CHAPTER I

INTRODUCTION

Fruit and vegetables are still alive after harvest (Watkins, 2003; Wills et al., 1998). Biological processes continue to occur within the fresh produce, which lead to degradation of quality. These biological processes are dependent on many factors including pre-harvest factors, harvest conditions, post-harvest factors, particularly temperature (Johnston et al., 2001c; Kopec, 1983) and storage conditions (Brackmann et al., 2002b; Bramlage, 1982; Brockmeulen, 2004). Change in the quality of fresh produce during storage involves a complex host of physical and physiological changes that are yet to be fully understood (Johnston et al., 2001b). Different genotypes of fresh produce respond differently to post-harvest conditions (Dris, 1999; Jobling and McGlasson, 1995), making study of specific cultivars necessary. Mathematical models for predicting change in the quality of fresh produce during storage is beneficial because it can elucidate the principal mechanisms of the actual processes occurring within the produce, and facilitate prediction of change in quality under various storage conditions, which can be used to improve marketing and product management.

'Cripps Pink' is a cultivar of apple grown widely in Australia. The apple was created in 1979 by crossing 'Golden Delicious' and 'Lady Williams' apples from the Margaret River area of Western Australia to combine the best features of both varieties. It has a brilliant pink colour, a sweet-tart flavour and a crunchy texture (Herna'ndez et al., 2005). Along with 'Golden Delicious', 'Gala'/'Royal Gala', 'Fuji' and 'Granny Smith', 'Cripps Pink'/'Pink Lady' apple is expected to enter the ranks of the top ten apple varieties, which are expected to provide about 60% of world apple production by 2015 (Wall,

2007). The most important market qualities of 'Cripps Pink' apples are background colour and firmness, which are related to ethylene and CO_2 production, and are influenced by storage conditions. There have been many studies related to changes in the quality of different apple cultivars during storage (Ertur et al., 2003; Skrzynski and Konopacki, 2003), but as yet little study of 'Cripps Pink' apple. An understanding of the responses of fruit quality attributes to storage conditions can help to improve post-harvest equipment and management, in order to minimise losses and increase profitability. Data can also be used to establish models for the prediction of changes in quality and keeping quality of fruit during storage. NA cold storage is used to reduce degradation in apple quality, particularly softening. In addition, a combination of cold storage and CA can have further enhanced benefit (Chapon and Bony, 1991; Meberg et al., 2000).

Therefore, the main aims of this study were to investigate changes in the weight loss, firmness, stiffness, ethylene production, respiration and background colour of 'Cripps Pink' apples during storage in a wide temperature range (0^oC to 30^oC) and under varying storage conditions, including NA cold storage and a combination of NA and CA storage. Based on the experimental firmness and stiffness data, mathematical models were established to characterise the softening process and predict changes in the flesh firmness and stiffness as well as the overall keeping quality of 'Cripps Pink' apples during storage.

The experiments were conducted using three different storage conditions. That is, NA cold storage at six temperatures (0^oC, 2.5^oC, 5^oC, 10^oC, 20^oC and 30^oC); NA cold storage at 0^oC followed by NA at the aforementioned six temperatures; and CA (2 kPa O_2 : 1 kPa CO_2, 0^oC) storage followed by NA storage at the six temperatures previously mentioned. Physical and biological changes in the apples during storage such as firmness, stiffness,

weight loss, background colour, ethylene and CO_2 production were monitored and analysed. Potentially mathematical models were determined and compared to select a model that best describe the change in quality; then, the model was developed based on the non-transformed firmness and stiffness data sets, as well as existing theories on the post-harvest softening of climacteric fresh fruit.

This thesis consists of six chapters as follows: (1) Introduction, (2) Literature review, (3) Changes in quality of 'Cripps Pink' apple under different storage conditions, (4) Prediction of change in firmness of 'Cripps Pink' apple, (5) Prediction of change in stiffness of 'Cripps Pink' apple, and (6) General discussion and conclusion.

CHAPTER II

LITERATURE REVIEW

This chapter starts with concepts of quality and defines some important quality attributes of fruit that will be used throughout the thesis. The purpose of this is to discriminate some terminologies, for example, firmness, stiffness and acceptable quality. The chapter then introduces the methods used to measure the main quality attributes in the experiments on apple. Pre-harvest and post-harvest factors affecting the quality of apples are also discussed generally. The chapter also discusses some basic methods for modelling changes in the quality of food materials and reviews some relevant major works.

2.1 Fruit quality and acceptable quality

Food products have many variable quality attributes, such as texture, colour, nutritional value and flavour. Information regarding quality has critical influence on purchasing decisions of consumers. The question arises, what defines acceptable quality for consumers? Or, what level of quality leads to rejection by consumers? The level at which a food is rejected varies, depending on the type of food, sensory expectations of consumers and their perceptions. Consumer rejection may be based on a poor external appearance of product, for example, yellowing of apple skin or shrinkage (dehydrated appearance) of orange fruit. Alternatively, consumers may reject food because of changes in the texture, taste or smell of food materials due to spoilage (McMeekin and Ross, 1996) or biochemical reactions, for instance, obvious reduction in the flesh firmness of

apples and peaches. Acceptable quality is defined as quality that is higher than the highest level leading to consumer rejection.

2.2 Weight loss and factors affecting weight loss of apples during storage

Water loss is known to reduce senescent breakdown in apple (Watkins and Thompson, 1992). The incidence of breakdown decreases linearly with an increase in weight loss of apples (Scott and Roberts, 1968). However, the extent of these reductions may vary with harvest time (Paull, 1999). Lowering the RH of storage air also helps to reduce low temperature injury (Scott and Roberts, 1968) and minimise coreflush in apples during storage (Grierson and Wardowski, 1978). However, reduction in RH to lower than 90% may cause a reduction in volume, leading to shrivelling of individual apples (Wilkinson, 1965). For example, shrivelling caused by high water loss occurred for 'Lobo' apples stored at 3^{o}C with low RH of 85% (Dris and Niskanen, 1999). Therefore, regardless of possible minor benefits, an application of low RH storage to apple should be avoided because it can increase weight loss, causing aging and shrivelling of the fruit (Bramlage, 1982). In contrast, a high RH can create an ideal medium for all kinds of micro-organisms during storage (Brockmeulen, 2004).

