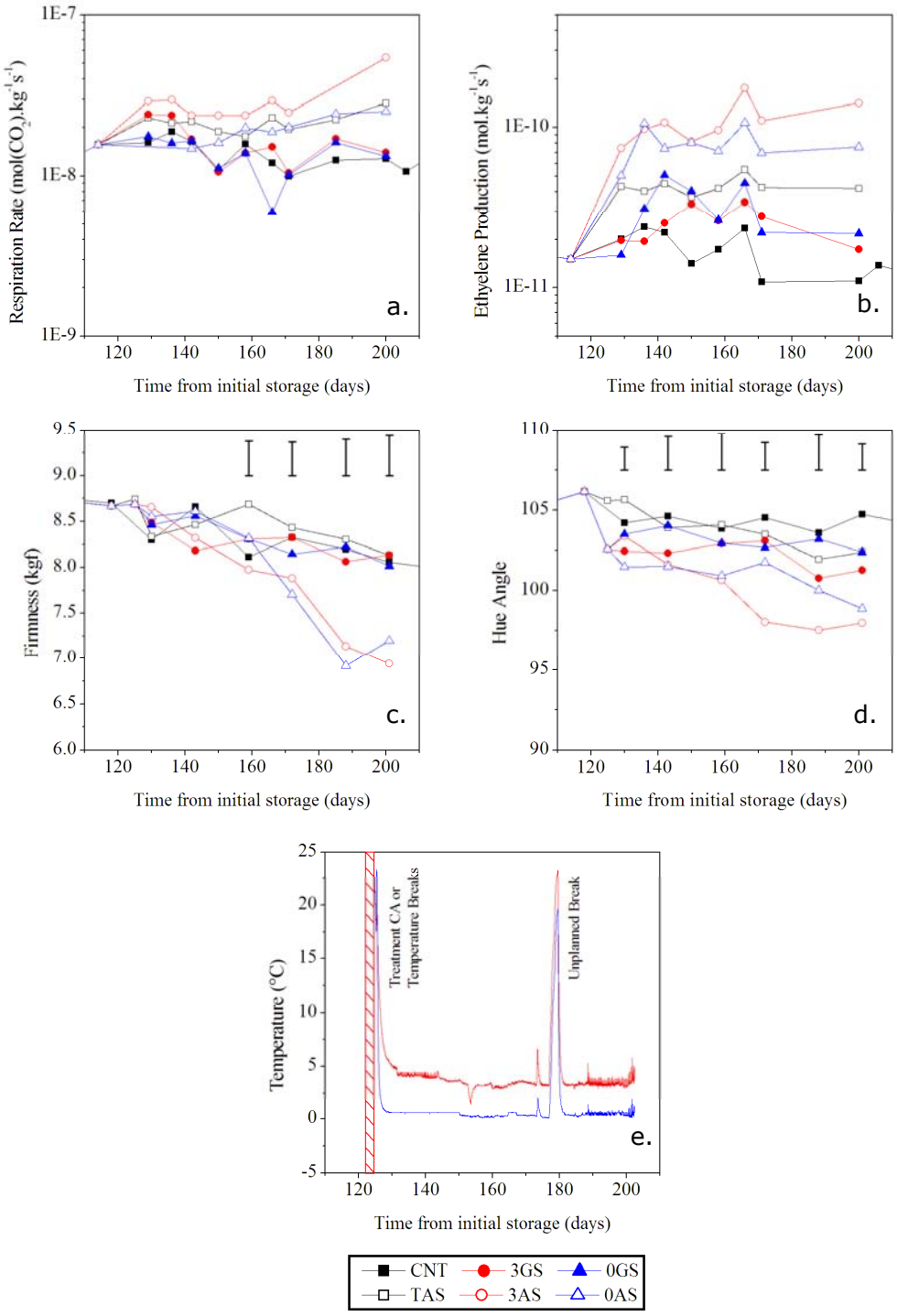

Figure 5.4, Effect of breaks in temperature control at time of load-out and choice of shipping atmosphere on 'Cripps Pink' (a) respiration rate; (b) ethylene production; (c) firmness and (d) background hue angle. All fruit were stored at 0°C in CA (2% O_2 and 1% CO_2) for 122 days. After this treatments are different in atmosphere and temperature (e), with CNT = 81 days at 0°C in CA; and TAS = 81 days at 0°C in RA; with all other treatments exposed to 3 days at 20°C in RA followed by for 3GS = 78 days at 3°C in CA; 3AS = 78 days at 3°C in RA; 0GS = 78 days at 0°C in CA; 0AS = 78 days at 0°C in RA. Bars shown for hue angle and firmness represent LSD ($P<0.05$). All treatments are not significantly different at times where no LSD bar is presented. Note the unplanned temperature variation that occurred at day 178 for 3 days in all treatments of the experiment (e).

not differ significantly from that of both treatments at 0°C in CA, whereas the respiration rate of fruit in the treatment at 3°C in air (3AS) was consistently higher than the treatments at 0°C in air (TAS and 0AS).

Gas conditions during the simulated shipping period and exposure to breaks in temperature control both influenced ethylene production (Figure 5.4b). As observed for respiration rate, treatments that returned to air storage (TAS, 3AS, and 0AS) were observed to produce ethylene at a consistently higher rate than those returned to CA (CNT, 3GS, and 0GS). Exposure to 20°C for 3 days resulted in increased rates of ethylene production on return to coolstorage temperatures, irrespective of the gas conditions applied. The rates of ethylene production of fruit exposed to 3 days at 20°C were approximately twice that of fruit held at 0°C, whether in air (compare treatments TAS and 0AS) or in CA (treatments CNT and 0GS). This result is similar to that observed for fruit stored in air prior to exposure to periods at 20°C (Figure 4.4b).

An increase in temperature from 0°C to 3°C resulted in slightly greater respiration rates and ethylene production rates in air (compare 3AS to 0AS), while no clear differences were observed for fruit in CA (compare 3GS to 0GS, Figure 5.4a-b). This result supports previous findings for the respiration rate of 'Golden Delicious' apples (Andrich et al., 1998) and ethylene production in tomatoes (Sanders and de Wild, 2003) and 'Conference' pears (de Wild et al., 2003) in which storage temperature was observed to have little effect on the metabolic rates while the fruit were stored in low oxygen atmospheres. CA appears to mitigate physiological differences that might be expected as a result of small differences in temperature.

5.3.2.2. Firmness

Significant firmness differences between treatments were not evident until 160 days (Figure 5.4c) which is equivalent to approximately 40 days of simulated shipping time. Both treatments stored in air (at 0°C or 3°C) and previously exposed to 3 days at 20°C (treatments 0AS and 3AS) were observed to decrease in firmness at approximately 0.02 $kgf.day^{-1}$ (prior to the unplanned temperature abuse), while all other treatments were not significantly different during the 80 days of simulated shipping (at 200 days, Figure 5.4c) and all softened at approximately 0.01 $kgf.day^{-1}$. Similarly, Brookfield et al. (1998) found that significant differences in apples exposed to breaks in temperature control and those not exposed only became evident after further storage and/or temperature abuse and Johnston et al. (2006) found that on transfer from CA to air atmospheres, 'Cox's Orange Pippin' apples have a 10 day delay period prior to softening at a more rapid rate.

Drake (1993) showed that a period of 30 days in RA following 30 days in CA could result in a significantly different apple firmness for one of 'Cripps Pink' parent cultivars, 'Golden Delicious'.

Ethylene production had no influence on rates of firmness change in CA shipped fruit. Treatment 0GS produced ethylene at twice the rate of non-temperature exposed CA shipped fruit (treatment CNT, Figure 5.4b) and yet both treatments softened at the same rate. This result contradicts the findings for 'Cox's Orange Pippin' apples, in which ethylene production in CA conditions are found to significantly influence rates of softening (Stow et al., 2000) and suggests that the response of apples to ethylene in CA is cultivar specific.

5.3.2.3. Colour

Differences in background colour between treatments exposed to 3 days at 20°C and those remaining in refrigeration were evident at the beginning of the simulated shipping period, with those treatments exposed to 3 days at 20°C having a substantially lower hue angle (being more yellow) than the other treatments (Figure 5.4d). In the subsequent 80 days of simulated shipping, the rates of hue angle change were also affected by gas storage conditions, with those treatments stored in CA changing in hue angle by 0.6 degrees on average, while those stored in air at 0°C lost 3.7 degrees of background hue over the same simulated shipping period.

5.4. FURTHER DISCUSSIONS AND CONCLUSIONS

5.4.1. The Effect of Breaking CA on Apple Physiology

The rate of change of the gas conditions within the fruit is governed by the diffusion properties of the flesh and skin of the fruit and the respiration rate as a function of the current gas concentration and temperature. When the external atmosphere is changed there will be a lag between the then prevailing respiration rate and the establishment of that expected at the new atmosphere.

In this experiment the change of atmosphere from CA to air was rapid, caused by breaking the seal of the storage barrel. However, in large storage facilities a longer time frame is required to remove the dangerous atmosphere from the room before workers can enter. Hence the results reported in this work should represent the fastest possible time to change between the initial and final physiological states.

Models that include the influence of flesh and skin diffusivity on respiration rates and subsequent internal gas concentrations in constant external gas conditions have

previously been created for apples (Hertog et al., 1998) and pears (Lammertyn et al., 2003). However, the adoption of these models to this unique scenario of removing the CA atmosphere for a short time period has not previously been published. The question still remains as to how long it takes for fruit to transfer from the homeostasis in CA conditions, to the point of homeostasis in the new atmospheric (RA) conditions. It is possible that if the time the fruit are exposed to an air environment is sufficiently short, that the fruit will not significantly shift to the new physiological status and hence it would be expected that these breaks in CA control would not have noticeable effects on fruit physiology or quality.

The time taken to shift from a point of homeostasis in CA to that in air is influenced by both apple cultivar and previous length of storage in CA (Johnston, 2001). 'Royal Gala' apples stored for 50 or 80 days at CA required a time in excess of 50 days in air storage at the same temperature (0.5°C) until the apples produced ethylene at a rate that was significantly higher than fruit that remained in constant CA. However increases in ethylene were immediate for 'Royal Gala' apples removed after 7 or 20 days in CA and for the 'Cox's Orange Pippin' apple cultivar stored under a similar scenario.

Improved knowledge of the time frames required for fruit to significantly change physiology may provide other operational advantages than the scenario that this experiment aimed to assess, i.e. the breaking of coolstorage CA in order to remove a portion of the fruit. Currently, CA generation, scrubbing and control systems are run 24 hours a day, 7 days a week during onshore fruit storage, with the exception of maintenance requirements. However, if significant lags exist between changes in the external atmospheres and physiological changes in the fruit, then it is possible that CA systems could be shut down for short time periods, potentially during peak power consumption, and hence reduce operating costs of the facility, with no marked adverse effect on the product quality.

The results obtained in this study indicate that breaks in CA of up to 1 day for postclimacteric 'Cripps Pink' apples in commercial coolrooms have no significant influence on fruit physiology and quality. Hence, there is no perceived disadvantage of having large CA rooms that required multiple loadout occasions, on the premise that loadout does not affect the temperature of the room. However, a significant body of research exists indicating that a delay in establishing CA immediately after harvest results in poorer apple quality for many cultivars after storage (Lau and Looney, 1982; Liu, 1986; King and Henderson, 1988; Johnston et al., 2006). In this case, large CA rooms are at a

disadvantage as they require larger amounts of fruit to be filled and hence there are larger time delays before CA can be established.

5.4.2. The Influence of Ethylene on Apple Quality

Apples that were not exposed to any break in temperature control and were shipped in air (treatment TAS) did not significantly differ in terms of colour and firmness from fruit shipped in CA over an 80-day period. In contrast, treatments that were exposed to 3 days at 20°C prior to shipping in air (3AS and 0AS) softened at a rate that resulted in significant firmness differences after 37 days (Figure 5.4c). As discussed previously, treatments exposed to 3 days at 20°C produce approximately double the ethylene of non-exposed treatments on return to coolstorage (Figure 5.4b). Consequently, it is not only the shipping atmosphere, but the induction of increased ethylene production due to exposure to room temperatures that also plays a significant role in the rate of quality loss during shipping. This result strengthens the knowledge that ethylene plays a significant role in the softening of some apple cultivars, as treatments which shared the same rates of respiration, but differed in endogenous ethylene production (treatments 0AS and TAS, Figure 5.5) softened at different rates (Figure 5.4c), while treatments with similar ethylene production (treatments TAS and OGS, Figure 5.5) and differing respiration rates softened at similar rates (Figure 5.4c).

Unfortunately, in this experiment, the largest change in fruit firmness occurred during the time in which all treatments were unintentionally exposed to higher temperatures (Figure 5.4e) and hence it is impossible to ascertain whether the change in firmness observed is attributed to the higher ethylene status of these treatments alone or the combination of the higher ethylene production and the secondary exposure to high temperatures. However, the cause of the change in firmness is not likely to be only the result of the high temperature itself, as treatment TAS (also in air) was not observed to lose firmness significantly during the unplanned temperature break. Furthermore, those treatments initially intentionally exposed to 3 days at 20°C after 122 days of CA storage (treatments 3GS, 3AS, 0GS and 0AS) did not have significantly different firmness to those not exposed (treatments CNT and TAS) on initial return to coolstorage (Figure 5.4c). Subsequently, it would seem that the increased ethylene production (and hence inferred increase of internal ethylene concentration) of the apples that had previously been exposed to 20°C and stored in air contributed to the subsequent higher rate of softening of these treatments, whether it was exacerbated by the unplanned high temperature exposure or not.

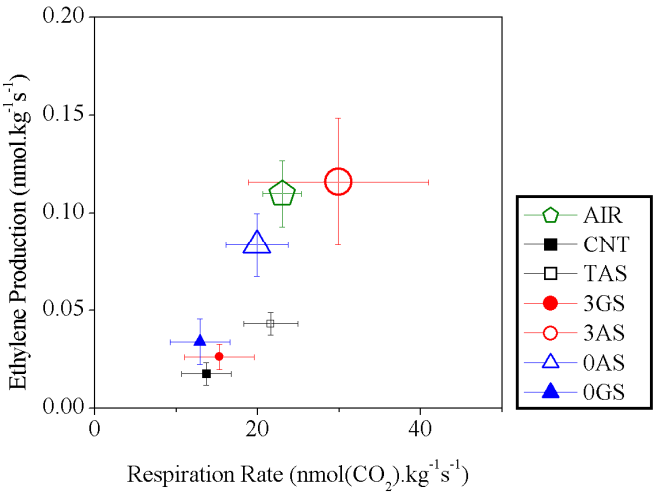

Figure 5.5, Physiological status at homeostasis during simulated shipping. Data presented is the average of data measured over the first 50 days of the simulated shipping period and presented in Figure 5.4a-b. Treatment AIR, the storage of fruit in air at 0°C from harvest, represents average data for postclimacteric fruit only (after 30 days of storage). All other treatments were previously stored in CA at 0°C for 122 days. Solid symbols represent treatments in CA (2% O_2 and 1% CO_2) whereas hollow symbols represent treatments in air. Red treatments were stored at 3°C whereas all other treatments were at 0°C. Treatments CNT and TAS, were immediately transferred to the storage conditions whereas all other treatments were exposed to 3 days at 20°C in air prior to movement to storage conditions. The size of the symbol represents the change in firmness over the 81 days in which the treatments differed after 122 days storage in CA. The size of the AIR treatment symbol represents the firmness change of the AIR treatment over a 79 day period (53 days to 132 days). Error bars represent ± 1 standard deviation.

The influence of ethylene on the rate of 'Cripps Pink' softening in air and CA (1% CO_2, 2% O_2) has previously been documented in the work of Golding et al. (2005). In that work, the researchers treated 'Cripps Pink' apples with aminoethoxyvinylglycine hydrochloride (AVG), an inhibitor of ACC and hence ethylene production (Dussi et al., 2002) prior to harvest and monitored ethylene production and softening rates during subsequent coolstorage in identical CA storage conditions to those used in this work. A one hundred fold reduction in the internal ethylene concentration was observed as a result of the application of AVG, with the untreated apples being significantly softer at the completion of the 8 month storage period in CA.

CA conditions have been shown to be effective in slowing quality changes for both non-climacteric and climacteric fruit. It is therefore reasonable to assume that atmosphere effects are mediated through suppressed metabolic changes that result in decreased respiration (Watkins, 2002). Previous studies have drawn a link between rates of gas exchange (respiration) and quality loss of produce (Tijskens and Polderdijk, 1996). In particular Tijskens et al. (1999) and Hertog et al. (2001) both used the respiration rate

of apples as a rate index for apple softening. However, in this research it was observed that apples with the same rate of respiration but different rates of ethylene production (Figure 5.5, treatments TAS and 0AS) softened at different rates (Figure 5.4c), suggesting that models for apple softening should incorporate ethylene production and/or concentration effects. Only the model of van der Sman and Sanders (2005) attempts to attribute softening of apples in part to the effects of ethylene.

The influence of ethylene on the firmness of CA-stored apples has been demonstrated in a number of ways in the past, strengthening the hypothesis that the major effect of CA is a result of reduced ethylene production and subsequent reduced stimulation of ethylene dependent quality attributes. Knee and Hatfield (1981) studied the effect of adding (1100 ppm) and removing ethylene (with potassium permanganate) from 'Bramley's Seedling' apples in CA storage. In both cases, the ethylene concentration that remained was found to have a significant effect on firmness retention with those treatments with higher ethylene concentration losing firmness at a faster rate. Liu (1977) also found that exogenous ethylene concentration during CA storage of 'McIntosh' apples influenced the fruit quality at the completion of storage. Similarly, research on the effects of delayed cooling and establishment of CAs prior to storage has observed that delays cause apples to produce more ethylene in storage and subsequently results in reduced apple firmness at the completion of storage (Liu, 1986; King and Henderson, 1988). In more recent times the application of 1-MCP prior to storage has been proven to reduced ethylene production in apples in CA resulting in substantial quality benefits (Watkins et al., 2000b; Rapusinghe et al., 2000).

5.4.3. The Influence of CA and Temperature on Range of Fruit Physiologies

During the simulated shipping period, treatments differed in storage gas conditions, temperature and whether or not they had been exposed to 20°C for 3 days during fruit load-out. These treatments resulted in a range of fruit metabolic states during the shipping period. Average respiration rate and ethylene production measured for the first 50 days of the simulated shipping period demonstrate a positive linear correlation between the two physiological measurements (Figure 5.5). The relationships between apples at 0°C, apples previously exposed to 20°C for 3 days at 0°C, and apples previously exposed to 20°C for 3 days at 3°C are similar for both gas conditions. However, in addition to having a more rapid metabolism (higher respiration rate and ethylene production), the spread of possible physiological status for apples in air is greater in terms of both respiration rate and ethylene production than for apples in CA. As a result, it would be expected that apples shipped in air would not only lose quality at a more rapid rate (as a result of the more rapid metabolism) but also have a larger

variation in quality of product on delivery (as a result of the larger range in fruit metabolic states) in comparison to apples shipped in CA.

Other studies in which fruit stored at different, but constant, gas conditions have also demonstrated the ability of CA to at least partially negate the effects of temperature on fruit physiology. Sanders and de Wild (2003) demonstrated the dependence of the rates of respiration and ethylene production of tomatoes on external oxygen partial pressures. At oxygen concentrations of 21%, temperature was observed to have a significant effect on the rate of respiration and ethylene production, yet at 2% O_2, temperature was observed to have little influence on either respiration rate or the rate of ethylene production. Furthermore, Andrich et al. (1998) modelled a similar effect of low oxygen levels on the respiration rate of 'Golden Delicious' apples and de Wild et al. (2003) observed a similar result for ethylene production of 'Conference' pears in low oxygen atmospheres. Jobling et al. (2003) observed no influence of temperature (0°C and 3°C) on rate of firmness change of 'Fuji' apples stored in CA for up to 6 months, while background hue angle remained influenced by temperature. Hence, it is likely that the effect of CA on reducing the range of physiological states in facilities with an inherent range of temperatures (i.e. a shipping container) may apply for a number of horticultural products.

In order to investigate these expected effects in a shipping container, data for individual fruit at 172 days (prior to the unplanned temperature exposure) for each gas condition was pooled from the two temperatures (0°C and 3°C) and compared using histograms (Figure 5.6) and the Kolmogorov-Smirnov test (Kirkman, 2005). As expected, the average values of the quality indices were observed to be slightly lower for apples transported in air. However, more notably, especially in the case of background hue angle, the variation in the quality aspects was larger as indicated by flatter and wider population profiles and a larger standard deviation (σ). The Kolmogorov-Smirnov test revealed that the populations were significantly different for both the firmness ($P = 0.023$) and background hue angle ($P = 0.001$). Comparison of the accumulated percentile plots for each combined population further demonstrates the differences between these two populations for both the quality measures (Figure 5.7).

5.4.4. Final Conclusions

Removing 'Cripps Pink' apples from CA to air without exposure to breaks in temperature control, causes increases in metabolism, although of the physical factors measured, only rates of background colour change are affected. Small breaks in CA (3 days) while at 0°C have no influence on fruit physiology or quality. Breaks in temperature control of

fruit results in the induced increase in ethylene production in subsequent coolstorage regardless of atmosphere. Those fruit stored in CA and exposed to a break in temperature control prior to storage in air, lose quality faster on return to coolstorage than fruit not exposed to a break in temperature. Analysis of the range of physiologies, potentially produced in a variable temperature (e.g. shipping container) environment showed that CA has the ability to not only reduce metabolic rate but also the variability in metabolism within the fruit population. This reduction in variability is transferred to quality parameters, causing fruit shipped in CA to be less variable in quality on delivery than equivalent fruit shipped in air atmospheres.