Under normal conditions of storage, weight loss in apples is caused mainly by water transpiration and partly by respiration (Wilkinson, 1965). It is dependent on relative humidity (RH), temperature and velocity of the storage air (Ozer et al., 2003).

Weight loss in apples increases linearly with storage time (Hertog et al., 2004) and water vapour pressure deficit (wvpd) (Paull, 1999). Wvpd, which is the difference in water vapour pressure between the surrounding air and the apples, is determined by the

interaction of temperature and RH of storage air. Weight loss can be decreased by reducing the wvpd via lowering the air temperature, increasing the RH or creating a barrier to water loss (Ben-Yehoshua, 1987; Grierson and Wardowski, 1978).

The rate of apple weight loss is constant at a certain temperature throughout the storage period (Nerya et al., 2001). Little difference in weight loss is reported for apples stored at -1^{o}C, 0^{o}C and 2.5^{o}C (Wills and McGlasson, 1971), when the fruit is in moisture equilibrium with the environment at 97% RH. The fruit may become desiccated or split at lower or higher RH, respectively.

Although water loss in apples caused by transpiration increases with velocity (turbulence) of the air flow in storage; under conditions of equilibrium between the apples and the storage air, the velocity of the air flow has very little influence on the weight loss of the fruit (Grierson and Wardowski, 1978).

Storage atmosphere also affects weight loss of apples. Because the respiratory process of apples oxidises the carbohydrates within the apples to create CO_2, water and heat, the process causes a reduction in weight of the apples. Therefore, weight loss of apples in CA storage which has lower oxygen (O_2) and higher CO_2 concentration compared with NA storage is lower than that in NA storage (Erkan et al., 2004; Ozer et al., 2003).

Weight loss of apples also varies with cultivars. In the same cold storage conditions of 0±0.5^{o}C and 90-95% RH, ‘Elstar’ apples had the highest weight loss compared to ‘Jonagold’ and ‘Granny Smith’ apples. The optimum storage periods for ‘Elstar’, ‘Jonagold’ and ‘Granny Smith’ apples were 150, 180 and 210 days, respectively (Ertur et al., 2003). Besides, coated fruit have less weight loss than uncoated fruit (Bai et al., 2001).

A previous study reported that 5% (Kaufman et al., 1956) was the acceptable maximum level of apple weight loss for consumers. In general, however, levels of about half those cited already cause visible shrivelling of the fruit (Hruschka, 1977).

2.3 Firmness and change in firmness of apples during storage

2.3.1 Quality and firmness of apples

Quality of apples can be determined by several quality attributes, such as flesh firmness, starch index, skin background colour, sugar and acid content, and flavour volatiles. Flesh firmness can directly influence consumer satisfaction and is used as a key quality criterion by wholesalers (Watkins, 2003). Firmer apples tend to be crisper, crunchier, juicer and less mealy than softer fruit (Abbott et al., 1984; Harker et al., 2002; Harker et al., 1997). Firm apples are also more resistant to physical damage, and are therefore often harvested immature to reduce damage during post-harvest transport and handling processes.

2.3.2 Apple texture and destructive firmness measurement

Many quality attributes are linked to the physical properties of fruit. It is therefore necessary to develop methods to evaluate fruit quality that are based on the measurement of its physical properties. Texture is an important and complex quality parameter for most fresh horticultural produces. The texture of fresh fruit is determined by the physical properties of cell walls, tissue structure and water status. These are interdependent factors that determine tensile strength, firmness and elasticity (Herppich et al., 2003). Change in firmness of fruit is generally assessed by measuring the force required to compress or

penetrate the fruit flesh by a defined depth (Vicente et al., 2007). Firmness measurements in fruit have been used for many years as a guide to the quality of fruit and are often the main test specified to establish the acceptability of the fruit for particular market or storage conditions (Dobrzanski and Rybczynski, 1999).

Firmness reflects the force required to bite into the sample (Harker et al., 2002). The puncture test using a penetrometer tool, which was proposed by Magness and Taylor (MT) in 1925, is an established standard in apple industries worldwide (Peleg, 1993). This is a destructive firmness tester that has also become the most widely used instrument for texture measurement and quality assessment of fruit and vegetables (Harker et al., 2002). A stainless steel conical or round probe is often used in conjuction with the firmness tester to determine the firmness of food materials. The stationary food sample must be penetrated by the probe to a certain depth at a certain velocity, about 0.5mm.sec^{-1}. The force required to achieve the defined deformation of the sample is used to compute its firmness. Firmness of horticultural products is determined as the maximum resistance force of the product flesh against penetration of the cylindrical plunger. The Instron Canton penetrometer measures firmness in one of two ways: it drives an 11mm diameter Effegi probe 8mm into the pared apple flesh at a speed of 240mm.min^{-1} (Harker et al., 2002); or it drives an 11mm diameter MT probe 7.9 mm into the pared apple flesh at a speed of 254 mm.min^{-1}. Maximum force is recorded, and firmness is calculated as the average of measurements on two opposing sides of the apple (Finney et al., 1978). Fruit firmness measured using the MT method is not influenced by water status of the fruit because it is mainly a measure of cell wall strength (Baritelle et al., 2001; Hertog et al., 2004).

The puncture test using the MT method to measure flesh firmness of apples is considered to be a standard, objective instrumental test. The puncture test has also been proven to correlate well with sensory evaluation of apples (Harker et al., 2006a; Harker et al., 2002), including the hardness, crispness and juiciness (Konopacka et al., 2003) of different cultivars, such as 'Elstar', 'Jonagold', 'Gloster' (Plocharski and Konopacka, 1999), 'Cox's Orange Pippin', 'Pacific Rose', 'Granny Smith', 'Royal Gala', 'Fuji', 'Red Delicious' and 'Braeburn' (Harker et al., 2002).

An understanding of the changing behaviour of the post-harvest firmness of apples can help to determine the appropriate type of firmness changing curve, structure of the mathematical model, and individual parameters of the model. Therefore, it is necessary to understand how firmness develops, how it changes, and which factors affect this change.

2.3.3 How is the firmness of apples formed?

The texture of agricultural produce is comprised of a combination of chemical and physical based forces, and archestructure forces. The first component of chemical and physical based forces, which are formed by special compounds and granules such as starch granules, turgor pressure inside the living cells, and adhesive forces between cells generated by the chemical composition and physical properties of the middle lamella and the pectin chains, changes during storage. In contrast, the second component of the archestructure forces, which are generated by the overall structure, shape and size of individual cells, overall structure and shape of tissue, and the distribution of vascular tissue, remains unchanged during storage (Tijskens et al., 1999).

The cell wall of plant materials is a complex structure and can be considered as a network of cellulose microfibrils embedded in a matrix of hemicellulose and pectin. It plays an important role in forming cell shape and rigidity in many plant tissues (Cosgrove, 2001; Rose and Bennett, 1999). On the other hand, the middle lamella of cell walls, which is constructed by pectic polymers, is also known to be an important region for maintaining cell-to-cell adhesion and cell packing in fruit tissues (Harker et al., 1997; Redgwell and Fischer, 2002; Wakabayashi, 2000).

Cell turgor regulates the tissue tension affecting structural firmness. The size of cells and the magnitude of the contact area between adjacent cells create another physical aspect to firmness. Fruits and vegetables will have different chemical, physical and archestructure forces depending on their specific natural properties, which determine the textural behavior and firmness of the produce. For apple, characteristics of cell structure such as shape, size, cell wall thickness, distribution and size intercellular spaces vary from cultivar to cultivar (Korniak et al., 2002). This explains differences in the firmness behaviour of different apple cultivars.

2.3.4 How does firmness of apples change after harvest?

It is commonly recognised that the softening of climacteric fruit such as apple is due to enzymatic degradation of cell walls (Johnston et al., 2001c). Analyses of pectin fractions in apples during ripening revealed that softening in apple is usually related to an increase in water-soluble pectin, reductions in galactose and arabinose residues (Knee, 1973), and some depolymerisation in any pectin fraction (Yoshioka et al., 1992). A number of cell wall modifying enzymes have been found in apple during ripening, such

as exo-polygalacturonase, endo-polygalacturonase and pectin methyl esterase (PME) (Johnston et al., 2001c). These enzymes, especially the two polygalacturonases (PG), are regarded as responsible for the pectin solubilisation leading to flesh softening (Atkinson et al., 1998; Bartley, 1978). Similar disruption of the middle lamella was found to occur in unripe apple tissues treated with PG (Ben-Arie et al., 1979).

In practice, it can be seen that fruit firmness will decrease to a certain level and then stabilise, depending on the natural properties of a given fruit, after harvest and post-harvest handling. According to the aforementioned analysis (see 2.3.3), changes in fruit firmness can be regarded as having two main components.

The first component of chemical and physical based forces will change with time due to decay caused by PME and PG enzymatic reactions. PME activity reduces the degree of methylation of the pectin chain, while PG reduces the length of the pectin chains. Both affect the middle lamella and cause a reduction in the adhesion among cells (Tijskens et al., 1999). Microscopic tests of fracture faces on tissue show that fracture faces often occur between cells on soft fruits in response to the impact of a foreign force, whereas the fractures predominantly occur through cells in firm fruit (De Smedt et al., 1998; Harker and Hallett, 1992). This can be explained by reference to intact tissue structure. In comparison with firm or freshly harvested fruit, soft fruit have tissues consisting of rounder cells, more cell separation, and larger intercellular spaces (De Smedt et al., 1998; Lapsley et al., 1992).

Conversely, the second component of the archestructure forces, which is linked to specific compounds in the cells, cell shape, cell wall structure, and linkage among the cells, can remain almost the same as at the time of harvest (Tijskens et al., 1998).

Therefore, apple softening during storage is tri-phasic in nature, similar to several melon cultivars (Aggelis et al., 1997), tomato (Sozzi et al., 1998) and early harvested kiwifruit (Macrae et al., 1989). Firmness reduction begins with an initial slow rate, continues with a rapid rate and follows by a final slow rate (Johnston et al., 2002a; Johnston et al., 2006; Johnston et al., 2001c; Johnston et al., 2003). However, apples preserved at high temperatures (12^{o}C to 35^{o}C) undergo only two phases of the softening process. The softening process begins immediately with a rapid softening rate and finally enters the slow rate softening phase (Johnston et al., 2001c).

2.3.5 Factors affecting change in firmness of apples

2.3.5.1 Pre-harvest conditions

As mentioned previously, besides pre-harvest factors such as air temperature, RH, rainfall pattern, soil type, nutrient supply and irrigation pattern, texture and firmness of apples are also influenced by time of harvest (Ferrandino et al., 2001; Johnston et al., 2002c; Tu et al., 1997). This factor influences firmness at harvest and post-harvest changes in firmness (Ertur et al., 2003).