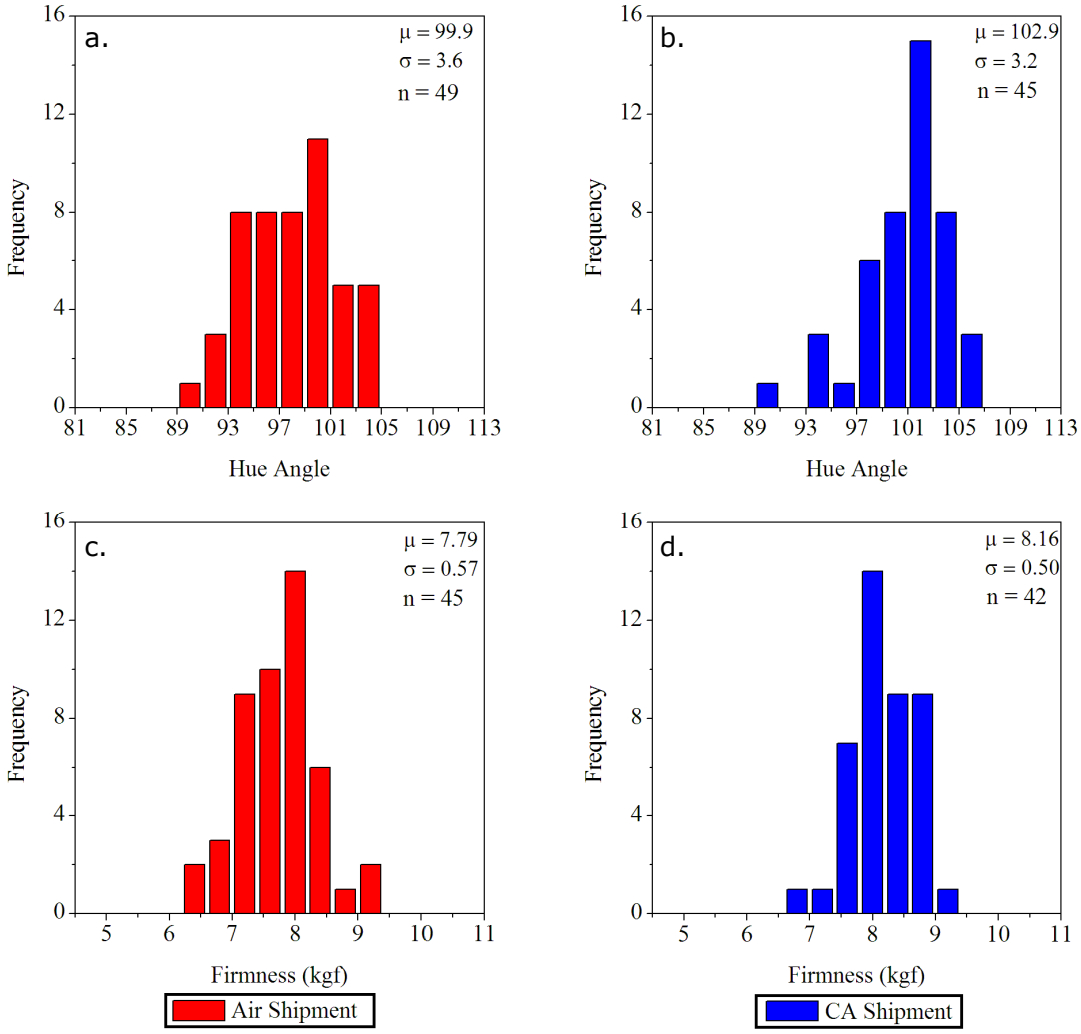

Figure 5.6, Histograms of quality attributes, (a, b) background hue angle; and (c, d) penetrometer firmness for pooled data from fruit shipped in air (treatments 0AS and 3AS) and controlled atmospheres (treatments 0GS and 3GS) at different temperatures (0 and 3°C). Note: μ = average; σ = standard deviation and n = number of individual fruit.

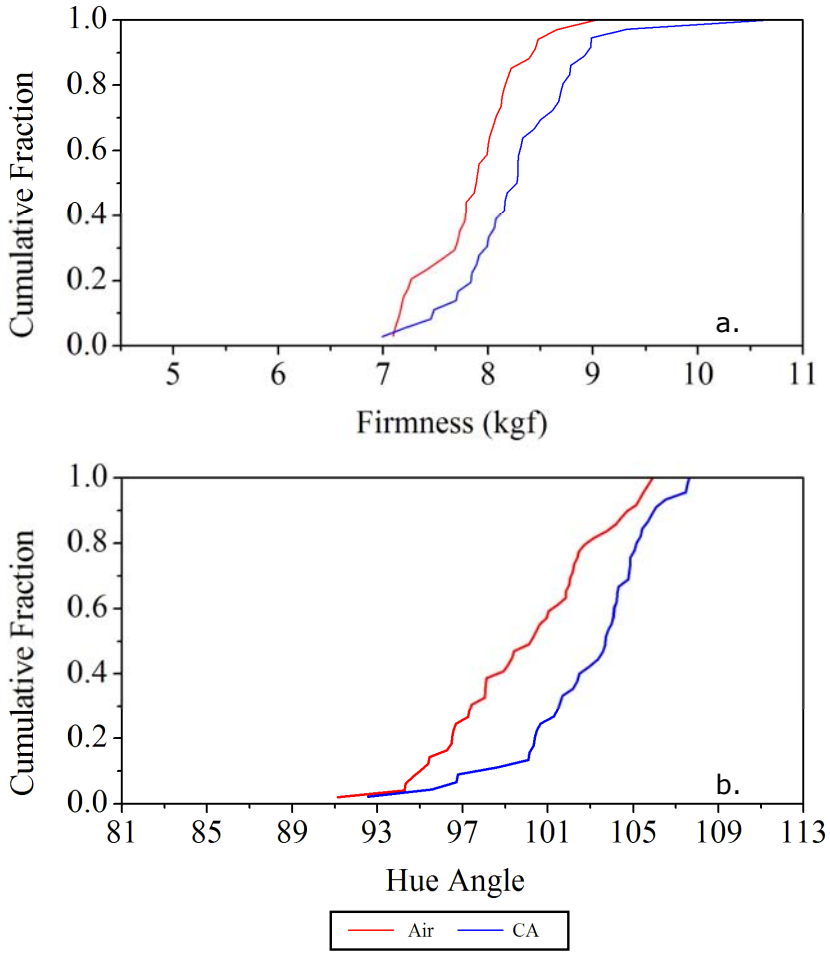

Figure 5.7, Cumulative fraction plots for (a) firmness and (b) hue angle for the combined populations of fruit shipped in air (treatments 0AS and 3AS) and CA (treatment 0GS and 3GS) at 0°C and 3°C after 172 days of the experiment.

6. The Effect of Shipping Atmosphere on 'Pink Lady™' Apple Quality

6.1. INTRODUCTION

Exporters transport refrigerated horticultural product in either air or CA conditions (at an additional cost). It is assumed that CA shipping will result in the delivery of a higher quality product. However, the total time to deliver product to the final consumer is relatively short (up to 6 weeks) in comparison to the previous time in storage (up to 6 months), and hence some doubt exists if CA has a significant influence over this time frame. Previous work with plums has shown that shipping in CA for 18 days lengthened shelf life by 2 to 3 weeks over fruit shipped in air (Mare et al., 2005).

In a laboratory-based simulation 'Cripps Pink' apples shipped in air were observed to have a different physiology than those shipped in CA (Figure 5.2). Furthermore, treatments in which fruit were exposed to a break in temperature control and shipped in air softened faster than those shipped in CA (Figure 5.4c). The objective of this study was to assess the influence of shipping atmosphere on 'Cripps Pink' apples in a commercial environment. Fruit from the same orchard and stored commercially in CA were shipped to UK markets in air and CA to allow a comparison between the two shipping techniques. All fruit were exposed to 24 hours of room temperature (approximately 20°C) between storage and shipment to induce an increased ethylene response (as per previous chapters). This was expected to simulate worst-case conditions. Fruit temperature measurements were made throughout the shipping period, allowing further investigation of the interactive effect of atmosphere and temperature variability on fruit quality.

6.2. METHODOLOGY

6.2.1. Fruit

Fruit were obtained from Donnybrook, WA, Australia in 2005. Fruit were harvested from 27/04/05 to 03/05/05 from a commercial orchard and stored in commercial CA conditions for 110-117 days. Following removal from storage, fruit were put through a commercial grading process, and packaged for export. Fruit were packed into standard 18 kg corrugated cardboard cartons, with layers of fruit (4 for count 120 (150 g), 5 for count 135 (133 g)) packed with moulded pulp fibreboard trays. Cartons were stacked on pallets (1 x 1.2 m), in 8 layers of 7 cartons per layer (Figure 6.1), arranged in alternate configurations for each layer.

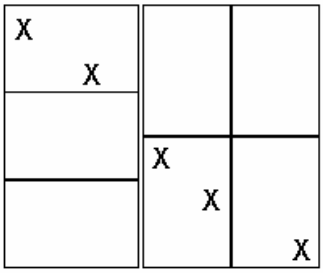

Figure 6.1, **Plan view showing location of monitored fruit and temperatures within each layer of cartons on the pallet.** Thermocouples were placed in the second layer (from the top) of the fruit within the cartons.

After the grading process, 1450 apples (of sizes 120 and 135) were isolated to be used in assessment of both initial and post-shipping quality. After initial quality assessment and labelling, the remaining 1200 fruit were reintegrated into the commercial apple boxes and shipped with commercially destined fruit from Donnybrook, WA, Australia to Pinchbeck, Lincolnshire, UK. On arrival at Pinchbeck, the labelled fruit were removed from the remaining commercial population.

6.2.2. Container Settings

Two commercial 40ft refrigerated containers (one air, the other CA) were used in this trial. A total of 20 pallets fitted into each of the containers with the door end floor space covered with loose cardboard to enforce good air circulation. The air container was set at 0°C, with a nominal air exchange rate of 15 m^3h^{-1} and did not have ethylene filters. The CA container was set at 0°C, 1% CO_2 and 2% O_2, with two ethylene filters at the air return. Defrost rates of both containers were set for 12 hour intervals.

6.2.3. Environmental Conditions Monitoring

6.2.3.1. Apple Grading and Packing

During the grading process, surface temperature data was captured with the use of small self-powered data loggers (I-buttons; Model DS1921Z, Dallas Semiconductors, USA) in order to obtain an estimate of a typical temperature break the apples were exposed to. The surface of an apple was cut out to allow placement of the temperature data logger, which was then secured with tape (Figure 6.2). This apple was then allowed to flow through the grading process and hence captured the temperature change of the apples during grading.

6.2.3.2. Shipping

The temperature of six pallets of fruit, 2 each located at the evaporator end, middle and door end of each of the 2 containers (CA and air) were monitored throughout the

shipping process (Figure 6.3). For each pallet, temperatures were measured in the second layer of fruit (from the top) in the cartons on the 1st (bottom), 3rd, 6th and 8th (top) layer, in a diagonal configuration (Figure 6.1). At each of these locations, 5 labelled apples from the experimental data set were also placed. The thermocouple was placed underneath (between the apple and molded cardboard tray) of one of these apples.

Figure 6.2, Placement of I-button temperature sensor for purpose of monitoring apple temperature during fruit grading and packaging.

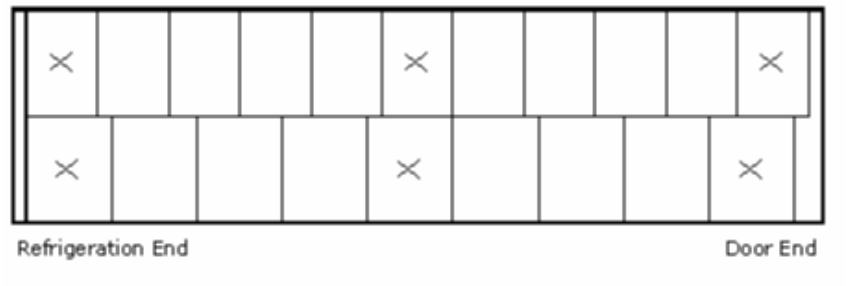

Figure 6.3, Location of monitored pallets containing monitored fruit and temperatures within the shipping containers.

In addition to the fruit surface temperatures, air inlet and outlet temperatures at the evaporator were monitored at 8 locations evenly spaced across the width of the container. All temperatures were monitored with type-T thermocouples (Kalestead, UK) and logged at 10-minute intervals with 64-channel Squirrel thermocouple dataloggers (Eltek, UK). Ice point calibration of the thermocouples was conducted prior to the experiment.

The oxygen levels were monitored in both containers with Ke25 oxygen sensors (Figaro, Japan), and voltage output logged with a 32-channel Squirrel voltage datalogger (Eltek, UK), or a Tiny Tag voltage logger (Tiny Tag, UK) at 10-minute intervals. Calibration of the oxygen sensor (to 20%, 10%, 6.75% and 1% β-standards, BOC gases, Australia)

was conducted prior to the experiment. Equipment malfunction prevented the collection of carbon dioxide levels during the experiment.

6.2.3.3. Shelf Life

On arrival to Pinchbeck, Lincolnshire (UK), the experimental fruit were removed from the commercial population and transported from Pinchbeck, to London (a 3 hour journey). On arrival the fruit were removed from the cartons and distributed along the floor of the sampling room overnight to reduce differences in the rate of warming to shelf life temperatures. Fruit were then placed back into cartons the following morning (after 8 hours). Fruit temperatures continued to be monitored from the time of removal from the commercial population and throughout the subsequent shelf life period (10 days) at room temperatures (18 ± 2°C).

6.2.4. Quality Assessment

6.2.4.1. Firmness

At the time of grading and packing (post-storage), 250 fruit were warmed to room temperature and then assessed for firmness with a Hortplus (New Zealand) electronic penetrometer fitted with an Effegi style 11mm probe. For each apple a 2 mm slice of skin and flesh was removed at two locations around the equator. The firmness value was taken as the average peak force required to penetrate the probe into the two locations.

On receipt of the fruit at Pinchbeck, the five fruit at each temperature monitored position were separated into two populations of equal size for each container. Each population represented all temperature monitoring locations (2 or 3 fruit from each location). One population was used to assess firmness as soon as practicable (off-boat), while the other was used to assess firmness after shelf-life. Assessment of firmness after shipping was conducted with an identical model of probe and technique as to that used to assess initial fruit firmness.

6.2.4.2. Colour

As colour can be assessed non-destructively, the same apples were measured prior to and after the shipping period and if applicable, after the shelf life period. Initial measurement of colour of individual fruits was assessed at room temperature with a chromameter (CR-400 calibrated with a white tile Y = 92.9, x = 0.3134, y = 0.3196; Minolta, Japan). Illuminant C was used for all colour measurements. Off-boat and after shelf-life colour was measured with a different chromameter (CR-300 chromameter, calibrated with a white tile Y = 93.38, x = 0.3135, y = 0.3196; Minolta, Japan). In all cases, a single measurement was taken at the greenest region of the apple that was

identified by marking this region with an indelible pen at the time of the initial measurement.

6.2.4.3. Superficial Scald

At the time of off-boat and shelf-life colour measurement, fruit were checked for presence of superficial scald and/or other marks. Affected fruit were not used for colour assessment.

6.2.5. Data Analysis

A one-way analysis of variance and Fishers LSD method was used to assess the significance of quality (firmness and hue angle) differences between each population ($P<0.05$, Minitab v13, USA). Assessment of the significance of scald incidence was determined with the chi-square statistic (Minitab v13, USA).

Comparison of distributions of data were conducted with the Kolmogorov-Smirnov test (Kirkman, 2005). This test is able to determine if the nature of the distribution of two populations is significantly different, while not requiring the distributions to be of any type.

6.3. RESULTS AND DISCUSSION

6.3.1. Environmental Conditions

6.3.1.1. Apple Grading and Packing

Temperature monitoring during the grading and packing process revealed that apple temperatures can change from their storage temperature (of 0°C) to approximately 14°C in a single hour. Apples remained at this temperature for approximately 2 hours, while whole pallets were assembled. For the experiment, the isolated apples (1450) fruit were then exposed to a further break in temperature control to allow initial colour measurement to take place and also to ensure induction of a changed physiology as a result of the temperature exposure. Notably, once returned to the coolroom environment, apples took approximately 12 hours to return to the coolstorage temperature. Therefore, it would seem that an exposure to temperatures above the optimum refrigerated temperature for approximately 12-15 hours is not unreasonable for apples during the usual commercial grading and packaging process. Currently, no knowledge exists as to whether the length of exposure to higher temperatures during commercial practice alters the subsequent 'Pink Lady™' apple ethylene production on return to coolstorage temperatures, although this work has shown that an exposure as short as 1 day can result in inducing increased ethylene production on return to coolstorage (section 4.3.1.2).

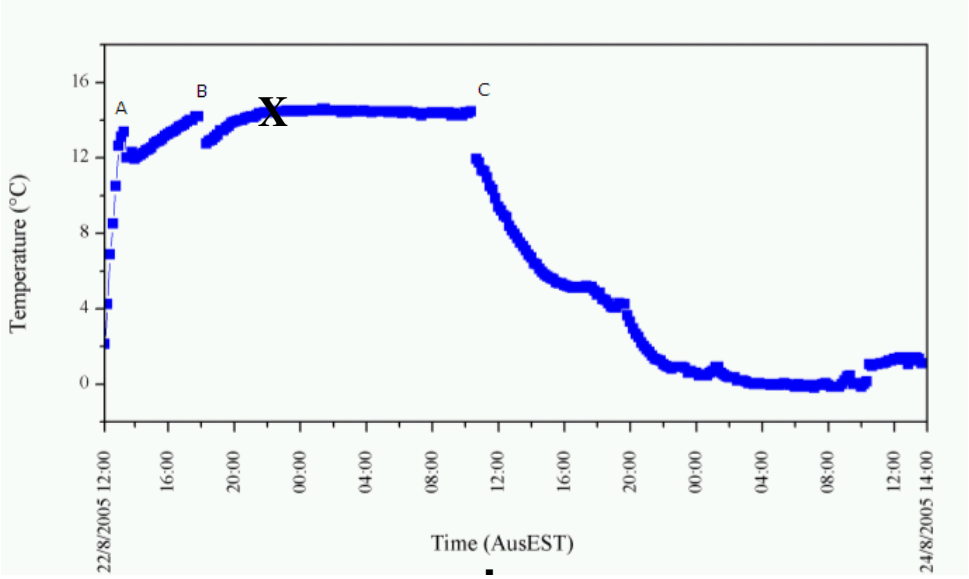

Figure 6.4, Temperature exposure from removal from coolstorage to container loading. Marks A, B and C indicates the completion of grading, time required to assemble one complete pallet and the completion of the extended delay time (for the experimental apples only), while the X mark indicates the time when initial quality measurement was started.

6.3.1.2. Shipping Conditions

Fruit surface temperatures in both containers were relatively constant throughout the 32-day shipping period (Figure 6.5). The average fruit surface temperature in both containers was 0.76°C (Figure 6.6). This temperature can be considered to be within specification, considering that fruit will always be warmer than the cooling air set point of the container (in this case 0°C) due to heat produced by the fruit as a result of respiration. The fact that the average temperature was the same for both containers allows for a reliable comparison between the apples from both containers. The distribution of temperatures in the CA container was slightly larger than that in the air container (Figure 6.6), although the small range of temperatures measured in both containers would be considered as a benchmark standard.

6.3.1.3. Shelf Life

The post-shipping phase began with a period of 12 hours where the apples were warmed from the shipping temperature (0°C) to approximately 4-8°C. During this time all the experimental (labelled) apples were being isolated from the commercial pallets held in a loading dock at 5-10°C. Upon removal from the coolstorage facility, fruit warmed quickly to approximately 16°C and they remained at 16-20°C throughout the 10-day shelf life period. Off-boat and shelf-life quality evaluations were conducted at the beginning and completion of this shelf-life phase, respectively (Figure 6.7).

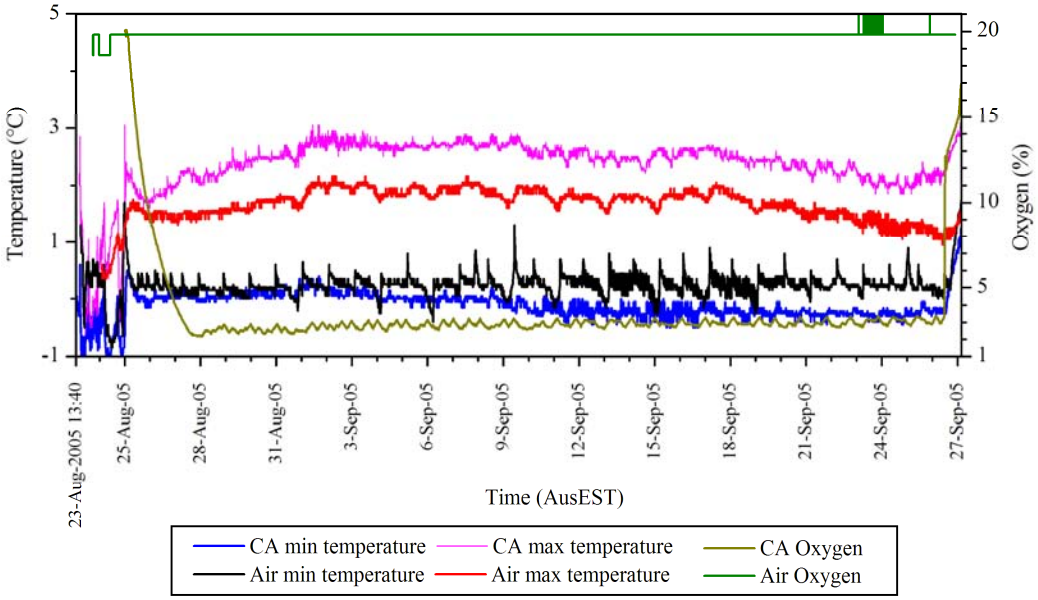

Figure 6.5, Minimum and maximum fruit surface temperatures and monitored oxygen conditions during shipment in the CA and air containers. Note: Dates indicate midday of that day Australian Eastern Standard Time (AEST).

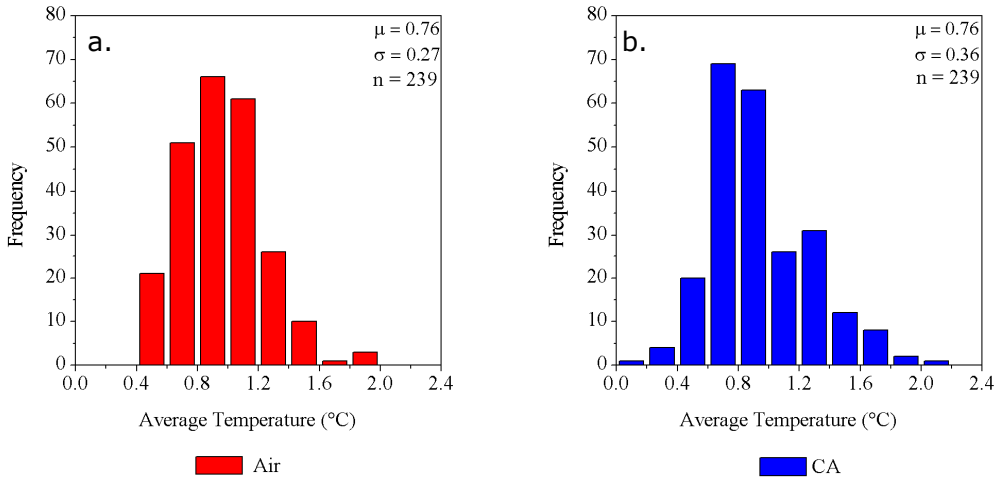

Figure 6.6, Distribution of average temperatures at each monitored location in the (a) air and (b) CA container. Note: μ = average; σ = standard deviation and n = number of temperature monitored locations.