The maturity of apples at harvest may also influence the post-harvest softening process. Regardless of NA or CA storage conditions, overmature fruit are often softer at harvest and after storage than less mature fruit (Ingle et al., 2000; Stow and Genge, 2000) and vice versa (Konopacka and Plocharski, 2002). Apples that are riper at harvest reach their climacteric sooner, even under CA conditions, and display more rapid decline in firmness during the marketing period (Tijskens et al., 2004b). For examples, after 248 days of CA (1.8 kPa O_2 : 2.8 kPa CO_2, at 1^{o}C) storage, the firmness values of late-

harvested ‘Golden Delicious’ apple are significantly lower than those of early and normal harvested fruits (Ferrandino et al., 2001); later picked ‘Cox’ apples tend to lose their firmness faster during post-harvest storage (Tu et al., 1997); later picked ‘Cox’s Orange Pippin’apples have less firmness and toughness after CA storage at 2 kPa O_2 (Knee and Smith, 1989); early picked ‘Cox’s Orange Pippin’ apples maintain higher firmness during NA at 0^{o}C and CA (1.25 kPa O_2 : 0.5 kPa CO_2, at 3^{o}C) storage (Tu et al., 1997). Another apple experiment conducted in CA storage (2.5-3 kPa O_2, 3-5 kPa CO_2, less than 1$\mu l.min^{-1}$ ethylene, 2.2 to 3.3^{o}C and 90-95% RH) concluded that apples harvested within 10 days of the onset of autocatalytic ethylene production lose less than 10% of their original firmness after 7.5 months of CA storage. These apples had very low ethylene production during the first four months of storage followed by gradually increased ethylene production, but this remained less than 0.5$\mu l.kg^{-1}h^{-1}$ for at least 7 months (Liu and Samelson, 1986). In addition, the duration of the initial slow softening phase may also be influenced by maturity of apples at harvest (Johnston et al., 2001c). Apples that are harvested less mature are firmer at harvest and have a longer initial slow softening phase than apples that are harvested at more mature stage (Johnston et al., 2002a).

However, early harvesting can have negative effects on volatile production of apples. It was reported that under conditions of NA or CA storage, ‘Gala Must’, ‘Elstar’ and ‘Jonagold’ apples, which were picked early, had lower volatile production at harvest time and up to 4 months after harvest than apples picked later. On the other hand, delayed harvesting can significantly reduce fruit quality (Miszczak and Szymczak, 2000) because harvest maturity has dramatic effects on the climacteric stage of apples. Flesh browning symptoms have been found to increase with delay in picking date for apples stored under

regular conditions at 0°C as well as under conditions of CA storage (Ben, 2001). Moreover, late harvested fruit stored in CA have higher rot, mealy pulp, cork breakdown and cracked fruit incidence compared with early harvested fruit (Brackmann et al., 2002a). In general, fruit harvested at the climacteric stage produce more volatiles than pre-climacteric fruit under all storage conditions and have higher acceptability to customers (Brackmann et al., 1993; Knee and Smith, 1989).

In addition, cultivar can also affect the physiology, ripening and softening of apples during storage. Different cultivars have different polysaccharide compositions, which may cause differences in softening rate and final firmness (Colgan et al., 2006; Johnston et al., 2001c). Overall change in firmness also varies with cultivar. This may be caused by differences in the amount of polysaccharides in the cell walls (Gheyas et al., 1997). The occurrence of a rapid softening phase in apples at ambient temperature (10-20°C) also varies among cultivars (Johnston et al., 2002b). In comparison with later maturing apple cultivars, earlier maturing cultivars may soften more rapidly during ripening because the fruit with larger cells and more intercellular spaces are generally less dense (Kahn and Vincent, 1990) and considered to have weaker tissues (Harker et al., 1997).

2.3.5.2 Treatments after harvest

Heat treatment after harvest can be used to disinfect and control diseases, delay ripening or decrease incidence of disorders in order to maintain the quality of fresh fruit (Watkins, 2002). Hot water treatment at 52°C for 5 minutes is optimal for apples. This treatment reduced loss of flesh firmness and weight, increased total soluble solids (TSS) content and reduced the titratable acidity of fruit stored in CA for 6 months and then at

room temperature for 5 days (Lunardi et al., 2002). The treatment also increased resistance to bruising (Tahir and Ericsson, 2003), but could also accelerate the rate of de-greening (Watkins, 2003).

Because the 'Cripps Pink' apples and the firmness data sets were independent of pre-harvest factors and treatments after harvest, only storage conditions should be considered during studying and modelling changes in the fruit quality.

2.3.5.3 Storage conditions

Many different factors affect changes in quality of horticultural produces during storage. Reduction in quality, which is visible in fresh produces like fruit, vegetables or flowers, can be caused by chemical and biochemical reactions. These reactions can result from the respiration process of living materials or a combination of different chemical elements, leading to changes in the concentration of chemical components as well as the structure or texture of the fresh produce. Degradation in cell walls and changes in the middle lamella structure of apple tissues during ripening cause reduction in firmness of the apples (Bartley and Knee, 1982). The main factors affecting firmness of apples during storage are temperature, RH, gas composition and ethylene (Johnston et al., 2002a; Watkins, 2003).

Temperature

Storage temperature is the most important factor affecting post-harvest quality and keeping quality of perishables (Kopec, 1983), including apple (Johnston et al., 2002a). Although the firmness created by the cellulose-based structure of the primary cell wall

can hardly be affected by temperature, the contribution of pectic compounds to firmness is affected by storage temperature (Tijskens et al., 1998). Besides, because the rates of chemical and biochemical reactions occurring within the fresh fruit increase with temperature, high storage temperatures can speed up ageing, and cause serious reductions in quality, firmness and shelf-life of the fresh produces (Tijskens et al., 1994). Studies on the effect of storage temperature on textural change in different commodities showed that inappropriate temperature can cause dramatically deleterious changes in fresh produces (Paull, 1999). Beside, temperature can also affect the softening pattern of apple fruit. It was revealed that apples stored at temperatures ranging from 20^{o}C to 35^{o}C do not undergo an initial slow softening phase (Johnston et al., 2001c). For example, 'Royal Gala' apples stored at 0-5^{o}C, 'Cox's Orange Pippin' stored at 2.5^{o}C, and 'Granny Smith' and 'Pacific Rose' stored at 0-12^{o}C have three typical softening phases, whereby the first phase of little or no firmness loss took place over the first 5-25 days of storage (Johnston et al., 2001c)**.** In contrast, 'Royal Gala' stored at 12-35^{o}C, and 'Cox's Orange Pippin' stored at 5-35^{o}C have only the last two softening phases, beginning immediately with the rapid softening phase (Johnston et al., 2001c).