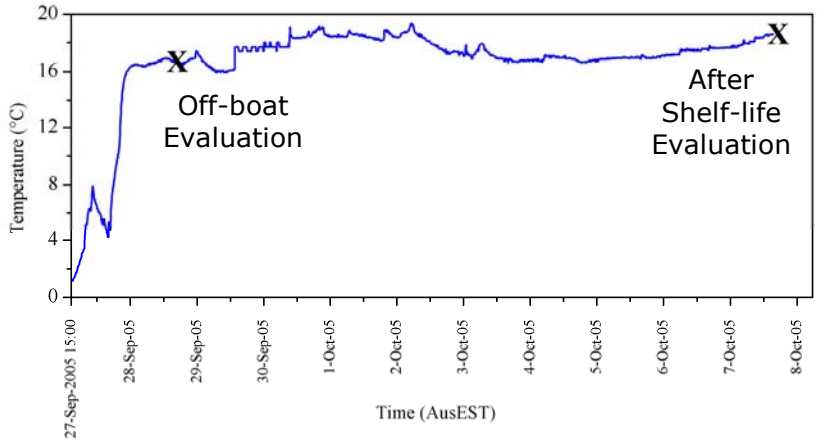

Figure 6.7, Temperature profile experienced during the post shipping period.
Note: X marks indicate times of quality evaluation.

6.3.2. Quality Assessment

6.3.2.1. Firmness

The firmness of 'Pink Lady™' apples post-storage (i.e. prior to load-out and shipping) and after the shipping period of 37 days (off-boat) in either atmosphere were not significantly different (Table 6.1, Figure 6.8a-c). These results confirm the previous reports that the 'Pink Lady™' apple cultivar is an excellent storing apple that has the ability to maintain high levels of firmness over extended periods of time (Drake et al., 2002; Golding et al. 2005; Gualanduzzi et al., 2005). The Kolmogorov-Smirnov test confirms that the distributions of the air shipped and CA shipped off-boat populations were not significantly different either to each other (P= 0.300) or with respects to the pre-shipping population (P = 0.245 and P = 0.061, respectively; Figure 6.9).

Table 6.1, Average hue and firmness of 'Pink Lady™' apples after storage, shipping and shelf-life.

Measurement		Hue Angle	N	Firmness (kgf)	N
Post-storage		100.04 ^{b}	834	8.28 ^{a}	251
Off-boat	Air	100.15 ^{b}	383	8.32 ^{a}	294
	CA	101.14 ^{a}	430	8.40 ^{a}	298
Shelf-life	Air	98.21 ^{c}	146	7.76 ^{c}	287
	CA	99.73 ^{b}	177	7.93 ^{b}	296
$LSD_{0.05}$		0.79	268	0.13	284

Different letters in columns indicate significant differences between treatments (P<0.05).

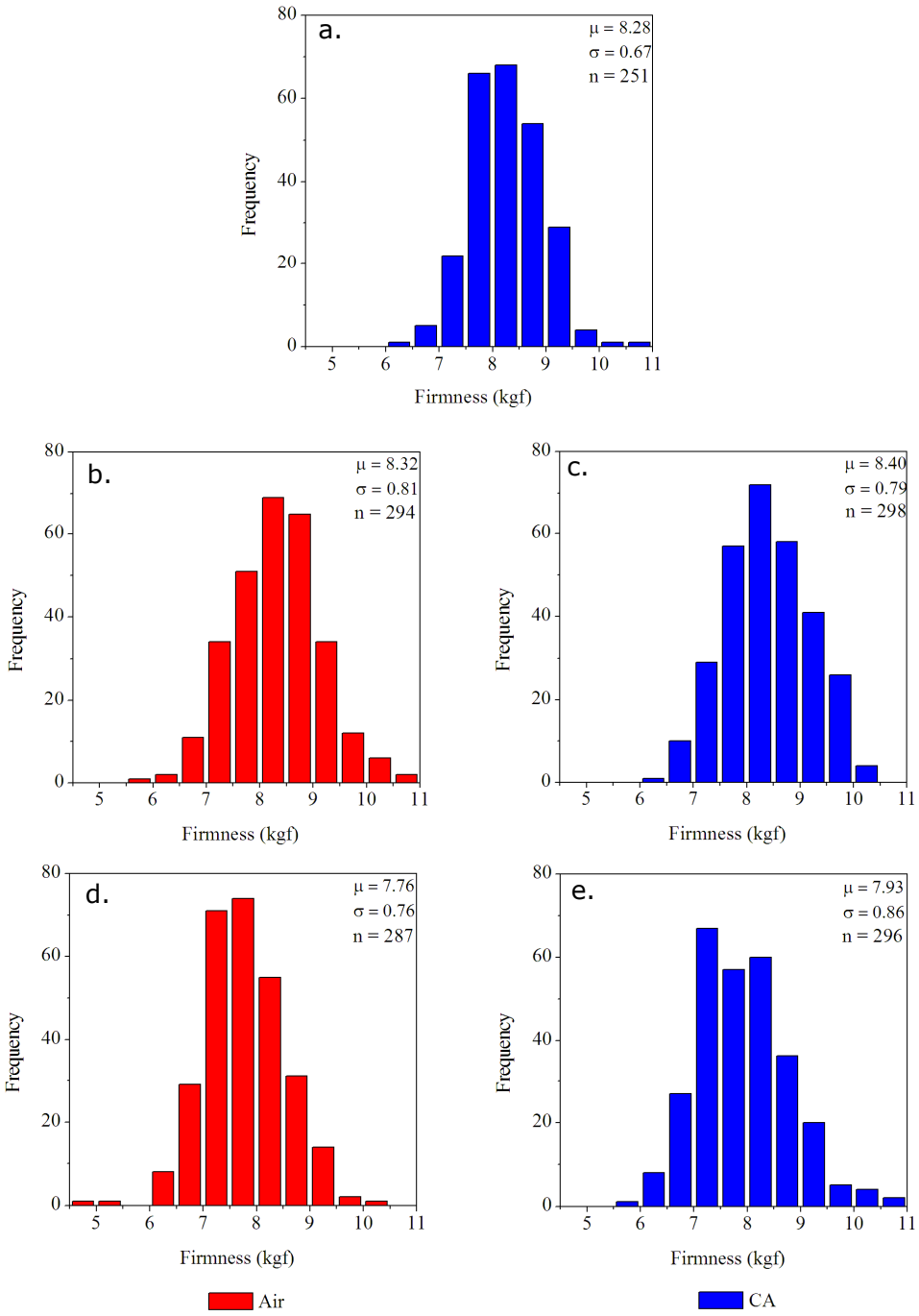

Figure 6.8, Firmness of apples (a) post-storage, (b, c) off-boat and (d, e) after shelf-life, after shipping in air and CA. Note: μ = average; σ = standard deviation and n = number of individual fruit.

After the shelf life period at 16-20°C for 10 days, fruit from both the air and CA containers had significantly softened (Table 6.1, Figure 6.8d-e) although all fruit were still well above the commercially acceptable firmness of 6 kgf (Gualanduzzi et al., 2005). Most notably, fruit shipped in air were significantly softer than those shipped in CA despite being not significantly different after shipping, and subsequently experiencing the same shelf life conditions. The Kolmogorov-Smirnov test, confirmed that the distribution

of firmness of the two after shelf-life populations were significantly different (P = 0.03; Figure 6.9a). Johnston et al. (2006) reported a slight delay in softening of 'Cox's Orange Pippin' and 'Royal Gala' immediately after removal from CA to shelf life temperature, before ripening at the faster rate expected at the shelf-life temperatures. If this behaviour also applies to 'Pink Lady™' apples, this would have caused the separation of the mean firmness of the two populations.

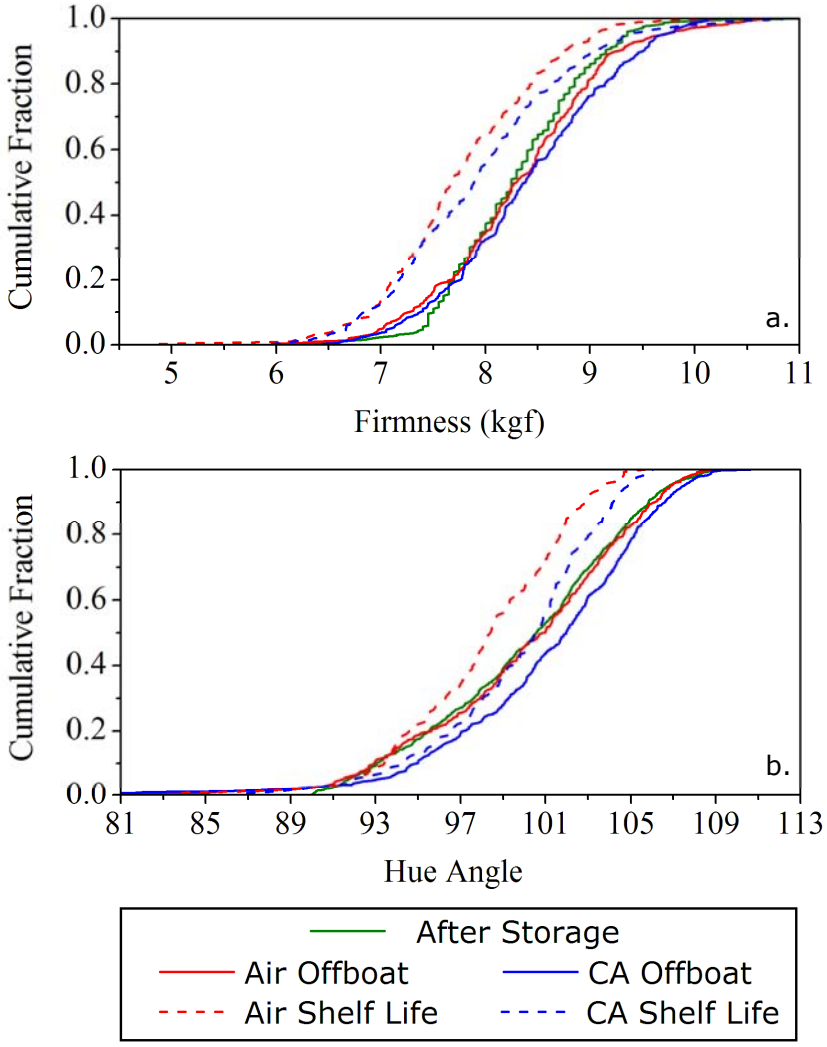

Figure 6.9,Cumulative percentile plots for (a) firmness and (b) hue angle for the populations prior to shipment, post shipping (in either air or CA) and after a shelf life period. Note: data used to construct these plots is identical to that used to create histogram in Figure 6.8 and Figure 6.10.

Although firmness differences between air and CA shipped fruit are statistically significant after 10-days of shelf life, it is unlikely to be noticed by commercial quality measurements, as far fewer fruit are used and hence the minimum detectable difference increases. Similarly, it is unlikely that this small difference in mean firmness (0.2 kgf) would have an effect on consumer acceptability of the product. Consumers find it difficult

to detect differences as small as 0.5 kgf (Harker et al., 2002), while a 1.2 kgf difference is required for one population to be recognised as firmer than the other on 95% of tasting occasions (Harker et al., 2006).

Results from laboratory based simulations of coolchains where 'Pink Lady™' apples stored in CA are shipped in air or CA after 3 days exposure to 20°C, were first observed to have significantly different firmness (0.42 kgf) after 55 days of simulated shipping (compare treatments 0AS and 0GS, Figure 5.4c). In the commercial based shipping trial, statistically different firmnesses were observed after 37 days of shipping and a further 10 days shelf life (0.20 kgf). It would therefore seem that the time frame of commercial shipping from the southern hemisphere producers to the northern hemisphere markets (approximately 40 days maximum) is too short to enable CA in shipping conditions to provide a commercially significant firmness benefit (at least 0.5 kgf) for 'Pink Lady™' apples upon delivery. These experiments also represent worst case scenarios where in the laboratory based simulations, fruit were exposed to non-refrigerated temperatures for 3 days, and in the commercial based fruit trial to a 24 hour exposure, whereas temperature breaks in industry may be in the order of 12-15 hours (as shown in Figure 6.4). This reduction in exposure time in the commercial situation may also have the effect of reducing the benefits of shipping in CA by extending the shipping timeframe required for differences between air and CA shipped fruit to be observed.

6.3.2.2. Colour

Off-boat hue angles for apples shipped in CA were found to be slightly, although significantly, greater than post-storage (Table 6.1). As apples do not become greener with storage, this irregularity is likely to be a result of using different instruments and calibration plates at each measurement location. However, the focus of this work, to compare fruit shipped in CA versus those shipped in air, is not compromised by this difference.

At both of the post-shipping quality assessments (off-boat and after shelf-life, Figure 6.10b-e) apples shipped in air were significantly less green than those shipped in CA, as assessed by Fishers LSD (Table 6.1) or by the Kolmogorov-Smirnov test (Figure 6.9, $P<0.01$). How this difference affects consumer acceptability of a batch of fruit which contains a large variability in hue angle is unknown.

The variation in hue angle (estimated by σ) after the shipping period was observed to be larger than that after storage, with the variation increasing slightly more in the air shipped apples (Figure 6.10). This result provides further evidence that the range of

physiological states that occurs in a shipping container as a result of temperature variation has the ability to increase product quality variability, and that the CA atmosphere has the potential to counteract these effects (section 5.4.3).

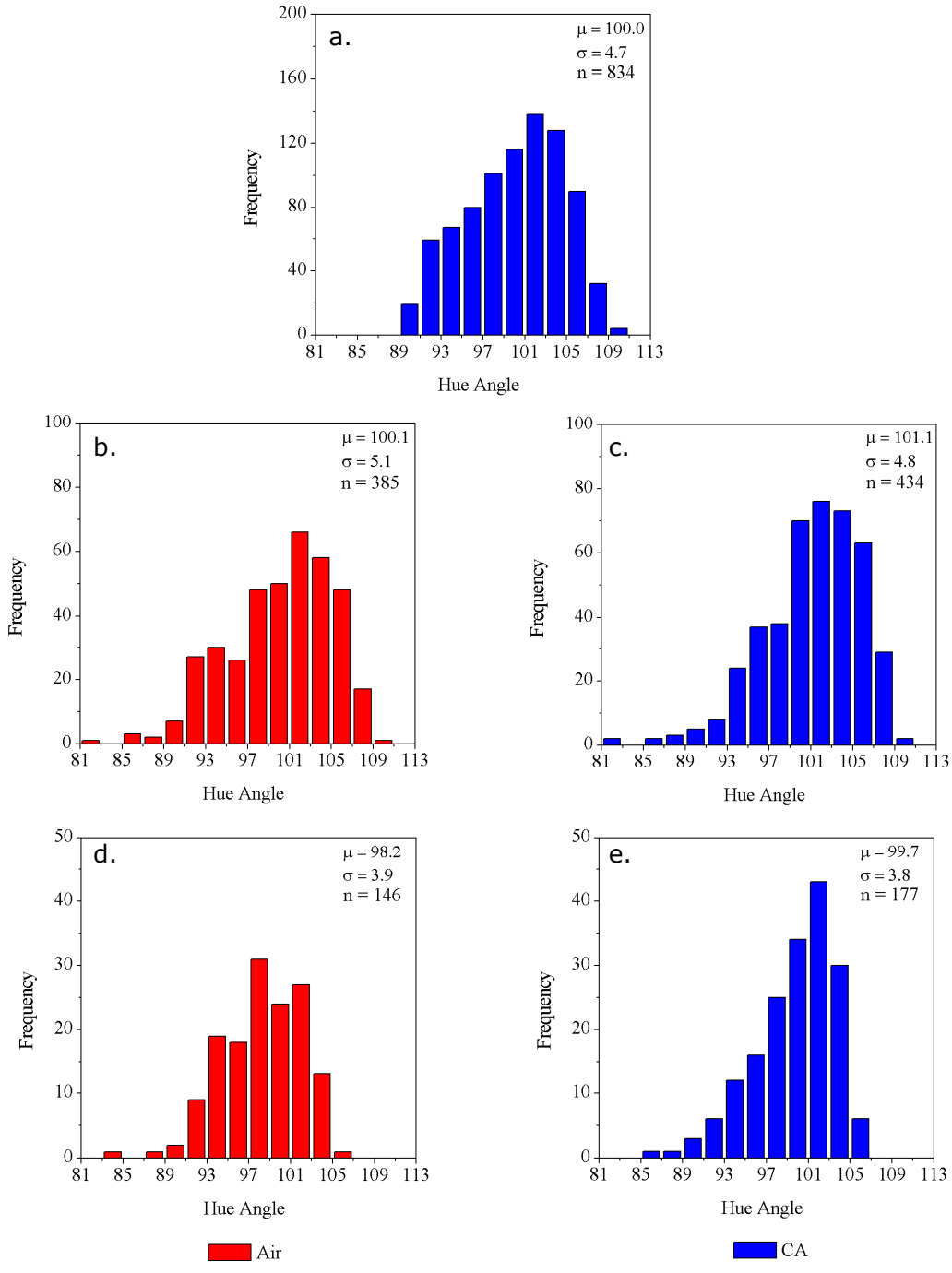

Figure 6.10, Hue angle (greenness) of the background colour of 'Pink Lady™' apples (a) after storage, (b, c) off-boat and (d, e) after shelf-life, after shipping in air and CA. Note: μ = average; σ = standard deviation and n = number of individual fruit.

6.3.2.3. Superficial Scald

Superficial scald (Figure 6.11) was observed on some apples from both the CA and air containers at both the off-boat and after shelf-life quality measurements. Superficial scald is a postharvest physiological disorder of some apple cultivars that manifests itself as browning of the skin with no influence on the flesh, and often only develops upon removal from coolstorage temperatures (Bauchot et al., 1995). The disorder is sporadic in nature, being affected by season, growing location and harvest. Commercial incidence of scald is often controlled with the application of the antioxidant, diphenylamine (DPA) after harvest, although this is less common in modern times due to concerns of market acceptability of the treatment (Watkins et al., 2000a). Incidence of superficial scald in 'Pink Lady™' apples is increased with early harvesting (section 3.3.4.3; Gaulanduzzi et al., 2005).

Figure 6.11, Example of severe superficial scald after the shelf-life period.

Although the mechanism of scald development is not precisely known, it is generally accepted that products of the oxidation of α-farnesene cause the browning. Since, the reaction is an oxygenation reaction, reducing the availability of oxygen (by application of CA) could be expected to reduce the reaction rate and hence browning development. Additionally, accumulation of α-farnesene concentrations have previously been linked to ethylene production (Du and Bramlage, 1994). Laboratory simulation of the cool chain has shown that air shipped apples produced more ethylene during shipping than the CA shipped fruit (Figure 5.2), especially if exposed to room temperatures between on-shore storage and shipping (Figure 5.4). Hence it would be expected that α-farnesene production would be greater in the fruit shipped in air, and subsequently scald development greater on exposure to shelf life temperature. The incidence of superficial scald after the shelf life period of 10 days was 12% and 16% for fruit shipped in CA and air respectively. This difference in scald incidence was not significantly different ($P > 0.05$) and hence suggests that CA during shipping does not aid the alleviation of superficial scald injury.

6.4. FURTHER DISCUSSION AND CONCLUSIONS

In this commercial shipping trial, firmness after shelf-life and background hue angle after both shipping and the shelf-life period were statistically superior in the 'Pink Lady™' apples shipped in CA in comparison to 'Pink Lady™' apples shipped in air. However these differences in quality between CA and air shipped apples were small and would be considered commercially insignificant. In summary, application of best-practice air shipping can reliably deliver high quality 'Pink Lady™' to distant markets following periods of on-shore CA storage.

6.4.1. Validation of Quality Variation Increase Effect

Physiology data from the laboratory simulation showed that the range of possible fruit physiological states in a CA container is far less than that in an air container (Figure 5.5). Hence the range of temperatures in a shipping container is expected to produce a larger range of quality responses in the apples shipped in air. Subsequently, the variation in the quality of apples shipped in air is expected to increase more than that of apples shipped in CA during the shipping period. This effect was demonstrated in the laboratory simulation by pooling data from the 0°C and 3°C treatments (Figure 5.6). This commercial based experiment provided a set of data, in which this benefit of shipping in CA could be assessed further.

Increases in variation of firmness (Figure 6.8) and hue angle (Figure 6.10) were observed for apples shipped in both atmospheres. However, the variation in the air shipped population did not suffer a substantially larger increase than those apples shipped in CA. This result was largely influence by the fact that fruit in either container did not alter quality substantially during the voyage, and hence dramatic changes in variation in quality were not possible.

7. Modelling Ethylene Production in 'Cripps Pink' Apples Exposed to Variable Temperature Scenarios

7.1. INTRODUCTION

The production of the plant hormone ethylene is often used in combination with respiration rate as an indicative measurement of product physiology in postharvest horticulture. The rate of ethylene produced by the product is dependent on fruit maturity (Jobling and McGlasson, 1995), storage temperature (Knee et al., 1983), gas conditions (Dadzie et al., 1996; de Wild et al., 2003), previous time-temperature history (Zhou et al., 2001; chapter 4), previous time-atmosphere history (chapter 5), and chemical treatments (Watkins et al, 2000b; Golding et al., 2005). Static measurement of ethylene production is dependent on the permeability of the flesh and the fruit skin. Assuming that the permeability properties of the product do not change substantially throughout the storage life, ethylene production rate is a non-intrusive measurement of ethylene concentration where a high rate of ethylene production infers a high internal ethylene concentration.

Increases in ethylene production subsequent to harvest, stimulating increased rates of respiration and senescence of fruit tissue, define climacteric fruit behaviour (Alexander and Grierson, 2002). In apples, after the development of the climacteric peak, the behaviour of ethylene production is cultivar dependent (Gussman et al., 1993). Johnston (2001) found that early season apple cultivars 'Cox's Orange Pippin' and 'Royal Gala' ethylene production rates reduced substantially after the climacteric peak, whereas in late season cultivars 'Granny Smith' and 'Pacific Rose™' ethylene production levels remained at rates relatively similar to that produced at the climacteric. Higher concentrations of ethylene in apples have previously been linked to increased rates of respiration (Saftner et al., 2003), more rapid rates of degreening (Dandekar et al.; 2004), softening (Johnston et al., 2002b; Moran and McManus, 2005), titratable acidity loss (Fan et al., 1999) and volatile formation (Defilippi et al., 2004; Mattheis et al., 2005). While not all quality changes are ethylene dependent, fruit softening and chlorophyll loss are ethylene dependent and the most important quality indices used in industry.

It has been observed that exposure of postclimacteric 'Cripps Pink' apples to periods where temperature increases to 20°C during storage results in a change in the physiology of the fruit on return to coolstorage temperatures, irrespective

of whether fruit are stored in air or CA prior to, or subsequent to, temperature exposure (chapters 4 and 5). Increased rates of quality change (firmness and hue angle) after temperature exposure were not observed for fruit stored in regular air prior to temperature exposure (Figure 4.8 and Figure 4.9). However, the rate of quality loss for fruit that were previously stored in CA, and exposed to a temperature break before a period of coolstorage in air was more rapid than for fruit not exposed to a break in temperature control but subjected to the same gas atmosphere change (Figure 5.4c, treatment 0AS). Subsequently, the results obtained in the experimental work of this thesis suggest that in order to adequately model quality changes of apples in variable gas and temperature scenarios, one must include the influence of ethylene and hence have the capability to predict ethylene production rates in variable storage condition scenarios.

The notion that ethylene plays a significant role in quality changes of apples (and other horticultural products) is well established. Previous research has shown that removal of ethylene from coolstorage can slow apple quality changes (Knee and Hatfield, 1981; Graell and Racasens, 1992), while supplying exogenous ethylene can enhance rates of quality loss (Tan and Bangerth, 2000). More recent research with ethylene action inhibitor 1-MCP has proven to slow quality changes significantly (Fan et al., 1999; Watkins et al., 2000b). More detailed research of firmness changes of apple continues to build further links to the influence of ethylene on firmness changes (Hertog et al., 2001; Johnston et al., 2002b; Johnston et al., 2006). Most recently, Dandekar et al. (2004) produced the first work with transgenic apples with reduced ACO and ACS ability and again proved that limiting ethylene production can reduce rates of quality loss.

Despite the strong evidence of the influence of ethylene on quality of horticultural produce, mathematical models that predict ethylene and subsequently the rate of quality change are rare. Many models exist to predict instantaneous respiration rates of apples in air and CAs (Andrich et al., 1991; Lee et al., 1991; Peppelenbos and van't Leven, 1996; Andrich et al., 1998; Hertog et al., 2001; Mahajan and Goswami, 2001) and others predict changes in respiration rate due to product maturation during storage (Uchino et al., 2004). Models that predict ethylene production on the basis of external atmosphere have been developed for apples (Dadzie et al., 1996) and tomato (Sanders and de Wild et al., 2003); while the work of Tijskens et al. (1999) attempts to model ethylene production of apples in storage as an empirical function of temperature and maturity and relates this to

quality changes. This work of Tijskens et al., (1999) failed to test or validate the ethylene prediction to any experimental data. Golias et al. (2001) also used an empirical model to describe ethylene production of apples after storage in different atmospheres. More recently, van der Sman and Sanders (2005) acknowledged the need to relate ethylene to apple quality changes by modelling ethylene as a "biological switch" controlling softening, although ethylene production itself was not modelled. Alternatively, Genard and Gouble (2005) recently developed and tested a model for ethylene production of apples prior to harvest.

The major pathway for ethylene biosynthesis in higher plants is methionine → S-adenosylmethionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene (Yang and Hoffman, 1984). The steps in the pathway are catalysed by SAM synthase, ACC synthase (ACS) and ACC oxidase (ACO), respectively. It was originally thought that ACS activity was the key step in controlling the production of ethylene, however, the role that ACO plays in regulating ethylene biosynthesis has only become apparent in recent years (Alexander and Grierson, 2002). Both ACS and ACO enzymes are encoded by multigene families and the expression of each gene is differently regulated by various developmental, environmental and hormonal signals (Jiang and Fu, 2000). Turnover of both ACS and ACO is rapid, allowing rapid control of both ACS and ACO and hence subsequent ethylene production by controlling the transcription of both enzymes (Fluhr and Mattoo, 1996). Additionally, ACC can be converted to N-malonyl-1-aminocyclopropane-1-carboxylic acid (MACC) in an irreversible reaction of unknown significance (Amrhein et al., 1983).

The aim of this work is to assess the ability of two recently published ethylene models (Tijskens et al., 1999; Genard and Gouble 2005) to predict the ethylene production of 'Cripps Pink' apples while in coolstorage and during subsequent exposures to breaks in temperature control and return to coolstorage. Additionally, a third model, based on the ethylene production pathway, is developed in an attempt to improve previous modelling. This chapter explores the difficulties of predicting ethylene production of apples, especially in temperature variable scenarios and aims to identify gaps in current knowledge that need to be filled to develop a successful model. For purposes of simplification, the influence of storage atmosphere on 'Cripps Pink' physiology was not considered, although it is acknowledged that a model with this capability would be required to adequately simulate commercial coolchain situations. No

attempt is made to relate the predicted ethylene production to rates of change in 'Cripps Pink' apple quality.

7.2. MODELS EVALUATED

For the purpose of modelling, an apple was assumed to be a homogeneous object (De Smeldt et al., 2002) with a permeable layer that controls the diffusion of ethylene to the environment. The internal apple environment was considered as a single entity that allows free movement of all components and contained no thermal heat capacity, hence removing the need for mass and heat transfer mechanisms. Only one independent variable exists (time), allowing the model to be created with ordinary differential equations (ODEs). In addition, as the model is to be validated against ethylene production data, a passive (diffusive) transport of ethylene from inside the apple to the external environment was modelled. The apple skin, with its protective waxy layer is assumed to be the major resistance to this diffusion.

The two models sourced from literature were slightly adapted to fit the current scenario of apples stored in air in variable temperature conditions. One further model was developed based on the current knowledge of the biochemical process of ethylene production. This section mathematically describes the models evaluated and provides reasons for any adaptations of the published models to fit the current scenario. A table of the notation used is presented at the end of the chapter.

The model of Tijskens et al. (1999) is empirical, whereas that of Genard and Gouble (2005) and the proposed model are developed based on the biochemical kinetics of ethylene production and transfer phenomena of the ethylene through the apple skin. How the state of the precursors and enzymes involved in ethylene production change over time is modelled by setting up mass balances for each of the components (Figure 7.1). This results in first order differential equations for each state variable. The accumulation of any compound in the system is a summation of production and consumption within the apple and the outflow via diffusion to the environment. Equations used commonly in some of the models are first presented and then the specifics of each model are described. In the models adapted and proposed, those rate constants considered dependent on temperature are marked with an asterisk (*).

7.2.1. Commonly Used Equations

7.2.1.1. Arrhenius' Equation

Generally, the rate of any chemical reaction responds to temperature following Arrhenius' law. Hence, the temperature dependence for those rate constants of reactions considered to be temperature dependent were modelled using Arhennius' equation, resulting for any rate ($k_{(n)}$) in:

$$k^{*}_{(n)} = k_{ref(n)} e^{\frac{E_{a(n)}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \qquad [7.1]$$

7.2.1.2. Diffusion of Ethylene to Calculate Measured Ethylene Production.

Ethylene produced within the apple will diffuse to the exterior of the apple to be measured as ethylene production. Ficks' law of diffusion states that the rate of diffusion is proportional to the concentration (C) difference across the membrane (apple skin), a factor describing the permeability of the substance through the barrier (D) and the surface area of the membrane (A), and inversely proportional to the thickness (d) of the membrane (equation 7.2).

$$\text{Rate of Diffusion} = \frac{DA([Eth] - [Eth_{Ext}])}{d} \qquad [7.2]$$

If we assume that the diffusivity and thickness of the skin are constant throughout storage of an apple and that the external partial pressure of ethylene is insignificant then equation 7.2 can be simplified to equation 7.3, resulting in an equation equivalent to that used by Maguire et al. (2000) to model water loss from apples.

$$\text{Rate of Diffusion} = k_{Dif} A[Eth] \qquad [7.3]$$

Where:

$$k_{Dif} = \frac{D}{d} \qquad [7.4]$$

As all of the mass of each apple contributes to the production of ethylene the rate of ethylene production is expressed on a per kg of fruit mass (m) basis. Assuming that an apple is spherical and has a homogeneous density then the following relationships hold for volume (V) and surface area (A):

$$V = \frac{m}{\rho} \qquad [7.5]$$

and

$$V = \frac{4}{3}\pi\ r^3 \qquad [7.6]$$

and

$$A = 4\pi\ r^2 \qquad [7.7]$$

Substitution and rearrangement of equations 7.5 and 7.6 in order to make radius the subject of the equation and substitution into equation 7.7, provides the relationship of apple surface area (A) to mass (m):

$$A = 4\pi\left(\frac{3m}{4\pi\rho}\right)^{\frac{2}{3}} \qquad [7.8]$$

The average mass of the fruit used in the experimental work was approximately 150g with a density (ρ) of 900 kg.m^{-3}. Substituting those values into equation 7.8 results in a calculated surface area of 0.0146 m^2. Substituting this value into equation 7.3 and dividing the result by the mass of the fruit provides a relationship for the rate of production of ethylene (Eth_{Prod}) by the average apple used in the experimental work.

$$Eth_{Prod} = \frac{k_{Dif}\,(0.0146)[Eth]}{0.15} \qquad [7.9]$$

7.2.2. Model of Tijskens et al. (1999)

Tijskens et al. (1999) modelled ethylene production (Eth_{Prod}) of apples with a simple logistic kinetics function (equation 7.10). Both the change in ethylene production rate (k_{Eth}) and the maximum rate of ethylene production (Eth_{Max}) are dependent on temperature as described by Arrhenius' equation (equation 7.1).

$$\frac{\partial Eth_{Prod}}{\partial t} = k^*_{Eth}.Eth_{Prod}\left(1 - \frac{Eth_{Prod}}{Eth^*_{Max}}\right) \qquad [7.10]$$

As there is one ordinary differential equation (ODE), one initial condition (Eth_{Prod0} = initial ethylene production; nmol.kg^{-1}s^{-1}) is required.

7.2.3. Model of Genard and Gouble (2005)

Genard and Gouble (2005) proposed a model for apple ethylene production while the apple remained on the tree, considering the influence of apple growth, internal gas atmosphere and temperature. This model was adapted and simplified, in order to remove the model dependencies on apple volume change during growth and internal gas conditions. The removal of these factors is applicable for the situation in which apples have been harvested as it can be assumed that there is no significant growth or shrinkage (change in volume) and the external atmosphere is constrained to regular air.

The model uses the knowledge of temperature dependence of respiration rate to stimulate ethylene production (Figure 7.1a). Methionine was assumed to be an unlimited resource (although not in constant concentration) due to the ability of the Yang cycle to recycle the components at the cost of energy (ATP). Subsequently it is assumed that as the availability of energy increases at higher temperature (due to increased respiration rate and production of ATP), the Yang cycle operates more rapidly, effectively providing a more readily available pool of methionine and hence the production of SAM is also more rapid. The temperature dependence of respiration rate was modelled using the Arrhenius equation (equation 7.1), and concentration of ATP available for the Yang cycle was assumed to be proportional to the respiration rate (equation 7.11).

$$[\mathrm{ATP}] = \lambda . \mathrm{RR}^{*} \qquad [7.11]$$

By assuming that the Yang cycle operates in steady state the rate of ACC from SAM is shown to be proportional to the amount of ATP, and subsequently the catabolism and anabolism of SAM does not need to be directly modelled (Genard and Gouble, 2005). As a result the production of ACC was modelled as a first order reaction dependent on ATP concentration (equation 7.12).

$$\mathrm{ATP} \xrightarrow{k_s} \mathrm{ACC} \qquad [7.12]$$

The metabolism of ACC was modelled with both known pathways, primarily the production of ethylene with ACO (equation 7.13) and secondarily the production of MACC (equation 7.14).

$$\mathrm{ACC} \xrightarrow{k_g} Eth \qquad [7.13]$$

$$\mathrm{ACC} \xrightarrow{k_4^*} \mathrm{MACC} \qquad [7.14]$$

The influence of ethylene on its own production (the autocatalytic response) is modelled by applying an ethylene effect to both rate controlling steps, the production of ACC from ATP, and ethylene from ACC (equations 7.15 and 7.16) with an empirically developed square root function that compares the current ethylene concentration to a reference ethylene concentration. Additionally, Genard and Gouble (2005) applied a generalised form of the Michaelis-Menten equation used by de Wild et al. (1999) for pears to apply an influence of gas atmosphere (oxygen [O_2] and carbon dioxide [CO_2]) on ethylene production during the transfer of ACC to ethylene (equation 7.16).

$$k_s = k_{ref(s)} \sqrt{\frac{[Eth]}{[Eth_{ref}]}} \qquad [7.15]$$

$$k_g = k_{ref(g)} \frac{[O_2]}{\left(K_{O_2} + [O_2]\right)\left(1 + \frac{[CO_2]}{K_{CO_2}}\right)} \sqrt{\frac{[Eth]}{[Eth_{ref}]}} \qquad [7.16]$$

Dadzie et al. (1996) showed that small changes in O_2 concentrations in atmospheres close to that of air had no significant influence on 'Cox's Orange Pippin' and 'Granny Smith' ethylene production. Subsequently, for the scenario being modelled (apples stored in air) the gas concentration effects for the conversion of ACC to ethylene could be ignored. This assumption results in the same form of function for both rates (k_s and k_g, equation 7.17).

$$k_{(n)} = k_{ref(n)} \sqrt{\frac{[Eth]}{[Eth_{ref}]}} \qquad [7.17]$$

The final Genard and Gouble (2005) based model, adapted for the scenario for apples stored in air, consists of 5 equations, two equations for estimating the content of energy available for the Yang cycle and subsequent production of ACC (equations 7.11 and 7.18) and three ODE equations describing the mass balances of the metabolites ACC, MACC and the internal ethylene concentration (equations 7.19 to 7.21).

$$RR^{*} = RR_{ref} e^{\frac{E_{a(RR)}}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} \quad [7.18]$$

$$\frac{\partial[ACC]}{\partial t} = k_{ref(s)}[ATP]\sqrt{\frac{[Eth]}{[Eth_{ref}]}} - k_{ref(g)}[ACC]\sqrt{\frac{[Eth]}{[Eth_{ref}]}} - k_4^{*}[ACC] \quad [7.19]$$

$$\frac{\partial[MACC]}{\partial t} = k_4^{*}[ACC] \quad [7.20]$$

$$\frac{\partial[Eth]}{\partial t} = k_{ref(g)}[ACC]\sqrt{\frac{[Eth]}{[Eth_{ref}]}} - \rho.Eth_{Prod} \quad [7.21]$$

As the accumulation of MACC does not have any direct influence on ethylene production, equation 7.20 was removed from the model optimisation and solution for simplification. This leaves two ODE equations, requiring two initial condition terms for ACC concentration [ACC_0] and ethylene production (Eth_{Prod0}) with the later being used to calculate the initial internal ethylene concentration ([Eth_0]) with rearrangement of equation 7.9.

7.2.4. The Proposed Model

The proposed model is based on the established knowledge of ethylene production (Figure 7.1b). For purposes of model simplification, the potential control of ACC content by the maloynation of ACC to MACC was ignored.

Methionine is a precursor not only for ethylene production, but for many other products (Yang and Hoffman, 1984). It is assumed that a constant pool of methionine is available for all reactions to use. The production of SAM from methionine is assumed to be a first order reaction, sensitive to temperature (as described by Arrhenius' equation; equation 7.22).

$$Meth \xrightarrow{k_1^{*}} SAM \quad [7.22]$$

The conversion of SAM to ACC is facilitated by the enzyme ACS in a process that does not influence ACS viability. Temperature and the availability of both SAM and ACS affect the rate of production of ACC (equation 7.23).

$$SAM + ACS \xrightarrow{k_2^{*}} ACC + ACS \quad [7.23]$$

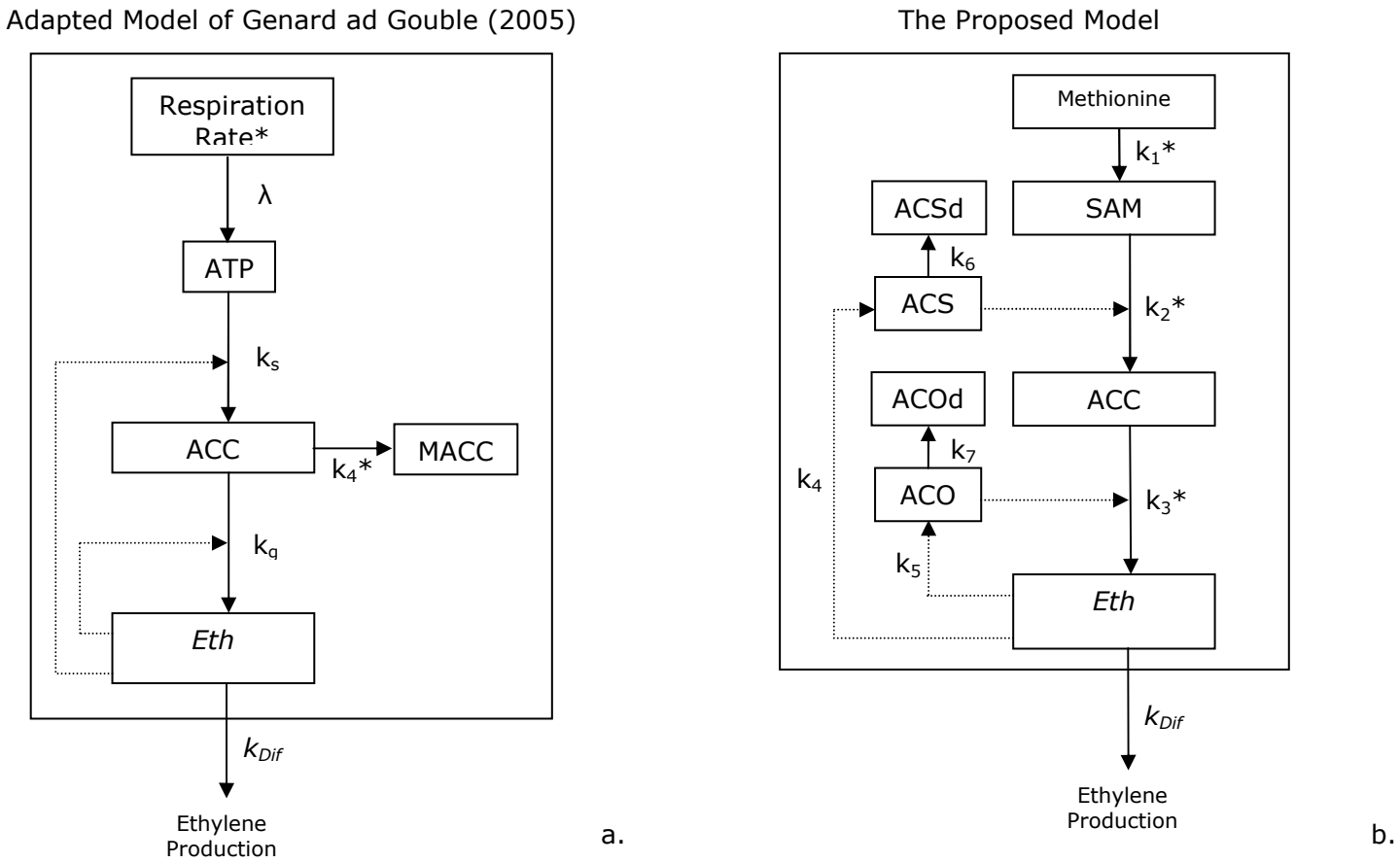

Figure 7.1, Schematic representation of the models evaluated. Full arrows denote reaction streams whereas dashed arrows represent regulatory effects. Note: Those with * are assumed to be temperature dependent as described by Arhennius' equation (1) while all other rates are assumed to be independent of temperature.

The conversion of ACC to internal ethylene (*Eth*) is facilitated by the enzyme ACO (equation 7.24) in an identically formulated mechanism to that discussed for the conversion of SAM to ACC.

$$\mathrm{ACC} + \mathrm{ACO} \xrightarrow{k_3^*} Eth + \mathrm{ACO} \qquad [7.24]$$

Production of the enzymes, ACS and ACO are both stimulated by the presence of ethylene (Jiang and Fu, 2000), aiding stimulation of ethylene production and ultimately resulting in the autocatalysis of ethylene production. McGlasson (1985) argued that ethylene itself induces the rise of climacteric ethylene, with low concentrations present in the preclimacteric fruit becoming effective in fruit as the tissue matures and the sensitivity of the tissue to ethylene increases.

$$Eth \xrightarrow{k_4} Eth + \mathrm{ACS} \qquad [7.25]$$

$$Eth \xrightarrow{k_5} Eth + \mathrm{ACO} \qquad [7.26]$$

Additionally, ACS and ACO are highly labile (Fluhr and Mattoo, 1996), resulting in the reduction of concentration of the active forms of both enzymes, represented in this model as a conversion into the inactive forms ACSd and ACOd respectively. For simplification, neither the autocatylytic stimulation of ethylene production, or the transformation into inactive forms of the enzyme were considered as temperature dependent.

$$\mathrm{ACS} \xrightarrow{k_6} \mathrm{ACSd} \qquad [7.27]$$

$$\mathrm{ACO} \xrightarrow{k_7} \mathrm{ACOd} \qquad [7.28]$$

Concentration balances for the ethylene precursors and enzymes on a nmol.m^{-3} basis were created, resulting in 5 ODEs (equations 7.29 to 7.33). Five initial conditions for each of the five components described by ODEs are required. As with the model of Genard and Gouble (2005), [Eth_0] was calculated from the prediction of Eth_{Prod0}.

$$\frac{\partial[\mathrm{SAM}]}{\partial t} = k_1^*[\mathrm{Meth}] - k_2^*[\mathrm{SAM}][\mathrm{ACS}] \qquad [7.29]$$

$$\frac{\partial[\mathrm{ACC}]}{\partial t} = k_2^*[\mathrm{SAM}][\mathrm{ACS}] - k_3^*[\mathrm{ACC}][\mathrm{ACO}] \qquad [7.30]$$

$$\frac{\partial[Eth]}{\partial t} = k_3^*[ACC][ACO] - \rho.Eth_{Prod} \quad [7.31]$$

$$\frac{\partial[ACS]}{\partial t} = k_4[Eth] - k_6[ACS] \quad [7.32]$$

$$\frac{\partial[ACO]}{\partial t} = k_5[Eth] - k_7[ACO] \quad [7.33]$$

7.3. METHODS

7.3.1. Treatment Selection

7.3.1.1. Model Development

All models were calibrated to fit averages of two treatments sampled from those collected during the course of the experimental work (Figure 4.1 and Figure 4.2, treatments 4B and 2BS) and labelled Chain 1 and 2 respectively. These treatments represent fruit from two different harvests (late harvest 2003, Hawkes Bay and 2004, Batlow). Measured physiological behaviour and time/temperature profiles of the selected treatments are presented in Figure 7.2.