Therefore, storage at low temperatures is a commonly used method for slowing the ripening process to allow transport over long distances to markets or for delaying the marketing of fruit in order to achieve higher returns (Wills et al., 1998). Refrigeration is the most common storage technology used to preserve the quality of horticultural produce (Kader, 1986). However, excessively low temperatures can cause chilling injury in the fruit (Tijskens and Polderdijk, 1994). 'Gala' apple should be stored at 0^{o}C in CA storage. This storage temperature can help the fruit to have higher titratable acidity after nine

months of storage and higher pulp firmness, lower mealiness and lower internal breakdown after seven days shelf-life at 20^{o}C (Brackmann et al., 2001). Furthermore, an interaction between storage temperature and apple harvest maturity also affects change in apple firmness (Watkins and Thompson, 1992).

RH of air and water loss

Undesirable reduction in MT firmness of apples during storage is influenced only by temperature and not by the wvpd between the fruit and the storage air ($p_a - p_s$) (see 2.3.2). Therefore, MT firmness may be unaffected by RH of the storage air and water loss from the apples.

Gas composition of storage

Storage atmosphere affects change in apple firmness during storage (Colgan et al., 2006). A combination between low O_2 concentration with high CO_2 concentration can slow down ethylene biosynthesis and action, and respiration rate, leading to decreases in cell wall disassembly and reduction in flesh softening (Burg and Burg, 1967; Dilley et al., 1989; Hertog et al., 2001; Siddiqui et al., 1996). Besides, similar to apples in NA cold storage, apples in CA storage also undergo three phases of softening (Johnston et al., 2002a). However, the fruit has a longer initial slow softening phase and a slower rate of softening during the rapid softening phase compared with the fruit in NA storage (Johnston et al., 2003). Therefore, CA storage is superior to NA storage in retarding softening and maintaining apple firmness (Drake, 1993; Erkan et al., 2004; Ferrandino et al., 2001; Lopez et al., 2007). Seven days after removal from storages, apples stored in

low-oxygen (LO) CA storage also have better firmness and quality compared with those in NA storage (Lopez et al., 2007). In spite of these, an elevation of CO_2 concentration compared with the standard CA conditions (2 kPa O_2 : 3 kPa CO_2 : 95 kPa N_2, at $0^oC \pm 1^oC$) confers no additional advantage in maintaining apple firmness (Barrett et al., 1991).

In addition, it has been found that storage of apples in low-oxygen controlled atmosphere (LO-CA) (1 kPa O_2 : 1.5 kPa CO_2) at 3^oC in the initial stage from 2 to 5 months, followed by standard CA storage (5 kPa CO_2 : 3 kPa O_2) at 3^oC, reduced the softening rate and maintained up to 90% of initial firmness; besides, it also reduced the rate of titratable acidity and the risk of flesh browning (Lidster et al., 1985).

Delay of CA establishment and slow initial cooling rate

The benefits of CA on reducing softening rate of apples can only be realised if CA conditions are applied during the initial slow softening phase. The apple softening rate in CA will be the same as that in NA storage if fruit have already entered the rapid softening phase by the time they enter CA storage (Johnston et al., 2002a). Any firmness benefits will be lost if the delay of CA establishment is more than 50 days (Johnston et al., 2003). In general, the quicker CA conditions are established, the better the CA storage is able to maintain apple firmness. Rapid establishment of CA storage to apples after harvest effectively maintains fruit firmness and juice acidity, and reduces the susceptibility of the fruit to bruising (Lau, 1983). Rapid establishment of CA is very important for long term (> six months) storage of apples (Watkins, 2003). Flesh firmness of 'Fuji' apple is higher when CA conditions are established on the first day compared with CA established later (Brackmann and Saquet, 1999; Brackmann et al., 2002b).

On the other hand, the initial cooling rate during NA and CA storage also affects subsequent reduction in apple firmness. A study on the effect of cooling time at the beginning of CA storage on the quality of 'Gala' apple reported that apples cooled more quickly are firmer at the end of CA storage and after seven days of shelf-life; and have a lower percentage of mealy fruit. Apples cooled more slowly have lower TSS, higher rot incidence and yellower peel colour than the other more quickly cooled apples (Brackmann et al., 2000; Brackmann and Saquet, 1999). Some research also indicated that apple cultivars with a lower respiration rate and longer shelf-life should be cooled within three days after harvest (Liu and Samelson, 1986).

In addition, there is also an interaction between establishment of CA conditions and cooling time. Establishment of CA conditions before fruit cooling causes scald in 'Gala' apples. Conversely, a delay of CA establishment results in a higher incidence of decay and more rapid yellowing of the apple background colour (Brackmann and Saquet, 1999).

Ethylene in storage

Role of ethylene

Ethylene, which is a simple gaseous hydrocarbon (C_2H_4), is one of several plant growth regulators affecting the growth, development, ripening and senescence of plants. It is the most important regulator for post-harvest ripening of climacteric fruit and has considerable effects on changes in background colour and texture; and on flavour development of horticultural produces (Watkins, 2002). It may induce and stimulate softening in apples by regulating expression of cell wall modifying enzymes (Johnston et

al., 2002a). It also plays an important role in regulating the transition between the initial slow and rapid softening phases of apple after harvest (Johnston et al., 2001c).