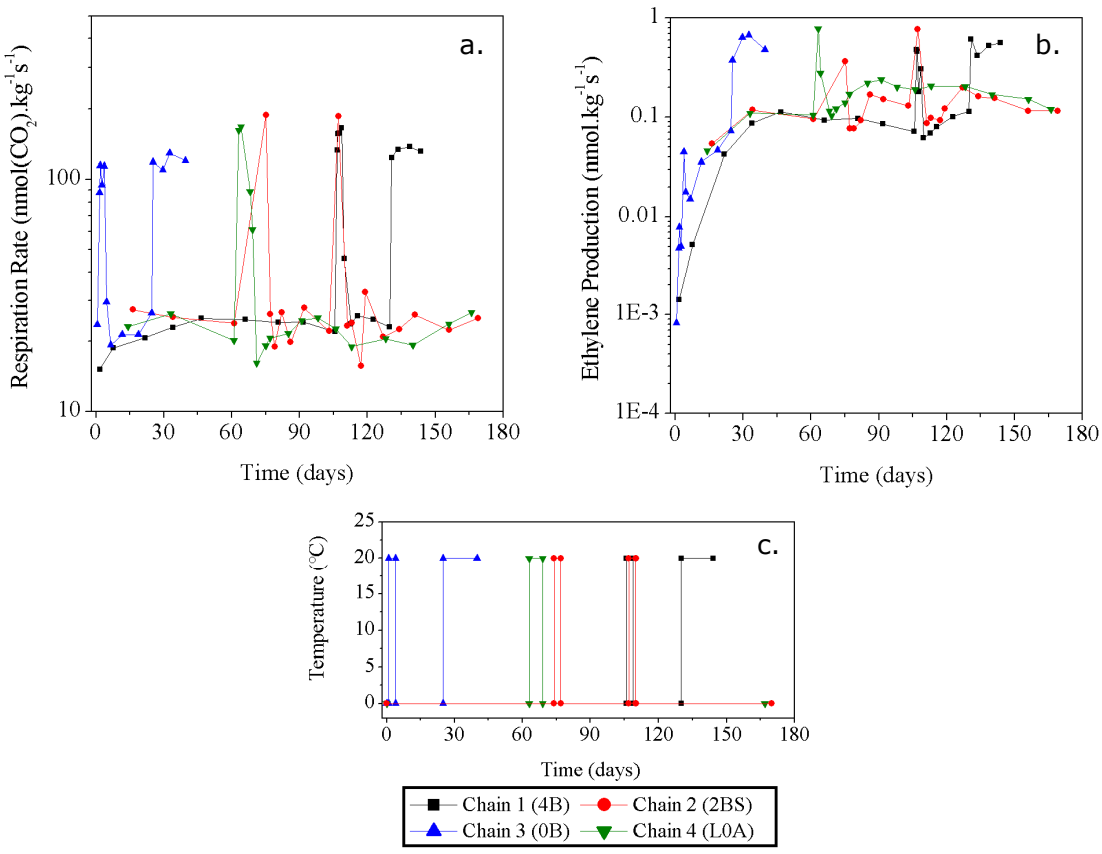

Figure 7.2, Measured (a) respiration rate and (b) ethylene production of four 'Cripps Pink' apple treatments during (c) variable temperature scenarios. Chains 1 and 2 were used to calibrate the models whereas all four were used to validate the models.

7.3.1.2. Model Testing

In addition to the two treatments used to calibrate the model, a further two treatments were use to validate the models by comparing the predictions against measured values not used in parameter optimisation. The addition of the two treatments for model validation allows assessment of the robustness of the model. One treatment (Chain 3) was selected from the 2003 late harvest (treatment 0B), while the other (treatment L0A, Chain 4) was taken from the 2004 experimental study, resulting in 1 other treatment from each of the harvests used to develop the models. Physiological behaviour and time/temperature profiles of these two treatments are also shown in Figure 7.2.

7.3.2. Model Parameter Optimisation

To aid parameter estimation all ethylene production values (modelled and predicted) were converted from SI units ($mol.kg^{-1}s^{-1}$) to $log(nmol.kg^{-1}s^{-1})$. This conversion allowed the model to consider differences in the lower range (in the first 30 days where fruit are preclimacteric) to be as significant as differences at 20°C. Without this conversion the experimental errors in assessing ethylene production at 20°C would be larger than the ethylene production values of preclimacteric fruit and hence the fitted models are unable to predict the preclimacteric fruit behaviour.

Model parameters were optimised with the use of the Optipa parameter optimisation software (Hertog, 2004), developed for use with MATLAB (v6.4, The Mathworks, USA). In all cases, all rate constant related parameters ($k_{(n)}$, $Ea_{(n)}$) were fitted to both chains. However, initial values (e.g. Eth_{Prod0}) were fitted to each chain individually to account for the differences that would occur due to at-harvest differences. Each chain used in model testing used the initial condition values of the chain from the same harvest.

Temperature was considered as a property of the environment that affected the behaviour of the apple. For simplicity, it was assumed that the apple had no thermal mass and hence the temperature of the fruit equalled that of the environment.

7.4. RESULTS AND DISCUSSION

Investigations of the influence of fluctuating temperature on 'Cripps Pink' physiology showed that, without exception, exposing apples to 20°C affected ethylene production of the apples on return to cool storage and at the time of a subsequent exposure (Chapter 4). In summary, the effects were:

a) Exposing preclimacteric apples to 3 days at 20°C caused apples to advance closer to climacteric production but did not influence ethylene production rates at the climacteric.
b) During 3 days of initial exposure of postclimacteric apples to 20°C, ethylene production was consistently higher on the 1st day than during the following 2 days of exposure.
c) On return to cool storage, ethylene production returned close to the level prior to the initial temperature exposure, and remained at this level for approximately 5 days before increasing to approximately 1.5-2 times the initial ethylene production rate over the following 10 days. The fruit maintained this increased level of ethylene production for the remainder of the storage period.
d) Length of exposure time influenced ethylene production on immediate return to 0°C with fruit exposed for 1 day producing less ethylene than prior to exposure, whereas fruit exposed for 6 days returned to levels similar to those prior to exposure.
e) Apples that had increased rates of ethylene production at 0°C (after an initial exposure to 20°C) also had increased rates of ethylene production at 20°C upon initiation of a second exposure (in contrast to fruit exposed for the first occasion).
f) Return to 0°C, after a second exposure to 20°C, reduced ethylene production to rates similar to that observed prior to initial exposure and then produced the induced increase in ethylene production as described under (c).

The three models created were evaluated on not only their ability to predict ethylene production as assessed by linear regression of predicted versus observed values (Minitab v13.31, Minitab Inc, USA), but also to describe the trends (a-f) outlined above.

7.4.1. Model of Tijskens et al. (1999)

Due to the limited number of parameters to be estimated, optimisation of the parameters for the adapted Tijskens model was relatively simple. The adapted logistic model was observed to be a good predictor of ethylene production (Figure 7.3, Table 7.1). Linear regression analysis of predicted versus observed for Chains 1 and 2 resulted in a R^2 of 0.65 ($P<0.001$) whereas for Chains 3 and 4, prediction was surprisingly slightly more efficient ($R^2 = 0.76$, $P<0.001$). Obvious deficiencies in the model include the prediction of more rapid development of the climacteric than was observed (Figure 7.3a and c) and the inability to predict the induced increase in ethylene production on return to 0°C (Figure 7.3b and d).

The model of Tijskens et al. (1999) is able to predict observed effect (a). The shift towards climacteric development in preclimacteric apples exposed to 20°C was predicted

(Figure 7.3c), although the predicted shift was greater than that observed in experimental results. All other effects observed in the experimental work were not predicted.

Table 7.1, Optimised parameters for the adapted model of Tijskens et al. (1999).

	$k_{Eth(ref)}$ s^{-1}	Ea_{kEth} $J.mol^{-1}$	$k_{EthMax(ref)}$ $nmol.kg^{-1}\ s^{-1}$	Ea_{EthMax} $J.mol^{-1}$	Eth_{Prod0} $nmol.kg^{-1}\ s^{-1}$
Late 2003	0.800	39700	0.381	57200	7.44e-4
2004					2.66e-4

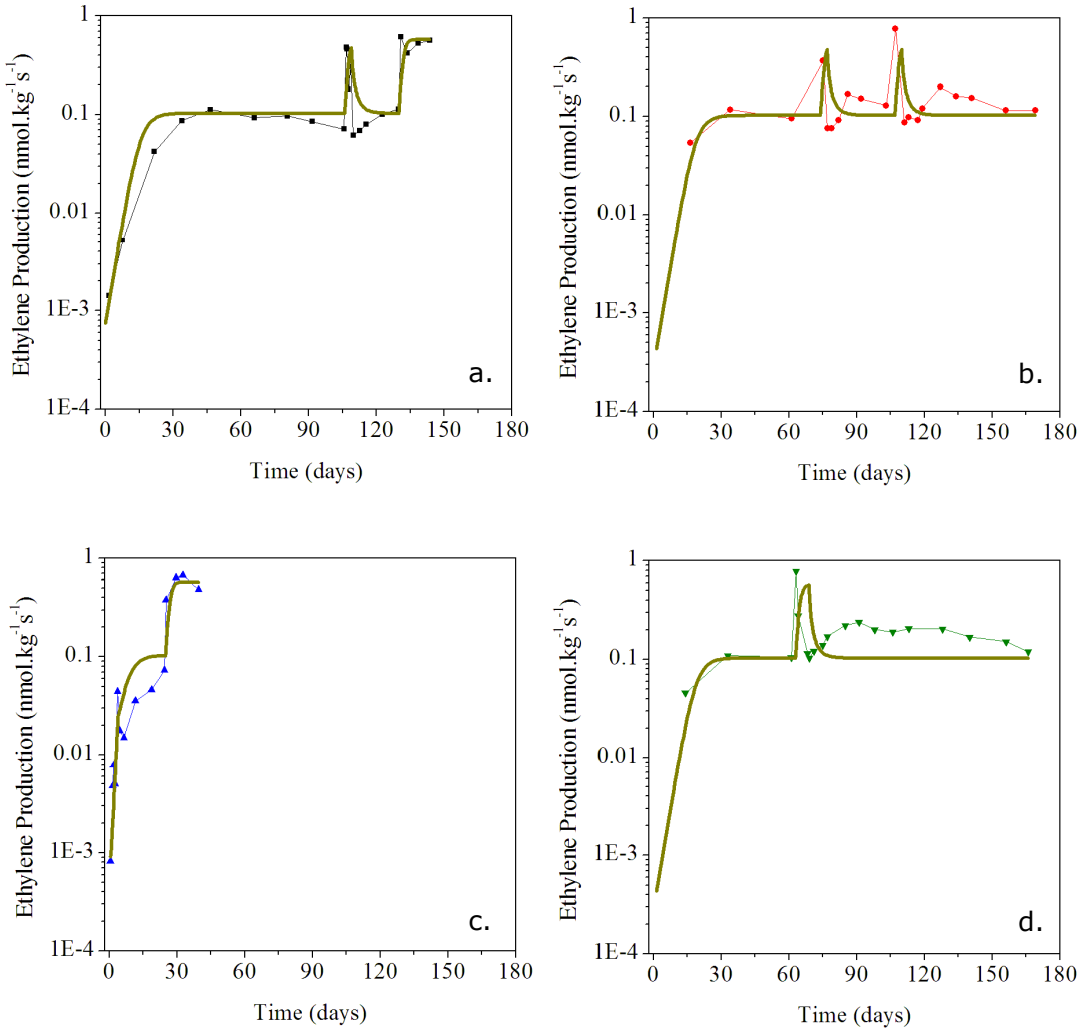

Figure 7.3, Predictive capability of the adapted logistic kinetics based model of Tijskens et al. (1999) for predicting ethylene production of 'Cripps Pink' apples in variable temperature scenarios. (a) Chain 1, (b) Chain 2, (c) Chain 3 and (d) Chain4.

7.4.2. Model of Genard and Gouble (2005)

Optimising the parameters in the adapted model of Genard and Gouble (2005) proved to be a difficult task, due to high correlation between some of the estimated parameters.

For instance, λ always occurs together with k_s (equations 7.11 and 7.19) making the estimation of both of them difficult. Subsequently, during parameter estimation, λ was fixed to 6 as it is considered that during oxidative respiration and assuming a respiratory quotient of one, every mole of CO_2 produced coincides with the production of 6 moles of ATP (Stryer, 1995). The model was also found to be extremely sensitive to the estimation of both [Eth_{ref}] and in particular k_{Dif}. Small adjustments to either of these parameters could result in significant shifts in the shape of the prediction of the model. Consequently [Eth_{ref}] and k_{Dif} were fixed (and 0.22 $m.s^{-1}$ respectively) during optimisation to allow easier estimation of the other parameters.

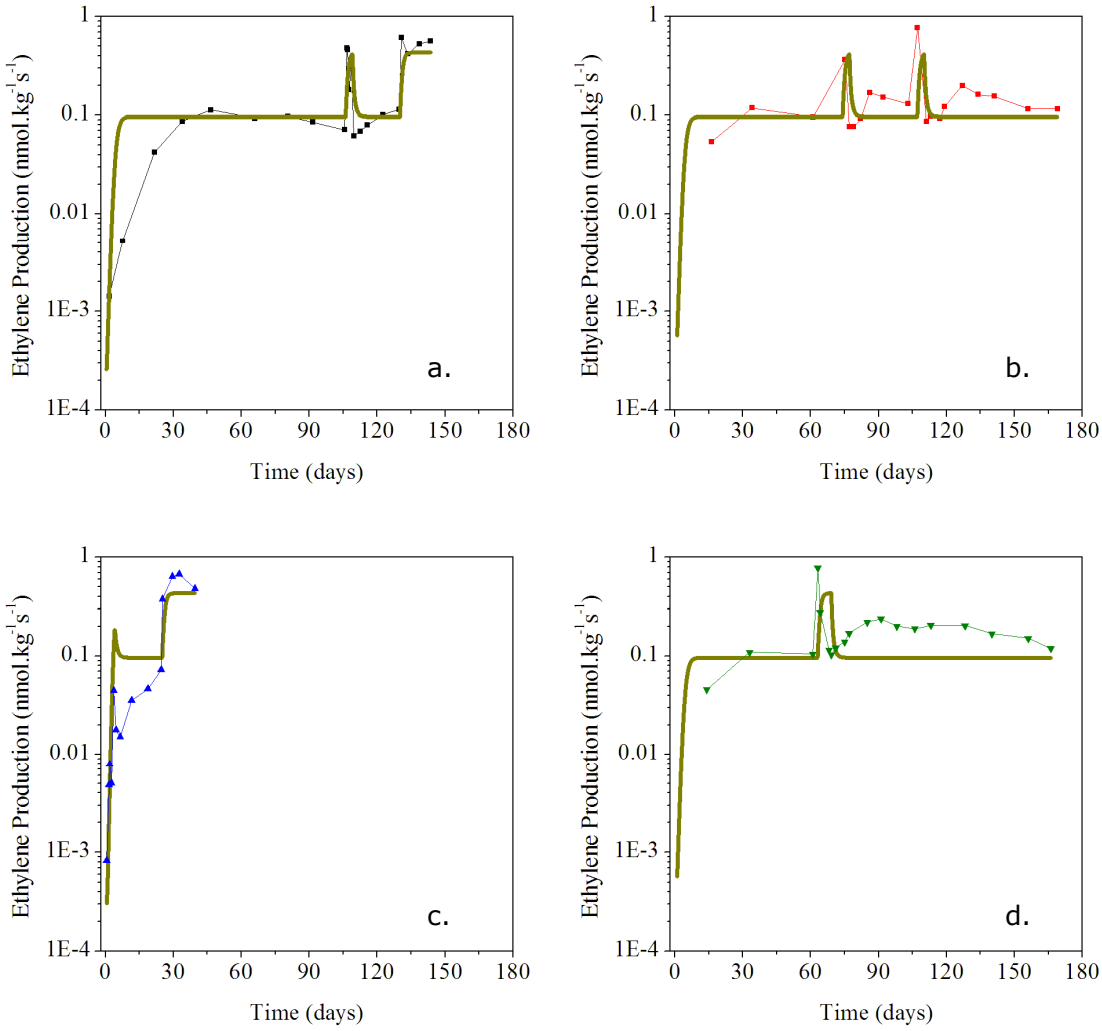

Figure 7.4, Predictive capability of the modified model of Genard and Gouble (2005) for predicting ethylene production of 'Cripps Pink' apples in variable temperature scenarios. (a) Chain 1, (b) Chain 2, (c) Chain 3 and (d) Chain4.

The optimised model showed a reasonable fit to the collected data (Figure 7.4, Table 7.2). Accuracy of prediction as determined by linear regression analysis was substantially less for the fitted Chains (R^2 = 0.34, P< 0.001) in comparison to the tested chains (R^2 = 0.60, P< 0.001). This difference in accuracy, is likely due to the prediction

of overly rapid development of the climacteric (Figure 7.4a). Observed weaknesses in the model are the overly rapid prediction of climacteric development (Figure 7.4a and c) and the inability to predict the induced increase in ethylene production (Figure 7.4b and d).

In addition to predicting ethylene production, the model provides a prediction of the change in ACC content throughout time (equation 7.19; Figure 7.5). ACC content of the chains mimics the shape of ethylene production, indicating that this model of ethylene production is not influenced by the ACC to ethylene process, contradictory to knowledge on ACO playing an integral role in controlling the rate of ethylene production rate (Fluhr and Mattoo., 1996; Jiang and Fu., 2000). More evidence of the inadequacies of this model are provided by the predicted reference rate values, where $k_{ref(s)}$ is 10^9 times greater than $k_{ref(g)}$. Clearly this model in its current form has some way to go to before becoming a reliable and robust predictor of ethylene production.

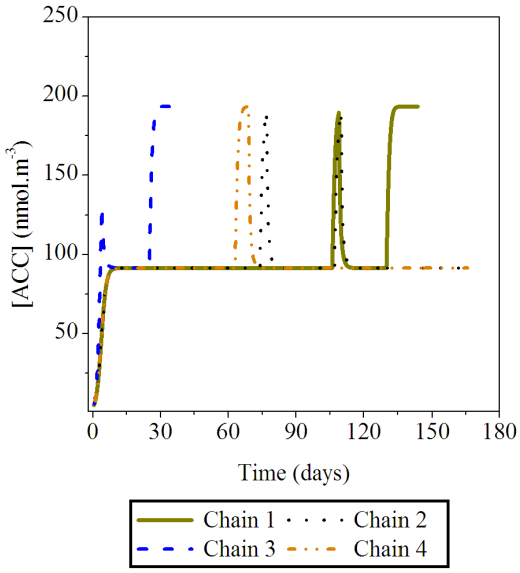

Figure 7.5, ACC prediction by the adapted model of Genard and Gouble (2005) predicting ethylene production of 'Cripps Pink' apples in variable temperature scenarios.

7.4.3. The Proposed Model

Optimisation was a time consuming task due to the large number of parameters dealt with (24 in total). In order to reduce complexity during optimisation, global parameters were fitted prior to optimisation of harvest specific parameters.

The optimised proposed model was found to be a very good predictor of ethylene production (Figure 7.6, Table 7.2). Linear regression analysis of predicted versus

observed for the fitted behaviours (Chains 1 and 2) resulted in a R^2 of 0.95 ($P<0.001$). As to be expected, Chains 3 and 4 were predicted with less accuracy (R^2 = 0.79, $P<0.001$). An initial over-prediction followed by a rapid reduction for the climacteric peak (at 30-45 days, Figure 7.6a and b) and a less accurate prediction of ethylene at 20°C (Figure 7.6a and c) provide room for improvement.

Table 7.2, Optimised parameters for the adapted model of Genard and Gouble (2005) and the proposed model.

Parameter	Units	Genard and Gouble		Proposed Model	
		Late 2003	2004	Late 2003	2004
$\mathbf{kRR_{ref}}$	$nmol(CO_2).kg^{-1}.s^{-1}$	8.73e-008		---	
$\mathbf{Ea_{RR}}$	$J.mol^{-1}$	61600		---	
$\mathbf{k_{4ref}}$	s^{-1}	0.039		---	
$\mathbf{Ea_4}$	$J.mol^{-1}$	651000		---	
$\mathbf{k_{ref(s)}}$	s^{-1}	3.20e8		---	
$\mathbf{k_{ref(g)}}$	s^{-1}	0.447		---	
$\boldsymbol{\lambda}$	1	6		---	
$\mathbf{[}\boldsymbol{Eth_{ref}}\mathbf{]}$	$nmol.m^{-3}$	1		---	
$\mathbf{[ACC_0]}$	$nmol.m^{-3}$	1e-6	1e-6	42.3	29.7
$\boldsymbol{Eth_{Prod0}}$	$nmol.kg^{-1}s^{-1}$	1.84e-2	1.82e-2	4.42e-4	3.67e-4
$\mathbf{k_{Dif}}$	$m.s^{-1}$	0.22	0.22	0.368	0.282
[Meth]	$nmol.m^{-3}$	---		138	170
$\mathbf{k_{1ref}}$	s^{-1}	---		1.646	
$\mathbf{Ea_1}$	$J.mol^{-1}$	---		42200	
$\mathbf{k_{2ref}}$	$m^3nmol^{-1}s^{-1}$	---		0.354	
$\mathbf{Ea_2}$	$J.mol^{-1}$	---		59500	
$\mathbf{k_{3ref}}$	$m^3nmol^{-1}s^{-1}$	---		0.573	
$\mathbf{Ea_3}$	$J.mol^{-1}$	---		64000	
$\mathbf{[SAM_0]}$	$nmol.m^{-3}$	---		16.7	15.7
$\mathbf{[ACO_0]}$	$nmol.m^{-3}$	---		0.081	0.220
$\mathbf{[ACS_0]}$	$nmol.m^{-3}$	---		5.90	13.0
$\mathbf{k_4}$	s^{-1}	---		6.20e-5	
$\mathbf{k_5}$	s^{-1}	---		8.82e-3	
$\mathbf{k_6}$	s^{-1}	---		2.64e-4	
$\mathbf{k_7}$	s^{-1}	---		1.76e-3	

The proposed model is able to predict effects (a) and (b) observed in the experimental data. The model was able to predict how exposure of preclimacteric fruit to 20°C not only resulted in advancing ethylene production towards the climacteric peak, but also resulted in a slight initial reduction in ethylene production on return to 0°C (Figure 7.6c). On exposure to 20°C, ethylene production was predicted to peak initially, and subsequently reduce while still remaining at 20°C (Figure 7.6a-b, d). Postclimacteric fruit, that were returned to 0°C after an exposure to 20°C, were consistently predicted to initially have a lower ethylene production rate than prior to the exposure to 20°C which subsequently is restored to its original levels. The model was unable to predict either (c) the induced increase in ethylene production or (d) the influence of the time at 20°C on ethylene production on immediate return to 0°C.

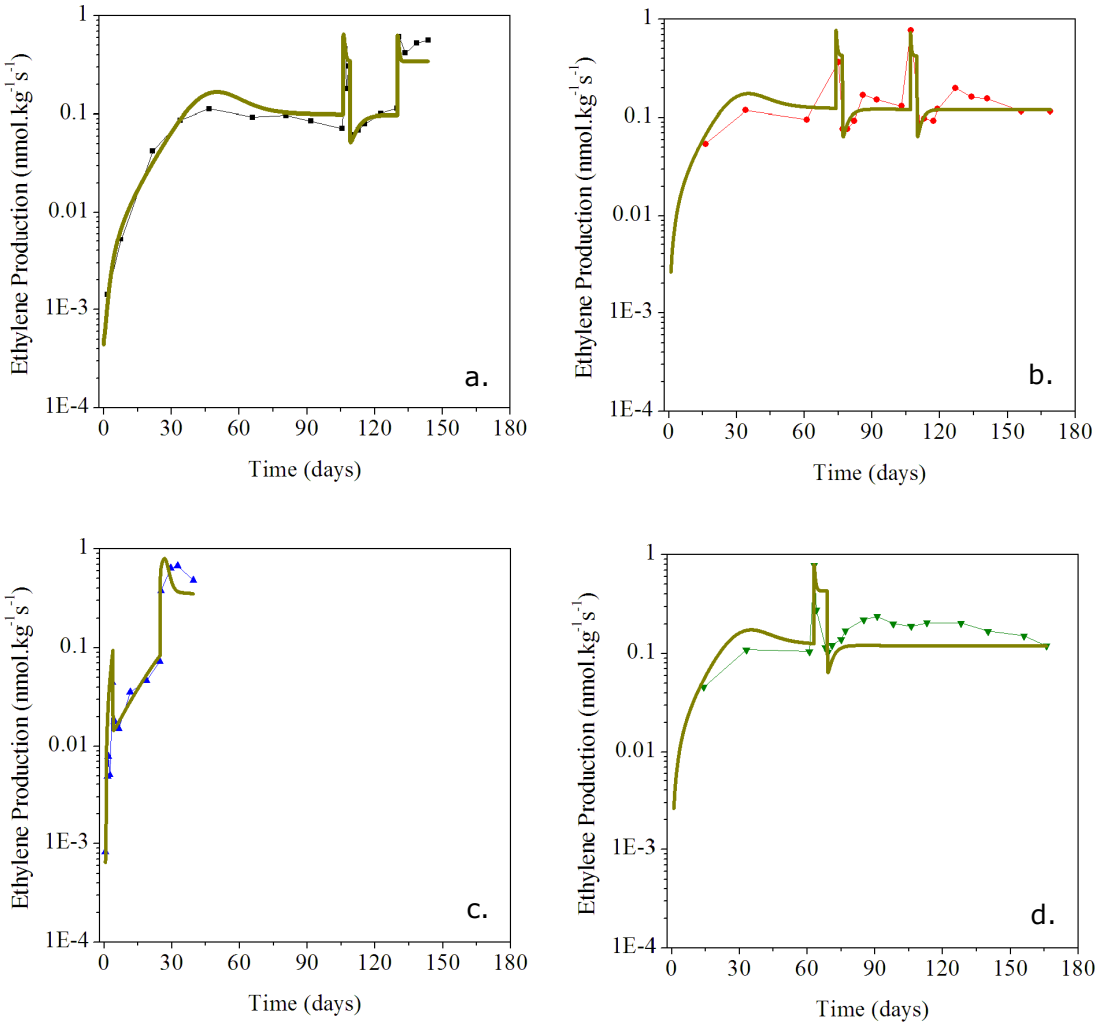

Figure 7.6, Predictive capability of the proposed model for predicting ethylene production of 'Cripps Pink' apples in variable temperature scenarios. (a) Chain 1, (b) Chain 2, (c) Chain 3 and (d) Chain4.

One advantage of the proposed model is the prediction of the behaviour of the precursors and enzymes in the ethylene production pathway in addition to the ethylene production rate (Figure 7.7). This allows access to valuable information that may provide clues as to the roles of the precursors and enzymes in the control of ethylene production in variable temperature scenarios. The change in the precursors and enzymes for both presented chains (Chain 1 and Chain 4) are similar.

The proposed model predicts ACO to increase throughout postharvest life (Figure 7.7a). At 20°C the increase in ACO was more rapid than at 0°C. This predicted increase in ACO agrees with the known behaviour of ACO during climacteric development (Brackmann et al., 1995; Tan and Bangerth, 2000, Figure 7.8) and changes in temperature (Jobling and

McGlasson, 1995; Lara and Vendrall, 2001; Tian et al., 2002) as observed in both 'Cripps Pink' parent cultivars 'Golden Delicious' and 'Lady Williams' and the other apple cultivars 'Fuji', 'Granny Smith' and 'Braeburn'. However it would be expected that the change in ACO would more closely match the pattern of ethylene production as demonstrated in 'Fuji' apples by Jobling et al. (2003).

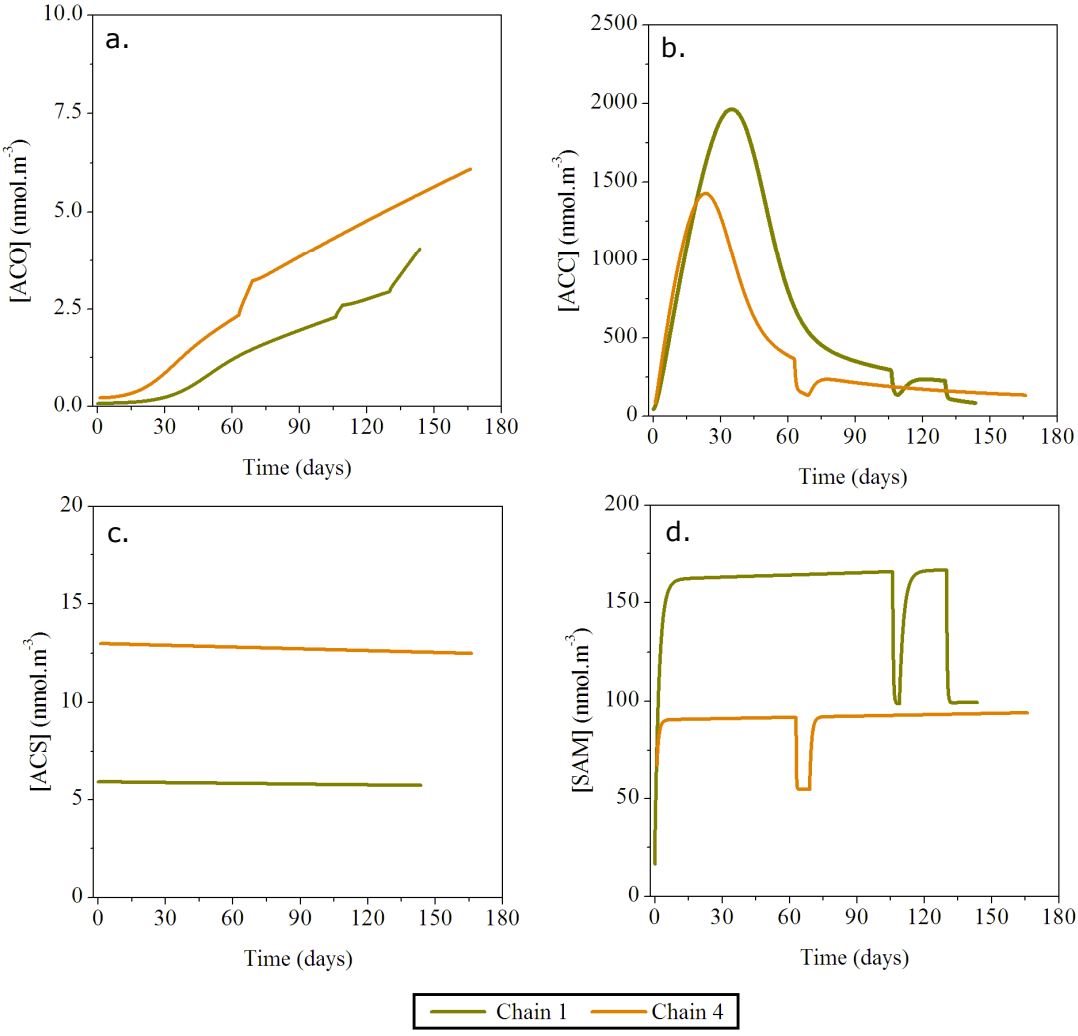

Figure 7.7, Enzyme and precursor concentration predicted by the proposed model when predicting ethylene production of 'Cripps Pink' apples in variable temperature scenarios.

The proposed model predicts an extremely large rise in ACC during climacteric development that reduces during the postclimacteric phase until reaching a point of near homeostasis (Figure 7.7b). The rise in ACC during climacteric development is expected, as the rise in ethylene production, matched by the simultaneous increase in ACC has been observed in both parent cultivars of 'Cripps Pink', 'Golden Delicious' (Knee et al., 1983; Tan and Bangerth, 2000; Figure 7.8b) and 'Lady Williams' (Jobling and McGlasson, 1995) and additionally the 'Fuji' (Jobling and McGlasson, 1995) and 'Cox's Orange Pippin'

(Stow et al., 2000) apple cultivars. At times when the apples are exposed to 20°C, the model predicts a reduction in ACC content which is rapidly recovered on return to 0°C, agreeing with work conducted by Jobling and McGlasson (1995) who observed that ACC accumulated in 'Lady Williams' apples stored at 0°C but decreased to low levels within 2 days after transfer to 20°C.

ACS levels were predicted to be relatively unresponsive to either fruit maturity or temperature by the proposed model (Figure 7.7c). The stimulation of ACS production by ethylene is predicted to be approximately 100 times less than that for ASO (compare k_4 to k_5). Similarly, the rate of degradation of the ACS is 10 times less than that of ACO (compare k_6 to k_7). These combined effects result in the model predicting ACS to be much more stable than ACO. This behaviour agrees with the results observed for peaches (Zhou et al., 2001, Figure 7.9b), where ACO responds more rapidly to increased ethylene concentration than ACS. However, the predicted non-responsive behaviour of ACS during ethylene climacteric development and temperature fluctuations is in vast contrast to the established knowledge that ACS is a rate controlling enzyme in the ethylene production pathway (Fluhr and Mattoo., 1996; Jiang and Fu., 2000).

SAM was predicted to be very responsive to both fruit maturity and temperature (Figure 7.7d), with sharp rise in SAM being observed to aid climacteric development, while reductions in SAM during periods of 20°C were also observed. The sensitivity to temperature of SAM is a result of both anabolistic and catabolistic reaction being modelled as a function of temperature. Validity of this response is unknown, as on one hand, the development of SAM is not considered as a rate controlling step in ethylene production, yet the production of SAM requires ATP and hence temperature may cause these predicted effects, due to higher respiration rates at higher temperatures.

7.5. FURTHER DISCUSSION AND CONCLUSIONS

The observed results for the proposed model prediction of precursor and enzyme behaviour confirm that in order to produce fundamental kinetic mechanistic-based models of postharvest fruit behaviour, information on all the precursors and the involved enzymes must be collected. Although purely fitting of what may seem a theoretically reasonable model can result in very good predictions (R^2 =0.95) of the predicted variable (ethylene production rate), the results for the intermediates can be in sharp contrast to current knowledge (Figure 7.7c). In this work, no effort was made to establish the validity of the values of the fitted parameters through either experimental data collection or comparison and extraction of already published data. Significant improvements in the accuracy of the models may be possible by undertaking this process. Only an

investigation where ethylene production, its precursors and the involved enzymes are tracked (e.g. Tan and Bangerth, 2000; Figure 7.8) will allow the development of a true model for ethylene production In this thesis, this work was not conducted as the aim of the work was to assess the influence of temperature breaks on fruit physiology and subsequently quality rather than, the influence of temperature breaks on the fruit physiology and what causes the changes in the fruit physiology.

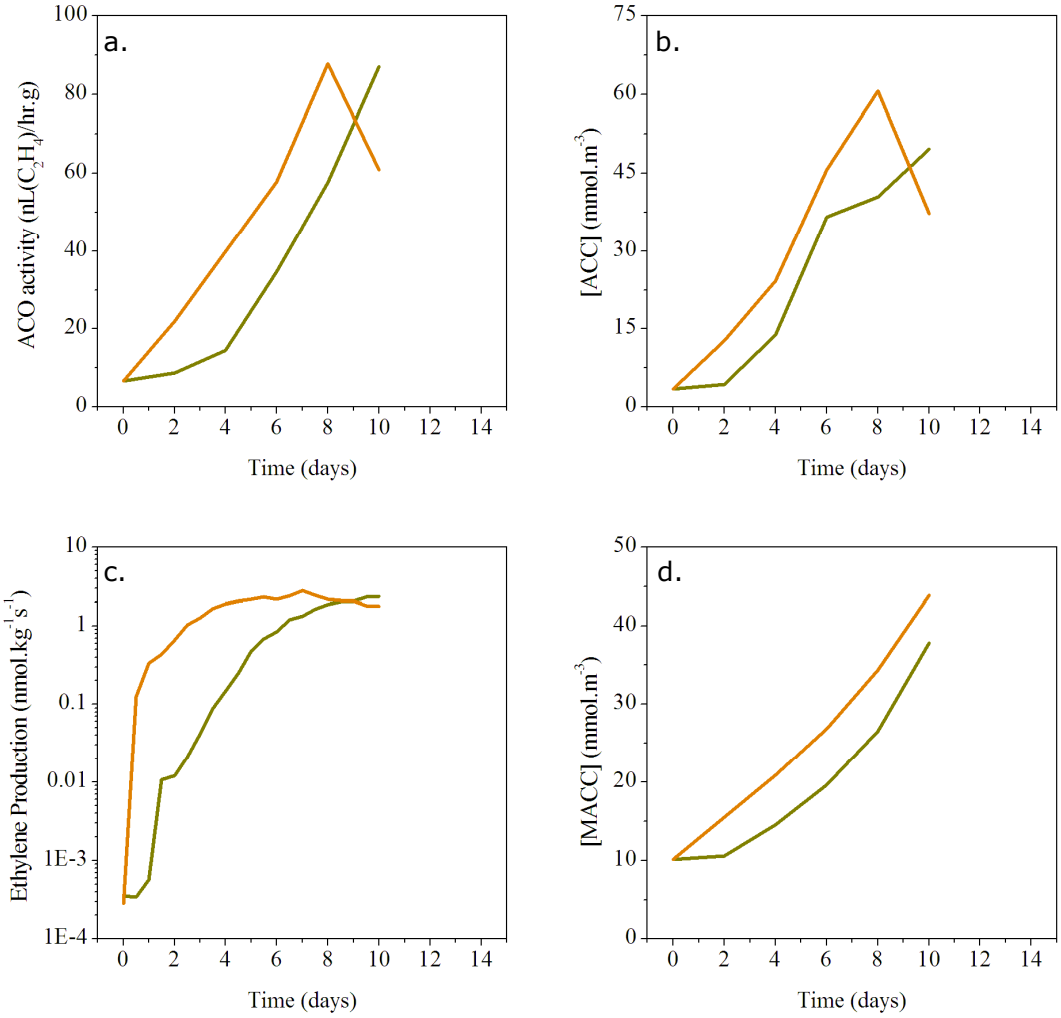

Figure 7.8, Change in (a) ACO activity, (b) ACC concentration, (c) ethylene production and (d) MACC for 'Golden Delicious' apples during postharvest maturation at 25°C and with exogenous ethylene treatment as measured by Tan and Bangerth (2000).

Probably the most detailed investigation of the type required during intermittent warming treatments is that of Zhou et al. (2001) in which the ethylene, ACS, ACC and ACO response of peaches was investigated (Figure 7.9). Peaches exposed to 20°C resulted in an increase in ethylene production (immediately) on return to storage at 0°C (Figure 7.9a). This increased ethylene production was linked to increased activity of both ACS and ACO activity (Figure 7.9b and d).

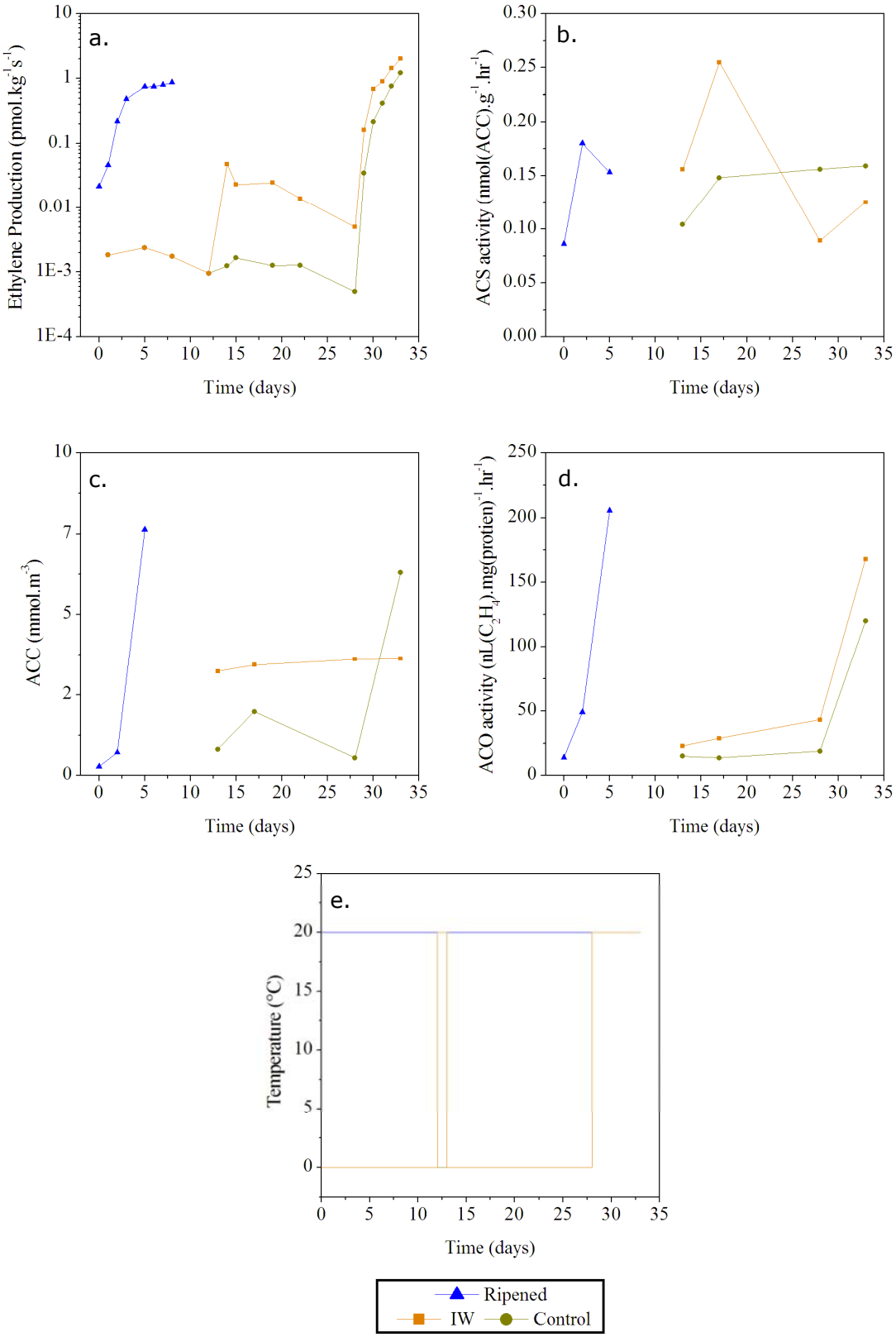

Figure 7.9, Change in (a) ethylene production, (b) ACS activity, (c) ACC, and (d) ACO activity of peaches subjected to (e) variable temperature treatments, including intermittent warming (IW) as found by Zhou et al. (2001).

Collection of a set of quality data of this type for the 'Cripps Pink' apple in variable temperature scenarios may yield significant knowledge of the control of ethylene production in apples. A smaller study than that conducted in this thesis is required. A single treatment of 'Cripps Pink' apples exposed to 20°C and monitored for ACC, ACO and ACS could be used to further tune the model. Once collected, the models presented in this work will be able to be further refined, identifying the mathematical description of the mechanisms of ethylene metabolism and production. Other factors not considered in the models created in this work such as CAs, harvest maturity, and application of 1-MCP may also need to be added to provide the ultimate ethylene predictor. When an accurate ethylene prediction model is established, models of the influence of ethylene on product quality and hence more accurate quality prediction in variable temperature scenarios may be possible.

7.6. NOTATION

Symbol	Unit	Definition
A	m^2	Surface area of an apple
[ACC]	$nmol.m^{-3}$	Concentration of ACC
[ACO]	$nmol.m^{-3}$	Concentration of ACO
[ASOd]	$nmol.m^{-3}$	Concentration of depleted ACO
[ACS]	$nmol.m^{-3}$	Concentration of ACS
[ACSd]	$nmol.m^{-3}$	Concentration of depleted ACS
[ATP]	$nmol.m^{-3}$	Concentration of ATP
$[CO_2]$	%	Atmospheric carbon dioxide concentration
d	m	Thickness of apple skin
D	m^2s^{-1}	Apple skin permeability to ethylene
$E_{a(n)}$	$J.mol^{-1}$	Apparent activation energy for reaction n
[*Eth*]	$nmol.m^{-3}$	Concentration of internal ethylene
$[Eth_{Ext}]$	$nmol.m^{-3}$	Concentration of external ethylene
Eth_{Max}	$nmol.kg^{-1}s^{-1}$	Maximum ethylene production
Eth_{Prod}	$nmol.kg^{-1}s^{-1}$	Ethylene production
$[Eth_{ref}]$	$nmol.m^{-3}$	Reference concentration of internal ethylene = 1
K_{CO_2}	$mol.m^{-3}$	Michaelis constant for CO_2 effect on ACO
k_{Dif}	$m.s^{-1}$	Diffusivity of ethylene through apple skin
$k_{(n)}$	s^{-1}	Rate constant for reaction n
K_{O_2}	$mol.m^{-3}$	Michaelis constant for O_2 effect on ACO
$k_{ref(n)}$	s^{-1}	Reference rate constant for reaction n
m	kg	Mass of an apple = 0.15
[MACC]	$nmol.m^{-3}$	Concentration of MACC
[Meth]	$nmol.m^{-3}$	Concentration of Methionine
n		Reaction identification number (1-7, s, g, Eth)
$[O_2]$	%	Atmospheric oxygen concentration
r	m	Radius of the apple
R	$J.mol^{-1}K^{-1}$	Ideal gas constant = 8.314
RR	$nmol(CO_2).kg^{-1}s^{-1}$	Respiration rate of apple
[SAM]	$nmol.m^{-3}$	Concentration of S-adenosylmethionine
T	K	Apple temperature
T_{ref}	K	Reference temperature
V	m^3	Volume of apple
ρ	$kg.m^{-3}$	Density of an apple = 900
π		Pi = 3.1415
λ	$kg.s.m^{-3}$	Proportional coefficient of RR to ATP concentration = 6

8. Overall Discussion and Recommendations

8.1. INTRODUCTION

Apples grown in Australia and New Zealand are harvested between January and May, and stored onshore with the aid of low temperatures; and in some cases, CAs and ethylene retarding (AVG) and blocking (1-MCP) technologies. These technologies allow distribution of apples to both local and export markets for an extended period of 6 – 12 months. In order to present the highest quality product to consumers, considerable effort is made to maintain optimal storage conditions. However, during commercial operations, optimal storage conditions may not always be maintained due to refrigeration system breakdown, facility constraints (e.g loading dock capacity), or management decisions (e.g. selection of transport mode). This research attempts to quantify the influence of these possible breaks in optimal storage conditions on fruit physiology and quality, both at the time of the break and in subsequent (optimal) storage. The 'Cripps Pink' ('Pink Lady™') apple cultivar was selected for study, as it is a high value cultivar of importance to the Australasian apple industry.

8.2. POSTHARVEST BEHAVIOUR OF THE 'CRIPPS PINK' APPLE CULTIVAR

In recent times, several investigations have been made of quality changes in 'Cripps Pink' as a result of postharvest treatments, including low temperature storage in both air and CA and in combination with the application of ethylene blockers (AVG and 1-MCP) (Drake et al., 2002; Crouch, 2003; Golding et al., 2005; Gaulanduzzi et al., 2005). Despite these studies a clear understanding of the postharvest behaviour of the cultivar had not yet emerged. This study generated information on the physiological response of the cultivar to different temperature and atmosphere conditions and the consequent quality changes and clarified expected behaviours.

Delays in harvesting 'Cripps Pink' apples are likely to cause a decrease in the hue angle, firmness and titratable acidity at the time of harvest and this difference is maintained during storage. Fruit harvested later are also more likely to develop internal browning and less likely to develop superficial scald.