Benefits of low-ethylene (LE) in storage

After harvest, apple firmness declines while ethylene production and concentration increases (Watkins et al., 2000; Yoshioka et al., 1995). Low-ethylene (LE) storage maintains apple firmness by inhibiting the initiation, rather than reducing the rate of flesh softening in apple fruit. Low-ethylene controlled atmosphere (LE-CA) storage with internal and external concentrations of <0.1 $\mu l.litre^{-1}$ (Stow et al., 2000) and <1 $\mu l.litre^{-1}$ (Liu, 1977), respectively, is regarded as a safe substitute for LO-CA storage in retarding flesh softening and other forms of senescence in apples (Knee and Hatfield, 1981; Stow et al., 2000). The onset of the rapid softening phase is found to be consistently associated with an internal ethylene concentration (IEC) exceeding 1.5 $\mu l.litre^{-1}$ in some apple cultivars (Johnston et al., 2002b; Johnston et al., 2002c; Johnston et al., 2001c).

Removal of ethylene in storage

Low-ethylene concentrations are to retard softening and reduce rot of apple during storage (Watkins, 2002). Ethylene concentration in storage can be reduced by ventilation, ethylene scrubbers, absorption onto carbon, oxidation using potassium permanganate, ozone; ultraviolet irradiation or catalytic burners (Watkins, 2003). Treatments with the ethylene inhibitor, aminoethoxyvinylglycine (AVG), in combination with various surfactants or LE-CA reduce the rate of CO_2 and ethylene production in 'Gala' apple

compared with the untreated control fruit. This method helps to slow down softening of the apples (Brackmann and Waclawovsky, 2001).

2.4 Stiffness and change in stiffness of apple during storage

2.4.1 Stiffness and non-destructive stiffness measurement

The properties measured by human cutting or biting tests are intuitively and fundamentally more related to the properties measured by the destructive MT puncture test. Although MT is an objective instrumental test, it can be prone to measurement errors if hand-operated devices are used (Harker et al., 1996). Besides, it is a destructive test in which measured samples are destroyed after each measurement. This causes difficulties to continuously monitor firmness of a given produce. Moreover, a large number of samples required for experiments lead to higher experimental costs. In addition, measurement using this method is also time and labour-intensive. Therefore, non-destructive methods for measuring texture are needed. The methods should allow fruit to be repeatedly monitored during ripening, and facilitate sorting into uniform batches of fruit that are more likely to meet consumer expectations.

According to the theories on sound generation and transmission, sound is an acoustic energy form resulted from the vibration due to interaction between two or more objects at a certain place and in a definite surroundings (Rauterberg et al., 1994). Three principal properties of sound are signal duration, amplitude and frequency. These properties are influenced by the properties of the interacting objects, such as stiffness, elasticity, density and inertia; as well as the properties of the surroundings. The properties of the sound source are also affected by the size and shape of interacting objects. Therefore, the

physical properties of an object can be characterised by sound properties (Carello et al., 1998). For example, the sound created by dropping a wooden ruler on the surface of a wooden table is different to the sound generated by dropping a rubber eraser from the same height onto the table. Applications of resonance frequency to determine the tissue rigidity and turgor pressure of biological materials have been reported by many plant physiologists (Abbott et al., 1992). During recent years, the acoustic impulse response method has been developed for non-destructive analysis of the elastic texture components of fruit and vegetables (Herppich et al., 2003). Measurement of stiffness index (S) using the sonic resonance technique is rapid, convenient, non-destructive and repeatable. Resonance frequency is mostly dependent on the mechanical properties of apple and the frequency of the second peak is sensitive mostly to apple texture, sonic resonance can be used to estimate the texture of apples (Abbott et al., 1992). Therefore, the Acoustic Firmness Sensor (AFS) provides a valuable means to objectively evaluate apple stiffness as an indicator of produce texture (Chen et al., 1992; Galili and Baerdemacker, 1996; Tu et al., 2000). The AFS-AWETA^{TM} (Nootdorp, The Netherlands) has been used for measuring non-destructive acoustic impulse response. A single measurement using AFS provides sufficient information for grading apples (De Ketelaere et al., 2006). The AFS is a useful tool for monitoring apple firmness in storage (Landahl et al., 2003). The ability of AFS to measure fruit firmness and monitor changes in firmness makes it worthy of consideration as a new standard for fruit texture evaluation, replacing the older destructive standard (De Ketelaere et al., 2006). AFS measurement is indicative of cell wall mechanical strength and tension caused by the turgor pressure of the tissues (Hertog et al., 2004), and has significant correlations with both firmness measured by a

penetrometer (Johnson and Dover, 2005) and sensory measurements of apple (Finney et al., 1978; Landahl et al., 2003).

The AFS-AWETA^{TM} measures the frequency of the sound generated and the mass of the apple. S is determined by the formula: $S = f^2W^{2/3}$.(Hertog et al., 2004; Landahl et al., 2003), where f is the resonant frequency of the first elliptical mode (Hz), or the natural frequency of the first local maximum obtained after fast Fourier transformation of the raw spectra, or the first peak frequency (Tu et al., 1997); and W is the weight of the apple (kg). To determine the stiffness of an intact apple, the apple is put on a cushioning material so that the direction of the fruit equator is vertical. The AWETA has a small plastic plunger that moves upward to strike the fruit from beneath. This impact causes the apple to response with a sound wave. The sound is captured by a small microphone located close to the impactor. A computer connected to the AWETA calculates the initial resonance frequency of the time domain signal by means of a Fast Fourier Transform. Apples with higher stiffness give higher resonance frequencies (Nicolai et al., 2003).