'Cripps Pink' apples display a typical climacteric ripening pattern, with a dramatically increased ethylene production and increased respiration rate in the first 30 days of storage at 0°C in air. Storage in CA causes ethylene production

to be decreased at the climacteric and delays the establishment of increased respiration rates. During storage at 0°C, the rates of firmness loss are relatively linear, whereas hue angle change is harvest dependent and °Brix does not change significantly. The loss of titratable acidity begins at the time of climacteric development and is subsequently linear. Storage in CA reduces the rate of firmness and hue angle change by 85% and 75% respectively. Changes in 'Cripps Pink' firmness have been found to be highly correlated to changes in weight loss, background hue angle and titratable acidity.

8.3. THE EFFECT OF BREAKS IN TEMPERATURE CONTROL.

Prior to this investigation, the use of variable temperature treatments prior to or during long-term storage had been shown to influence pome fruit quality both positively (Klein and Lurie, 1992; Watkins et al., 2000a) and negatively (King and Henderson, 1988; Brookfield et al., 1998) depending on the factor of interest. In the Australian and New Zealand apple industries, it is common practice to sort, grade and pack apples previously stored for a long-term period (in bins), prior to shipment to foreign marketplaces. During these operations an increase in product temperature is common and the influence of this on the delivered product quality (prior to this study) was unknown. This research assessed the influence of breaks in temperature control of 'Cripps Pink' apple physiology and quality, both during the temperature break and during subsequent optimal storage conditions. The influence of harvest maturity, time in storage before temperature break, length of break, and multiple exposure to breaks in temperature control were investigated.

8.3.1. Apple Physiology

In this study, respiration rate was found to be dependent on fruit maturity (climacteric status) and temperature, but not influenced by previous fluctuating temperatures. In contrast and without exception, exposing postclimacteric 'Cripps Pink' apples to 20°C resulted in significant changes in the ethylene production of the fruit on its return to cool storage and initially on its later return to a subsequent period of high temperature storage. In general, on return to cool storage, ethylene production returned close to that prior to the initial temperature exposure, and remained at this level for approximately 5 days but then increased to approximately 1.5-2 times the initial ethylene production rate over the following 10 days and maintained this increased level of ethylene production for the remainder of the storage period. This was termed the "induced increase in ethylene production". Harvest maturity and multiple exposures had no clear

influence on the response. The length of temperature exposure influenced ethylene production upon immediate return to coolstorage, with those fruit exposed for longer returning to a greater ethylene production. The time in storage prior to the break also had a significant effect on the response, with those apples exposed while preclimacteric not exhibiting an induced increase in ethylene production but rather the break accelerated the fruit towards the climacteric as previously found for other apple cultivars delayed before cooling (King and Henderson, 1988; Johnston, 2001).

The induced increase in ethylene production was observed to be both delayed (by approximately 5 days at 0°C on return to coolstorage) and sustained (for a period of up to 80 days after the initial break in temperature control) suggesting that the response is not a result of stress but rather a shift in metabolic control and altered homeostasis of ethylene production. Induced increases in ethylene production on return to cool storage have been observed in other investigations of variable temperature treatments for other apple cultivars (Alwan and Watkins, 1999; Watkins et al., 2000a ; Johnston, 2001) and other fruit (Cabrera and Salviet, 1990; Zhou et al., 2001) suggesting that an increase in ethylene production at coolstorage temperatures after exposure to non-refrigerated temperatures may be a widespread response of many climacteric and non-climacteric fruit. Evaluation of the response of other apple cultivars and horticultural products to similar characteristic temperature breaks may be used as an indictor of the importance of maintaining optimal coolstorage conditions throughout the coolchain.

8.3.2. Apple Quality

When held at 20°C, the loss of quality of postclimacteric 'Cripps Pink' apples as measured by destructive firmness, stiffness (acoustic firmness), hue angle, and titratable acidity was more rapid than at the cool storage temperature of 0°C. This more rapid rate of loss of hue angle, and stiffness at 20°C generally resulted in significant differences between fruit exposed to the higher temperature and control fruit on the former's return to cool storage. This result is replicated in a number of other studies applying intermittent warming treatments to other apple cultivars (Brookfield et al., 1998; Alwan and Watkins, 1999; Johnston, 2001) and tomatoes (Artes et al., 1998). Although ethylene has been well established as an influencing factor on rates of loss of some quality indices (in particular firmness and background hue angle) the induced increase in ethylene production as a result of a break in temperature control was not observed to significantly increase

rates of quality loss on return to coolstorage temperatures, for those fruit stored in air.

8.4. BREAKS IN CONTROLLED ATMOSPHERES

Prior to this investigation, the benefits of storing apples in CA had been well established, as had the need to establish CA rapidly to obtain the greatest benefit (King and Henderson, 1988; Hertog et al., 2001). However the influence of commercial CA practices; i.e. the breaking of CA in large storerooms in order to remove some of the fruit followed by the re-establishment of CA, and the subsequent selection of air or CA atmosphere during refrigerated shipping, had not been systematically investigated. This research investigated the influence of these practices on fruit physiology and quality, and in combination with the influence of breaks in temperature control.

8.4.1. Permanent Removal from CA

It was demonstrated that a change in atmosphere from CA to air results in a change in apple physiology. At a constant storage temperature (of 0°C), the respiration rates of CA stored apples increased from that of apples stored in CA to that of apple permanently stored in air. However, while ethylene production increased by 145%, it remained at 40% of that produced by apples permanently stored in air. Hence storage in CA for long periods (in excess of 50 days) not only reduces ethylene production during CA storage but also in subsequent air storage at refrigerated temperatures.

On transfer from CA to air at 0°C, rates of hue angle change accelerated to be equivalent to fruit stored permanently in air. In contrast, rates of firmness change showed little response despite the change in both major physiological parameters.

8.4.2. Breaks in CA while Remaining at 0°C

A break in CA for a period of 3 days while the apples remained at 0°C, caused no significant changes in either physiological indicator (ethylene production or respiration rate) either during, or subsequent to, the break in CA. On return to CA, fruit behave similarly to fruit that had not been exposed to a 3-day break in CA. This result indicates that adaptions in physiological response as a result of a change from CA to air at 0°C take a significant time (days) to occur.

Breaks in CA for up to 3 days while fruit remained at 0°C also did not result in any significant differences in fruit quality from the control. Hence, there is no perceived disadvantage of having large CA rooms that require multiple loadout occasions (with the associated loss of atmosphere) on the premise that loadout does not affect the temperature of the room. It should be noted however, that a significant body of research exists indicating that a delay in establishing CA immediately after harvest results in poorer apple quality after storage (Liu, 1986; King and Henderson, 1988; Johnston, 2001). In this case, large CA rooms can be disadvantageous as they require larger amounts of fruit to be filled and hence potentially larger delay times before CA can be established.

8.4.3. Breaks in CA in Combination with Breaks in Temperature Control.

An exposure to a break in temperature control (3 days at 20°C) in combination with a break from CA storage promoted the induced increase in ethylene production. The fruit behaviour was identical for that observed for apples stored in air and exposed to breaks in temperature control. An approximate doubling of ethylene production was observed whether apples were in air or in CA prior to or after temperature exposure. Respiration rates were found to be dependent purely on the current environmental conditions.

The firmness and background hue angle of fruit that were exposed to breaks in both CA and temperature and returned to air storage decreased at a faster rate than fruit that were either returned to CA storage, or fruit that were transferred from CA to air storage without a break in temperature control. Fruit that were exposed to a break in CA and temperature control and then returned to CA storage did not lose quality at a significantly different rate to fruit constantly stored in CA, despite ethylene production rates of approximately double the rate of the control fruit.

In the commercial shipping trial, firmness after shelf-life and background hue angle after both the shipping and shelf-life periods were statistically superior in the apples shipped in CA in comparison to the apples shipped in air. However these differences in quality between CA and air shipped apples were small and would be considered commercially insignificant.

8.4.4. Physiology and Quality Variation Suppression Effect of CA

One of the weaknesses of the use of the refrigerated shipping containers (in comparison to the refrigerated vessel) is that the control in temperature

throughout the load is not as consistent, largely as a result of the relatively large surface area to volume ratio of the shipping container (Tanner and Amos, 2003). Consequently, during the simulated shipping period, the implication of different storage gas and temperature conditions, and whether or not fruit had been exposed to 20°C for 3 days during fruit loadout, were investigated. Physiological data showed that the magnitude and range of possible fruit physiologies in a CA container is far less than that in an air container. As a result, apples shipped in air can not only be expected to lose quality at a more rapid rate (as a result of the more rapid metabolism) but also to exhibit a larger variation in quality amongst the population in comparison to apples shipped in CA. This hypothesis was validated by data collected in the laboratory trials. This effect of CA on fruit physiology had previously been demonstrated (Andrich et al., 1998; Sanders and de Wild, 2003; de Wild et al., 2003), although not specifically focused upon, as in best practice horticultural storage scenarios it is assumed that the temperature within a facility is homogeneous. It is possible that the effect of CA in reducing the variation in physiological processes where there may be an inherent range of temperatures (as in a shipping container) is universal for horticultural products. This influence of CA should be further considered when investigating and implementing control strategies across the supply chain to optimise fruit quality at outturn.

8.5. THE INFLUENCE OF ETHYLENE ON APPLE QUALITY

While the focus of this thesis was on the influence of typical commercial practices on delivered fruit quality, perhaps the most significant result was the observed induced increase in ethylene production in coolstorage subsequent to an exposure to a break in temperature control and the mixed influence that this exerted on rates of quality change. Consequently this thesis provides further information to aid understanding of the influence of ethylene on apple quality changes.

The influence of ethylene on the rate of 'Pink Lady™' softening in air and CA (1% CO_2, 2% O_2) has previously been documented in the work of Golding et al. (2005). In that work, the researchers treated 'Pink Lady™' apples with aminoethoxyvinylglycine hydrochloride (AVG), an inhibitor of ACC and hence ethylene production prior to harvest and monitored ethylene production and softening rates during subsequent coolstorage in identical CA storage conditions to those used in this work. A one hundred fold reduction in the internal ethylene concentration was created as a result of pre-harvest application of AVG, with the untreated apples being significantly softer at the completion of the 8 month

storage period in CA. Similarly, Crouch (2003) found that 1-MCP treatments significantly reduced softening of 'Pink Lady™' apples.

Despite this clearly established influence of lowered ethylene production on rates of firmness change, in this study, when fruit were stored in air and exposed to 20°C, inducing an increase in ethylene production on return to coolstorage, no increases in subsequent rate of firmness change were observed (Figures 4.8 and 4.9). Similarly, the 1000-fold increase in ethylene production at the onset of the climacteric did not result in any obvious increase in the rate of softening, although titratable acidity decreased (Figure 3.4b and Figure 3.7). It is hypothesised that the lack of response of the measured quality parameters to the induced increase in ethylene production is because 'Cripps Pink' apples stored at 0°C are already producing ethylene such that the concentration exceeds the saturation levels for influencing the rates of quality change. The lack of response to climacteric development is an indicator of cultivar insensitivity to ethylene in general.

Apples stored in CA also showed mixed responses to changes in ethylene production (Figure 5.2) during shipping. Apples that were not exposed to any break in temperature control and were shipped over an 80-days in air showed a significantly change in terms of colour, but not in firmness from fruit shipped in CA. This response was observed in parallel with a 145% increase in ethylene production for the fruit shipped in air. In contrast, treatments that were exposed to 3 days at 20°C prior to shipping in air and subsequently produced ethylene at 100% greater than fruit not exposed to a temperature break and shipped in air (and 300% greater than fruit shipped in CA) but shipped in air, softened at a rate which resulted in significant firmness differences after 37 days (Figure 5.4). Subsequently, it is not only the shipping atmosphere, but the inducement of an increased ethylene production due to exposure to room temperatures that also played a significant role in the rate of quality loss of CA stored apple during subsequent shipping. This result strengthens the knowledge that ethylene does play a significant role in the softening of the 'Cripps Pink' cultivar, as treatments which shared the same rates of respiration, but differed in endogenous ethylene production softened at different rates.

It is possible that the differences in the mixed response of rates of quality changes in comparison to changes in ethylene production are caused by the non-linearity between ethylene and the quality responses. When Flores et al. (2004)

studied the influence of ethylene on quality responses on non-ethylene producing melons, they found that each quality response (firmness and colour change) had a different point of sensitivity and saturation threshold. The existence of both zones below which the fruit is not sensitive to and zones above which the response is saturated, suggests a quality response curve as hypothesised in Figure 8.1a. To test this hypothesis, data from both the air storage (Chapter 4) and CA storage (Chapter 5) trials where ethylene production was approximately constant was analysed to calculate rates of quality loss (penetrometer firmness and hue angle) assuming constant linear rates and no influence of atmosphere (air or CA) (Figure 8.1b-c). The data extracted resembles the hypothetical model, with zones in which increases in ethylene result in no dramatic change in rates of quality loss, especially for firmness change at ethylene production levels below 75 $pmol.kg^{-1}.s^{-1}$ and for colour change at ethylene production levels above 75 $pmol.kg^{-1}.s^{-1}$

While there is no doubt that ethylene has significant effects on apple fruit quality changes, the relationships between ethylene production, respiration rate and quality changes are far from being clearly established. Defining minimal threshold and saturation values for ethylene's influence on each quality parameter, the relationship between these two values and how these values are affected by fruit maturity, and external temperature and atmosphere conditions is a substantial but important task.

8.6. MODELS OF ETHYLENE PRODUCTION

Prior to this research, only the models of Tijskens et al. (1999) and van der Sman and Sanders (2005) attributed softening of apples to ethylene effects. This research attempted to develop a mathematical model of ethylene production that included the temperature dependent physiological response observed in the experimental work. Two recently published models of ethylene production in apples (Tijskens et al., 1999; Genard and Gouble, 2005) were adapted to the variable temperature storage scenario and compared to the performance of a new proposed model. None of the models presented were able to predict all of the consistent behaviours of ethylene production observed during the experimental work, indicating that more knowledge of the ethylene production pathway is required, before modelling of ethylene and subsequently apple quality can be conducted successfully. The predicted precursor and enzyme behaviours differed considerably from data in literature and confirmed that in order to produce

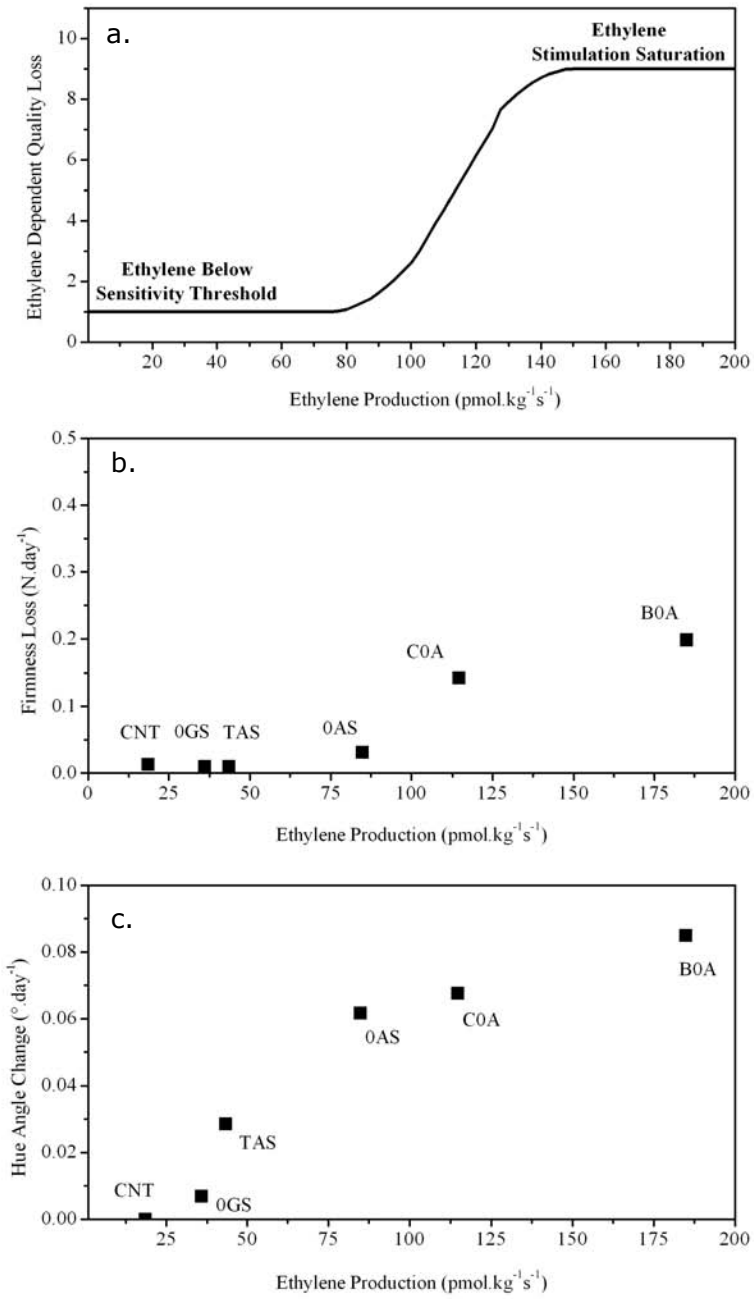

Figure 8.1, Quality response of 'Cripps Pink' apples to ethylene, (a) hypothetical response, (b) firmness loss and (c) change in background hue. Data for treatments CNT (CA at 0°C), 0GS (CA at 0°C after 3 days at 20°C in air), TAS (0°C in air after a period of 0°C in CA) and 0AS (0°C in air after a period of 0°C in CA and 3 days at 20°C in air) were retrieved from average ethylene production rates and straight line rate estimation of quality loss for data from day 130 to 200 in Figure 5.4. Hue angle data for treatments C0A (0°C in air) and B0A (0°C in air after 3 days at 20°C) were retrived in similar fashion between days 10 and 110 from Figures 4.5b (for average ethylene production) and 4.11a (for straight line rate of hue angle loss). Firmness loss for C0A was estimated from data extracted from Figure 5.2 (presented as AIR in Figure 5.2) between days 30 and 150. Rate of firmness loss for B0A was calculated from the difference between the destructive firmness of B0A at the completion of storage (48.8 N after 173 days, data previously not shown) and the firmness of air stored fruit prior to temperature exposure (69.8 N after 67 days, from treatment AIR, Figure 5.2).

mechanistic kinetic models of postharvest fruit behaviour, information on the precursors and enzymes involved in the metabolic pathway should be collected.

For a more accurate model of ethylene production, data of changes in SAM, ACC, ACO and ACS should be collected in conjunction with ethylene production data to gain a better understanding of the mechanisms of control of ethylene production.

8.7. FUTURE OPPORTUNITIES

8.7.1. Further evaluation of the Induced Increase in Ethylene Production

'Cripps Pink' apples were shown to produce an induced increase in ethylene production in response to a break in temperature control. However, with the exception of fruit stored in CA and then returned to air, rates of quality loss were not observed to be dramatically affected by the increase in ethylene production, despite strong links between ethylene and apple fruit quality.

8.7.1.1. Further Opportunities with the 'Cripps Pink' Apple Cultivar

While many possible coolchain scenarios were investigated in this study, investigation of whether related factors influence the subsequent ethylene production may further aid understanding of the response. These factors could include the minimum time at 20°C required for the response to be induced, the influence of lower temperature exposures (e.g. 10°C) or higher storage temperature, the effects of different rates of temperature change and whether or not fruit treated with 1-MCP respond in the same way.

8.7.1.2. Response of Other Apple Cultivars and Horticultural Products

Application of the results reported in this work for 'Cripps Pink' apples to other apple cultivars is difficult due to cultivar differences in ethylene response (Drake, 1993) and ethylene sensitivity (Liu, 1977; Gussman, et al., 1993). Whether other cultivars or horticultural products either produce the induced increase in ethylene production and a quality response to the increased ethylene is unknown. Such a study, would isolate whether the response is cultivar specific or more widespread.

8.7.1.3. Potential for Increased Volatile Production

A quality attribute of apples that was not measured in this study and is known to be influenced by ethylene is volatile production (Lurie et al., 2002; Mattheis et al., 2005). Song and Bangerth (1996) showed that rates of ethylene production were closely correlated to rates of aroma production for 'Cripps Pink' parent

cultivar 'Golden Delicious' apples. Consequently it is possible that the induced increase in ethylene production may prove to be beneficial in the production of volatile compounds during storage. An exposure to a short period at 20°C prior to shipping may be beneficial in producing volatiles and so improve the perceived sensory value of the fruit, with little adverse influence on other quality characteristics.

8.7.1.4. Use of Documented Response to Gain Understanding of Ethylene Control Systems.

Studies on ethylene production in horticultural products, and the influence of ethylene on quality changes have been facilitated by a number of application and response methods. Application of exogenous ethylene, ethylene removal agents, CA and temperature all create responses in fruit that allow the researcher to gain some understanding of the importance of ethylene to fruit physiology. In more recent times the use of the ethylene blocker 1-MCP and new transgenic cultivars provide further information. Both the induced increase in ethylene production and the mixed quality response of the fruit to the increase in ethylene observed for 'Cripps Pink' apples in this study, provide further targets for the fruit physiologist to study these processes to elucidate the control systems of the ethylene production pathway and the response systems of the ethylene dependent quality changes. In particular, it is speculated that the cause of the induced ethylene production may be a result of either triggering of a specific ACO/ACS gene expression or activity and/or changes in plasma membrane fluidity and subsequent functionality. The response detailed in this work could be used as a basis to investigate these processes and further elucidate mechanisms which control and influence ethylene production and quality responses in fruit.

8.7.2. Evaluation of Other Temperature Fluctuation Scenarios

This thesis investigated a dramatic, short time span and large temperature fluctuation. On the other end of the scale, the influence of cyclical temperature changes at an average temperature (as experienced by fruit maintained in mechanical refrigeration systems) may further prove to have an influence on long-term fruit quality (section 2.1.1.1.8). Research into the effect of such scenarios has yet to be thoroughly investigated in postharvest science.

8.7.3. Consequences of the Induced Increase in Ethylene Production on Laboratory Technique

This work has demonstrated that even small periods (1 day) at non-refrigerated temperatures can alter the physiology of a fruit on return to coolstorage, although quality indices may not be substantially different. This result suggests that in all postharvest experiments, breaks in temperature control should be avoided, whether due to measurement protocol or other circumstances as these breaks may influence the results of the experiment itself. This conclusion provides the researcher with a difficult quandary, as frequently experiments are designed in which fruit stored at different temperatures are to be compared by measurements taken at one (usually room) temperature. Basic scientific technique suggests that we should measure all treatments at the same temperature, as calibration differences will no doubt occur if treatments are measured at different temperatures. Temperature of measurement has an influence on both firmness (Johnston et al., 2001) and colour (this study, unpublished data). However, moving fruit to a single temperature for the purpose of measurement may influence the future response of that fruit.

8.7.4. Comparison of Apple Cultivars to Gain Understanding of Ethylene Control and Quality Influences.

Comparing the behaviour of 'Cripps Pink' apples to that of other cultivars suggests that the 'Cripps Pink' apple is relatively insensitive to ethylene. Ripening processes that are generally accepted as influenced by ethylene, include respiration rate, and loss of firmness, hue and titratable acidity. Differences in cultivar responses to ethylene between cultivars can be attributed to differences in reception and signal transduction of ethylene rather than the presence of ethylene itself. Detailed investigations into the roles of different ethylene receptors and/or ethylene response signal transduction pathways, possibly aided by the comparison of the different responses observed in different apple cultivars, will allow the isolation of critical regulatory factors and aid in future successful breeding and/or genetic manipulation of new commercial cultivars with enhanced storability.

8.7.5. Optimisation of Controlled Atmosphere Operations

The rate of change of the gas conditions within the fruit is governed by the diffusion properties of the flesh and skin of the fruit and the respiration rate as a function of the current gas concentration. When the external atmosphere is

changed there will be a lag phase between this time and when the fruit respire at the rate expected at the new gas atmosphere.

Knowledge of the time frame required for fruit to significantly change physiology, may provide operational advantages in addition to the scenario in which this thesis has aimed to assess, the breaking of coolstorage CA in order to remove a proportion of the fruit. Currently, CA generation, scrubbing and control systems are run 24 hours a day, 7 days a week during onshore fruit storage, with the exception of maintenance requirements. However, if significant lags exist between changes in the external atmospheres and physiological changes in the fruit, then it may be entirely possible that CA systems could be managed to shut down for short time periods, most beneficially during periods of peak power consumption, and hence reduce operating costs for the facility, with no (or little) effect on the product physiology or quality. In this study a break of 3 days resulted in no significant influence on physiology or quality.

Models that include the influence of flesh and skin diffusivity on respiration rates and subsequent internal gas concentrations in constant external gas conditions have previously been created for apples (Hertog et al., 1998) and pears (Lammertyn et al., 2003). However, the adaption of these models to this unique scenario of removing the CA atmosphere for a short time period has not been published. The question still remains as to how long it takes for fruit to transfer from homeostasis in CA conditions to homeostasis in the new atmospheric (RA) conditions. It is possible that if the time the fruit are exposed to an air environment is sufficiently short, then the fruit will not significantly shift to a new physiological state.

8.7.6. Economical Evaluation of the Benefit of CA Containers

The decision to use CA containers over regular air refrigerated containers introduces further cost to the product distributor. In this work, it was shown that CA shipping of 'Cripps Pink' apples resulted in significantly better apple quality, although perhaps not substantial enough to result in a difference that would be detected either by standard QA procedures or the consumer. Whether the faster deterioration of quality of produce shipped in air as opposed to CA is commercially significant or not, is determined by the cultivar specific rate of quality loss. A significant part of the commercial success of the 'Cripps Pink' apple cultivar (marketed as 'Pink Lady™') is its ability to store well. It would seem that in this commercial trial the rate of quality loss is too slow to cause a

commercially significant difference. However, this does not rule out the fact that other cultivars and horticultural products may benefit from CA shipping. When Mare et al. (2005), investigated the influence of shipping in CA (for a shipping period of 18 days) on shelf life of four plum cultivars, they found that shipping in CA provided a further 2 weeks of shelf life.

The need for postharvest science to evaluate technologies on a profit basis rather than a quality basis remains. All new technologies are only worthwhile in commercial scenarios, if the cost of their implementation and use is outweighed by the added value that is provided to the product or brand. Models that relate product quality to product value are not available for the postharvest researcher, hence reducing the ability of the researcher to judge the true impact of their work. Others would argue that economic evaluation is not the domain of the postharvest scientist at all. There is however, no doubt that if the postharvest scientist can communicate the results of their work on a triple bottom line basis (economic, environmental, and sociological), impact of the science developed would be more successful as would the reciprocal interest in the use of postharvest science to add value to horticultural industries.

8.7.7. Creation of Models that Predict Fruit Physiology and Subsequent Quality Changes.

While there is no doubt that ethylene plays a role in quality changes of apples and even the 'Cripps Pink' cultivar, clear understanding of the interplay of ethylene on rates of quality loss have not been established. Models that predict rates of quality loss as influence by ethylene and other physiological and environmental factors are required to bridge the gap between apple physiology and quality changes during storage and shelf life. Establishment of these models will aid assessment and economic optimisation of ethylene restrictive tools such as 1-MCP and ethylene scrubbers. This work provides an example of three models which could be further developed to predict ethylene production. Improvement of the current models is able to be achieved through the collection of data to enable better estimation of initial conditions and rate constants and tune the model to the changes in the ethylene production pathway. Once this knowledge is established, the established models for ethylene production will be able to be redefined in order to provide prediction of the data collected and the resulting ethylene production.

9. References

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10. Appendix

Model input files for use with Optipa parameter optimisation software (Hertog, 2004) are presented. Blue script indicates values required for successful operation of the optimisation process and hence are not altered between models. All green script (following a %) are comments to aid model development and understanding and are ignored during model implementation.

10.1. ADAPTED MODEL OF TIJSKENS ET AL. (1999)

```
function [out1, out2]= ethylene(t,y,param,currentcond)
%-----------------------------------------------------------------------
% Ethylene production model for temperature fluctuated 'Cripps Pink' apples
% based on the model published by Tijskens et al. (1999).
%-----------------------------------------------------------------------
if t=='__init___'
        %---------------------------------------------------------------
        % output variable definition.  First two columns are: 'EXP' and
        %'TIME' by default
        %---------------------------------------------------------------
          output_var={'EthProd'};
        %---------------------------------------------------------------
        % Definition of all model parameters, prior to optimisation.
        % One parameter per row.
        % param_DEF={'label'; Lower Bound;  Start Value;  Upper Bound };
        %---------------------------------------------------------------
        param_DEF = {  'kethref'   1e-9   0.67902   1e4 ;... %1 (1/s);(c)
                   'Eak'      1e-6   63192       1e9 ;... %2 (J/mol);(c)
                   'Ethmaxref' 1e-13  0.26037   1e6 ;... %3 (nmol/kgs);(c)
                   'EaEthmax'  1e-6   65000      1e9 ;... %4 (J/mol);(c)
                   'EthProd0'  1e-16  8.53778e-4    1e6 };  %5 (nmol/kgs);(h)

out1=output_var;
out2=param_DEF;
elseif t=='____y0___'
        %---------------------------------------------------------------
        % defining starting values for ODEs (definition of ODE's at bottom)
        % these initial conditions are initialised again for each experiment
        % so you can make use of experiment specific conditions or
        % parametervalues
        %---------------------------------------------------------------
          y0 = [abs(param(5))];  %EthProd0 (nmol/kgs)
        %---------------------------------------------------------------

out1=y0;
elseif t=='transdata'
expdata=y;
        %---------------------------------------------------------------
        % manipulation of original experimental data. Only transformation of
        % existing variables is allowed, not the creation of additional
        % variables
        % expdata:    Col1   Col2   Col3   Col3   ....
        %           EXP    TIME   dat(1) dat(2) ....
        % If not used, the unchanged values will be returned
        % These transformations are calculated per experiment simulated,
```

```
        % so you can make use of experiment specific conditions or parameter
        % values
        %------------------------------------------------------------
        %Conversion of experimental ethylene data from mol/kgs to
log(nmol/kgs)
        %------------------------------------------------------------
        expdata(:,3) = log10(expdata(:,3)*1e9);   %EthProd log(nmol/kgs)
        %------------------------------------------------------------


out1=expdata;
elseif t=='transform'
modelvalues=y;
        %------------------------------------------------------------
        % manipulation of modelvalues from ODE's per experiment simulated
        % Transformations, creating additional variables etc.
        % Make sure any additional column is included in output_var (see at top)
        % modelvalues:     Col1    Col2    Col3   ....
        %                TIME    y(1)   y(2)    ....
        % If not used, the unchanged modelvalues from your ODE's will be
        % returned. These transformations are calculated per experiment
        % simulated, so you can make use of experiment specific conditions
        % or parametervalues
        %------------------------------------------------------------
        % Conversion of model predicted ethylene production values
        % mol/kgs to log(nmol/kgs)
        %------------------------------------------------------------
        modelvalues(:,2) = log10(modelvalues(:,2));
        %------------------------------------------------------------

out1=modelvalues;
else
        %------------------------------------------------------------
        % MODEL ODEs
        %------------------------------------------------------------
        % defining local model constants
        %------------------------------------------------------------
           Tref=15;
           col_Time=2; %Time from condition file
           col_Temp=3; %Temperature from condition file
        %------------------------------------------------------------
        % defining experimental conditions using linear interpolation
        %------------------------------------------------------------
           Temp=interp1(currentcond(:,col_Time),currentcond(:,col_Temp),t);
        %------------------------------------------------------------
        % calculating temperature dependent rate constants with Arrhenius
        % karr is a function that calls on Arrhenius with:
        % The syntax for this is: karr(kref, Ea, Temp, Tref)
        %------------------------------------------------------------
           keth = karr(abs(param(1)),abs(param(2)),Temp,Tref);
           Ethmax = karr(abs(param(3)),abs(param(4)),Temp,Tref);
        %------------------------------------------------------------
        % defining model
        % dEthProd/dt = keth*EthProd*(1-(EthProd/Ethmax))
        %------------------------------------------------------------
              dydt  = [keth*y(1)*(1-(y(1)/Ethmax))];
```

```
  out1=dydt;
end
```

10.2. ADAPTED MODEL OF GENARD AND GOUBLE (2005)

```
function [out1, out2]= ethylene(t,y,param,currentcond)
%--------------------------------------------------------------------------
% Ethylene model for temperature fluctuated 'Cripps Pink' apples
% based on the model of Genard and Gouble (2005)
%--------------------------------------------------------------------------
if t=='__init___'
        %---------------------------------------------------------------
        % output variable definition. First two columns are: 'EXP' and
        % 'TIME' by default.
        %---------------------------------------------------------------
          output_var={'ACC' 'Ethc' 'RR' 'EthProd' 'ATP'};
        %---------------------------------------------------------------
        % Definition of all model parameters. One parameter per row.
        % param_DEF={'label'; Lower Bound; Start Value; Upper Bound };
        %          %number; (units) (common/harvest)
        %---------------------------------------------------------------
        param_DEF = {'kRRref' 1e-9   9.6674e-008  1e6 ;... %1 (1/s) (c)
                 'EaRR'   1e-6   6.1369e+004  1e9 ;... %2 (J/mol) (c)
                 'k4ref'  1e-9   0.002        1e6 ;... %3 (1/s) (c)
                 'Ea4'    1e-6   50000      1e9 ;... %4 (J/mol) (c)
                 'ks'     1e-9   0.01       1e12 ;... %5 (1/s) (c)
                 'kg'     1e-9   0.04        1e6 ;... %6 (1/s) (c)
                 'ACC0'   1e-15  2.4         1e6 ;... %7 (nmol/m3) (h)
                 'EProd0' 1e-15  5e-4        1e9 ;... %8 (nmol/kgs) (h)
                 'kDif'   1e-9   0.22        1e9 ;... %9 (m/s) (h)
                 'gamma'  1e-9   6     1e15 ;... %10 (1)
                 'Ethcref' 1e-15  1      1e9 }; %11 (nmol/m3)

out1=output_var;
out2=param_DEF;
elseif t=='_____y0___'
        %---------------------------------------------------------------
        % defining starting values for ODEs (see definition of ODE's
        % at bottom)
        % these initial conditions are initialised again for each experiment
        % so you can make use of experiment specific conditions or
        % parametervalues
        %---------------------------------------------------------------
        % [ACC0; Ethc0 calculated from EthProd0]
        %---------------------------------------------------------------
          kDif = abs(param(9));
          EthProd0 = abs(param(8));
          y0 = [abs(param(7)) 0.15*EthProd0/(kDif*0.0146)];
        %---------------------------------------------------------------
out1=y0;
elseif t=='transdata'
expdata=y;
        %---------------------------------------------------------------
        % manipulation of original experimental data. Only transformation
        % of existing variables is allowed, not the creation of additional
        % variables
        % expdata:     Col1   Col2   Col3   Col3   ....
```

```
        %            EXP    TIME   dat(1) dat(2) ....
        % If not used, the unchanged values will be returned
        % These transformations are calculated per experiment simulated,
        % so you can make use of experiment specific conditions or
        % parameter values
        %---------------------------------------------------------
        % Conversion of experimental ethylene data from mol/kgs to
        % log(nmol/kgs).
        % Conversion of experimental respiration rate data from mol/kgs
        % to log (nmol/kgs).
        %---------------------------------------------------------
        expdata(:,3) = log10(expdata(:,3)*1e9);
        expdata(:,4) = log10(expdata(:,4)*1e9);
        %---------------------------------------------------------

out1=expdata;
elseif t=='transform'
modelvalues=y;
        %---------------------------------------------------------
        % manipulation of modelvalues from ODE's per experiment simulated
        % Transformations, creating additional variables etc.
        % Make sure any additional column is included in output_var (see at top)
        % modelvalues:    Col1    Col2    Col3   ....
        %              TIME    y(1)   y(2)    ....
        % If not used, the unchanged modelvalues from your ODE's will be
        % returned.  These transformations are calculated per experiment
        % simulated, so you can make use of experiment specific
        % conditions or parametervalues
        %---------------------------------------------------------
        % Linear interpolation of temperature from experimental values
        %---------------------------------------------------------
           Tref=15;
           col_Time=2;
           col_Temp=3;
           Temp =
interp1(currentcond(:,col_Time),currentcond(:,col_Temp),modelvalues(:,1));
        %---------------------------------------------------------
        % calculating temperature dependent rate constants with Arrhenius
        % karr is a function that calls on Arrhenius with:
        % The syntax for this is: karr(kref, Ea, Temp, Tref)
        %---------------------------------------------------------
           kRR = karr(abs(param(1)),abs(param(2)),Temp,Tref); %(mol/kgs)
        %---------------------------------------------------------
        % Calculation of model predicted respiration rate and
        % converted to log(nmol/kgs).
        % Calculation of model predicted ethylene values from Ethc
        % values (modelvalues(:,3)) in nmol/m3 and converted to
        % log(nmol/kgs).
        % Calculation of model predicted ATP concentration from
        % respiration rate (nmol/m3).
        %---------------------------------------------------------
           kDif = abs(param(9));
           gamma = abs(param(10));
           modelvalues(:,4)=[log10(abs(kRR*1e9))];
           modelvalues(:,5)=[log10((modelvalues(:,3)*kDif*0.0146/0.15))];
           modelvalues(:,6)=[kRR*gamma];
        %---------------------------------------------------------
```

```
out1=modelvalues;
else
        %-------------------------------------------------------------
        % MODEL ODEs
        %-------------------------------------------------------------
        % defining local model constants
        %-------------------------------------------------------------
          Tref=15;
          col_Time=2;
          col_Temp=3;
        %-------------------------------------------------------------
        % defining experimental temperature conditions using linear
        % interpolation
        %-------------------------------------------------------------
          Temp=interp1(currentcond(:,col_Time),currentcond(:,col_Temp),t);
        %-------------------------------------------------------------
        % calculating temperature dependent rate constants with Arrhenius
        % karr is a function that calls on Arrhenius with:
        % The syntax for this is: karr(kref, Ea, Temp, Tref)
        %-------------------------------------------------------------
          kRR = karr(abs(param(1)),abs(param(2)),Temp,Tref);
          k4 = karr(abs(param(3)),abs(param(4)),Temp,Tref);
        %-------------------------------------------------------------
          ks = abs(param(5));
          kg = abs(param(6));
          kDif = abs(param(9));
          gamma = abs(param(10));
          Ethcref = abs(param(11));
          ATP = kRR*gamma;
        %-------------------------------------------------------------
        % defining model fluxes
        %-------------------------------------------------------------
        % y1, dACC/dt= ks*(Ethc/Ethcref)^(1/2)*ATP -
kg*(Ethc/Ethcref)^(1/2)*ACC - k4*ACC
        % y3, dEthc/dt: kg*(Ethc/Ethcref)^(1/2)*ACC -
density*area*Ethc/volume
        %-------------------------------------------------------------------------------
------          dydt  = [ATP*ks*((y(2)/Ethcref)^(1/2)) -
y(1)*kg*((y(2)/Ethcref)^(1/2)) - k4*y(1)
                y(1)*kg*((y(2)/Ethcref)^(1/2))- y(2)*900*(kDif*0.0146/0.15)];

  out1=dydt;
end
```

10.3. THE PROPOSED MODEL

```
function [out1, out2]= ethylene(t,y,param,currentcond)
%-------------------------------------------------------------------------
% Ethylene model for temperature fluctuated 'Cripps Pink' apples
%   East, A.R
%-------------------------------------------------------------------------
if t=='__init___'
        %-------------------------------------------------------------
        % output variable definition.  First two columns are: 'EXP' and
        %'TIME' by default
        %-------------------------------------------------------------
```

```
            output_var={'ACO' 'Ethc' 'ACC' 'ACS' 'SAM' 'EthProd'};
        %------------------------------------------------------------
        % Definition of all model parameters, prior to optimisation.
        % One parameter per row.
        % param_DEF={'label'; Lower Bound;  Start Value;  Upper Bound };
        %          % number;  (units); (common/harvest).
        %------------------------------------------------------------
        param_DEF = {'Meth'     1e-6   30      1e9 ;... %1 (nmol/m3)   (h)
                 'k1'       1e-6   0.25     1e9 ;... %2 (1/s)       (c)
                 'Ea1'      1e-6   75000      1e9 ;... %3 (J/mol)     (c)
                 'SAM0'     1e-6   30   1e9 ;... %4 (nmol/m3)   (h)
                 'k2ref'    1e-9   0.25     1e6 ;... %5 (1/s)       (c)
                 'Ea2'      1e-6   75000      1e9 ;... %6 (J/mol)     (c)
                 'ACC0'     1e-6   30     1e9 ;... %7 (nmol/m3)   (h)
                 'k3ref'    1e-9   0.125     1e6 ;... %8 (1/s)       (c)
                 'Ea3'      1e-6   65000      1e9 ;... %9 (J/mol)     (c)
                 'kDif'     1e-9   8e-3     1e4 ;... %10 (m/s)      (h)
                 'ACO0'     1e-6   0.45      1e9 ;... %11 (nmol/m3)  (h)
                 'ACS0'     1e-6   2     1e9 ;... %12 (nmol/m3)  (h)
                 'k4'       1e-9   0.1     1e6 ;... %13 (1/s)      (c)
                 'k5'       1e-9   0.08    1e6 ;... %14 (1/s)      (c)
                 'k6'       1e-6   0.03    1   ;... %15 (1/s)      (c)
                 'k7'       1e-6   0.03      1   ;... %16 (1/s)      (c)
                 'EthProd0' 1e-16  4.126e-4 1e6}; %17 (nmol/kgs) (h)

out1=output_var;
out2=param_DEF;
elseif t=='____y0___'
        %------------------------------------------------------------
        % defining starting values for ODEs (see definition of ODE's at bottom)
        % these initial conditions are initialised again for each experiment
        % so you can make use of experiment specific conditions or
parametervalues
        %------------------------------------------------------------
        % [ACO0; Ethc0 - calculated from EthProd0; ACC0; ACS0; SAM0]
        %------------------------------------------------------------
            kDif = abs(param(10));
            EthProd0 = abs(param(17));
            y0 = [abs(param(11)) 0.15*EthProd0/(kDif*0.0146) abs(param(7))
abs(param(12)) abs(param(4))];
        %------------------------------------------------------------

out1=y0;
elseif t=='transdata'
expdata=y;
        %------------------------------------------------------------
        % manipulation of original experimental data. Only
        % transformation of existing
        % variables is allowed, not the creation of additional variables
        % expdata:     Col1   Col2   Col3   Col3   ....
        %            EXP    TIME   dat(1) dat(2) ....
        % If not used, the unchanged values will be returned
        % These transformations are calculated per experiment simulated,
        % so you can make use of experiment specific conditions or parameter
        % values
        %------------------------------------------------------------
        % Conversion of time data from days to seconds
```

```
        % Conversion of experimental ethylene data from mol/kgs to
        % log(nmol/kgs).
        % Conversion of weight data from g to kg
        %---------------------------------------------------------
        expdata(:,3) = log10(expdata(:,3)*1e9);
        %---------------------------------------------------------

out1=expdata;
elseif t=='transform'
modelvalues=y;
        %---------------------------------------------------------
        % manipulation of modelvalues from ODE's per experiment simulated
        % Transformations, creating additional variables etc.
        % Make sure any additional column is included in output_var (see at top)
        % modelvalues:     Col1    Col2    Col3   ....
        %                TIME    y(1)   y(2)    ....
        % If not used, the unchanged modelvalues from your ODE's will be
returned
        % These transformations are calculated per experiment simulated,
        % so you can make use of experiment specific conditions or
          % parametervalues
        %---------------------------------------------------------
        % Creation of model predicted ethylene production values from
        % Ethc values (modelvalues(:,3)) in nmol/m3 and conversion to
        % log(nmol/kgs)
        %---------------------------------------------------------
           kDif = abs(param(10));
           modelvalues(:,7)=[log10((modelvalues(:,3)*kDif*0.0146/0.15))];
        %---------------------------------------------------------

out1=modelvalues;
else
        %---------------------------------------------------------
        % MODEL ODEs
        %---------------------------------------------------------
        %---------------------------------------------------------
        % defining local model constants
        %---------------------------------------------------------
           Tref=15;
           col_Time=2;
           col_Temp=3;
        %---------------------------------------------------------
        % defining experimental conditions using linear interpolation
        %---------------------------------------------------------
           Temp=interp1(currentcond(:,col_Time),currentcond(:,col_Temp),t);
        %---------------------------------------------------------
        % calculating temperature dependent rate constants with Arrhenius
        % karr is a function that calls on Arrhenius with:
        % The syntax for this is: karr(kref, Ea, Temp, Tref)
        %---------------------------------------------------------
           k1 = karr(abs(param(2)),abs(param(3)),Temp,Tref);
           k2 = karr(abs(param(5)),abs(param(6)),Temp,Tref);
           k3 = karr(abs(param(8)),abs(param(9)),Temp,Tref);
        %---------------------------------------------------------
           k4 = abs(param(13));
           k5 = abs(param(14));
           k6 = abs(param(15));
```

```
    k7 = abs(param(16));
    kDif = abs(param(10));
    Meth= abs(param(1));
%-------------------------------------------------------------
% defining model fluxes
%-------------------------------------------------------------
% y1,dACOdt= k5*Ethc-k7*AC0
% y2,dEthcdt= k3*ACC*ACO-density*EthProd
% y3,dACCdt = k2*ACS*SAM - k3*ACC*ACO
% y4,dACSdt = k4*Ethc - k6*ACS
% y5,dSAMdt = k1*Meth - k2*SAM*ACS
%-------------------------------------------------------------
 dydt  = [ k5*y(2)-k7*y(1)
         k3*y(1)*y(3) - 900*y(2)*(kDif*0.0146/0.15)
         k2*y(4)*y(5) - k3*y(1)*y(3)
         k4*y(2)-k6*y(4)
         k1*Meth - k2*y(5)*y(4)];


  out1=dydt;
end
```