The upward moving speed of the plunger is fixed for all measurements, so the plunger strikes the surface of all apple samples with the same force. The force can be calculated using Newton's Second Law of Motion, F = - m*a, where F is the force impacting on the surface of the apple (Newton, N); m is the mass of the plunger (kg); and a is the acceleration of the plunger ($m.sec^{-2}$). The external active force created by the kinetic energy of the plunger causes a deformation on the apple and exerts an internal restoring force of the apple. The internal restoring force builds up a potential energy in the apple. When the external force is removed, the potential energy of the apple transforms into kinetic energy. According to Newton's Third Law of Motion, the reaction

force (restoring force) caused by the kinetic energy of the apple has the same size with the opposite direction to the acting force generated by the plunger. The restoring force from the apple, in turn, creates a deformation of the air underneath the apple and generates another restoring force in the air space. The potential energy of the air space is then transformed into kinetic energy to help the surface layers of the apple to move back to its original position. The cycles of deformation and restoration of the apple and surrounding air space are repetitive. The surface layers of the apple swing through their original positions with a gradual reduction in the amplitude of swing due to loss of energy caused by friction between the molecules traveling within the apple flesh and within the air space. The swing of the apple surface causes a vibration of the apple. The vibration of the apple continues until the initial energy generated by the plunger is lost completely. The vibration creates a sound that is irradiated to and captured by the microphone of the AFS-AWETA. Because properties of the plunger and the surroundings are maintained the same at all measurements, the signal duration, amplitude and frequency of the sound wave captured by the microphone are dependent only on the properties of the apple, such as elasticity module E, density ρ and size of the apple.

2.4.2 Factors affecting change in stiffness of apples during storage

2.4.2.1 Pre-harvest conditions

Change in stiffness of apples during storage is also affected by harvest date and cultivars. Because elastic texture and cellular strength of apples are dependent on harvest date, apple stiffness is also influenced by harvest date (see 2.4.1). Besides, change in stiffness of apples is also dependent on cultivars because different apple cultivars differ

in cell structure (size and shape) (Kahn and Vincent, 1990; Korniak et al., 2002), density and rigidity (cell orientation, intercellular spaces and cellular adhesion) (Vincent, 1989). Late season cultivars have smaller cells and thicker cell walls, and tend to be stiffer and tougher than early season cultivars (Khan and Vincent, 1993).

2.4.2.2 Storage conditions

Storage conditions such as temperature, RH and velocity of air also affect change in stiffness of apples (Ozer et al., 2003). Reduction in apple stiffness may result from both reduction in cell wall strength caused by biochemical reactions, and water loss caused by wvpd (Hertog et al., 2004).

After harvest and during storage, because of conditions of the surrounding environment, moisture from inner layers of apples moves outward to its surface and evaporates to the air. This causes water loss for the apples, reduces the pressure exerting on the apple cell walls and results in a reduction in stiffness of the fruit. This reduction in stiffness was affected by the air velocity and the difference in water vapour pressure between the fruit and the surrounding air. The difference of water vapour pressure is, in turn, dependent on RH and temperature of the air. At the same storage temperature, higher RH creates a smaller difference in water vapour pressure between the fresh produce and the storage air, leading to a reduction in water loss and therefore, a reduction in stiffness loss. Apples stored at 94% RH suffer more shriveling after leaving storage compared to those stored at 97% RH. RH ranging from 90% to 95% is an indispensable minimum to maintain flesh stiffness of apples expected from consumers (Skrzynski and Konopacki, 2003). The optimal RH of storage for maintaining apple stiffness is about

95% (Skrzynski and Konopacki, 2003). In addition, the turgor pressure of apple cells, and hence stiffness, is also dependent on velocity of the air circulating within the storage. Higher air velocity leads to higher water loss and consequently a greater reduction in apple stiffness (Ertur et al., 2003).

On the other hand, biochemical reactions occurring within cell walls of the fruit weaken the strength of cells and rigidity of the fruit tissues leading to a reduction in stiffness. Besides, the rate of these biochemical reactions also increases with storage temperature. Therefore, higher temperature and lower RH cause a greater reduction in stiffness of the apples. In addition, CA storage can reduce further weight loss and stiffness losses compared to NA cold storage (Erkan et al., 2004; Ozer et al., 2003).

2.5 Ethylene and CO_2 production of apples during storage

2.5.1 Role of ethylene and relationship with CO_2

Ripening is a process involving a host of physical, metabolic and biochemical changes. It is initiated by ethylene, and involves loss of background green colour, softening of fruit tissue, and development of characteristic aroma and flavour (Wills et al., 1998). Products that produce ethylene are also sensitive to ethylene (Abeles et al., 1992). High concentrations of ethylene accelerate the ripening and decay of fresh produce (Brockmeulen, 2004). The rate of ethylene production in ripe climacteric fruit varies widely while that of non-climacteric fruit, by definition, remains low (Watkins, 2002). An increase in the CO_2 production of apples is closely linked to production of ethylene at almost any stage (except in the post-climacteric state) (Wills et al., 1998).

Ethylene and CO_2 production are influenced by many factors including cultivar, maturity and storage condition.

2.5.2 Factors affecting production of ethylene and CO_2

2.5.2.1 Pre-harvest conditions

Early maturing apple cultivars such as 'Royal Gala' and 'Cox's Orange Pippin' usually produce much higher levels of ethylene, ripen more quickly, and have a shorter storage life compared with later maturing cultivars such as 'Granny Smith' and 'Pacific Rose' (Hansen, 1945). The difference in ethylene physiology between the four apple cultivars is also reflected in their maximum IEC at different temperatures. For example, the IEC peaks of 'Granny Smith' and 'Pacific Rose' apples occur at 5-12^{o}C, while those for 'Royal Gala' and 'Cox's Orange Pippin' occur at 20-24^{o}C (Johnston et al., 2001c).

2.5.2.2 Storage conditions

After being harvested, horticultural produces still need O_2 for respiration and other chemical, biochemical reactions. Respiration in fresh produce is a process in which the more complex materials within the cells such as starch, sugars and organic acids are oxidised by O_2 and broken down into simpler molecules such as CO_2 and water (Wills et al., 1998). This process also generates vital heat. Thus, the respiration rate of horticultural produces is a function dependent on temperature, O_2 and CO_2 concentrations of the storage atmosphere.

There are many studies on the effects of storage atmosphere on ethylene production and respiration. CA, which has lower O_2 concentration and higher CO_2 concentration

compared with NA, has long been recognised as a tool to slow down respiration, as well as the chemical and biochemical reactions occurring in fresh produces. CO_2 production of apples in CA storage can decrease 2.5 to 3 folds compared with that in NA storage at 0^oC. Once O_2 concentration in air around fruit is reduced to less than 10 kPa, the respiration rate of apples decreases with further reduction in O_2 concentration (Kader, 1986).

On the other hand, CA storage conditions can also affect ethylene production of apples during and after storage. The high CO_2 and low O_2 concentrations influence the activity of enzymes involved in ethylene biosynthesis, particularly 1-aminocyclopropane-1-carboxylic acid oxidase (ACC-O), which requires both CO_2 and O_2 for activity (Poneleit and Dilley, 1993; Yip et al., 1988). In general, CO_2-enriched atmospheres have been reported to reduce ethylene biosynthesis in climacteric fruit including apple (Gorny and Kader, 1996), which helps to delay ripening and senescence. CA storage with a high CO_2 concentration or low O_2 concentration can severely suppress ethylene biosynthesis in climacteric fruit tissue, leading to inhibition or retardation of fruit ripening and senescence (Gorny and Kader, 1996). If O_2 levels are reduced below 8 kPa, ethylene biosynthesis is significantly inhibited in fresh fruit and vegetables (Kader, 1986). For example, CA storage with 2.5 kPa O_2 suppresses the IEC and 1-aminocyclopropane-1-carboxylic acid (ACC) accumulation of 'Golden Delicious' apples (Lau et al., 1984). Recovery in ethylene biosynthesis is often slow after removal from CA (Watkins, 2002). This residual effect of CA storage can be used to further maintain fruit quality after storage. Therefore, controlled or modified atmosphere storage is a further supplement to

refrigeration to maintain quality of the fresh produce and extend its storage life (Bramlage, 1982; Kader, 1986).

2.6 Background colour of apples and change during storage

2.6.1 Formation and change of apple background colour

Colour is a critical quality attribute for apples, especially during grading. Apples with better colour command better prices (Ritenour and Khemira, 2006). Apple contains many compounds, such as anthocyanins, chlorophyll, carotenoids and flavonols which blend together to produce colour. Among these compounds, anthocyanins are the most important compounds for red colouration in apple (Ritenour and Khemira, 2006). The anthocyanins are glycosides of anthocyanidins, which are part of a group of C_{15} compounds known as flavonoids. Analysis of anthocyanins in apple indicated that apple skin is composed of various glycosides of cyaniding, including cyaniding-3-galactoside, 3-glucoside, 3-arabinoside, 3-xyloside and 7-arabinoside (Timberlake, 1980).

Anthocyanins generally are located in the apple skin. Large peaks of anthocyanin accumulation are reported to characterise two development stages in apple, that is, the early development stage of intense cell division and the late development stage that encompasses fruit ripening. During the first stage, the biosynthesis of red pigments in apple depends on exposure to the sun. During the second stage, when anthocyanins can increase more than 5-fold in some apple cultivars (Ritenour and Khemira, 2006), anthocyanin synthesis requires low temperature. The accumulation of anthocyanins is closely associated with the phenylalanune ammonia-lyase (PAL) enzyme activity, which only occurs in red parts of the apple skin (Aoki et al., 1970). Therefore, PAL may be

involved in controlling the rate of anthocyanin synthesis in the apple skin overall (Faragher and Chalmers, 1977). PAL activity is closely related to the availability of sugars (Ritenour and Khemira, 2006).

2.6.2 Factors affecting change in background colour of apples

2.6.2.1 Pre-harvest conditions

Harvest maturity may also affect post-harvest colour development in apple. Apples harvested before the onset of colour change have a greater probability of inhibited ripening than fruit harvested after the onset of colour change (Watkins, 2002).

2.6.2.2 Storage conditions

Temperature

Although increase in temperature from 20^{o}C to 25^{o}C does not retard anthocyanin accumulation (Pan and Shu, 2007), the anthocyanin concentration of apples irradiated at 37^{o}C for 144 h decreases by over 50% (Marais et al., 2001a).

The effect of temperature on colour development in apples is also dependent on the cultivar, stage of fruit development (Ritenour and Khemira, 2006), climacteric status and other factors. The optimum temperature for maximum anthocyanin accumulation varies widely. The optimum constant temperature for maximum anthocyanin accumulation in mature fruit ranges from 16^{o}C to 24^{o}C (Creasy, 1968; Curry, 1997; Faragher, 1983; Proctor, 1974; Reay and Lancaster, 2001).

Although each apple cultivar has an optimum temperature for maximizing anthocyanin accumulation, fluctuating storage temperature can further improve the red

colouration of the fruit. A sequence of cool nights followed by warm days has been found to stimulate color formation in apple fruit (Reay, 1999). Fluctuating storage temperature also results in better colour and higher anthocyanin concentrations for 'Cripps Pink' apples during storage (Marais et al., 2001b). Low constant temperature of 6^{o}C improves colour formation in apples compared with a high constant temperature of 20^{o}C, while alternating temperatures of 20^{o}C and 6^{o}C has even better colour development than a low constant temperature of 6^{o}C (Marais et al., 2001a).

Gas composition

The effect of high CO_2 concentration atmospheres on anthocyanin biosynthesis in different fruits has been studied (Remo'n et al., 2004). Generally, high CO_2 concentration reduces (Holcroft and Kader, 1999) or inhibits (Gil et al., 1997; Holcroft et al., 1998) post-harvest anthocyanin biosynthesis in fruit. CA storage has a similar effect on anthocyanin synthesis and colour development in apple. Recent studies showed that CA storage delays loss of chlorophyll and yellowing of apple skin (Erkan et al., 2004). A study on 'Starkrimson' apples indicated that high CO_2 levels destabilize cyaniding derivatives (Lin et al., 1989). A decrease in O_2 level to 1 kPa can delay colour changes in apple (Bohling and Hansen, 1985). After nine months of storage, 'Granny Smith' apples from CA storage conditions (1 kPa CO_2 : 2 kPa O_2; 2 kPa CO_2 : 2 kPa O_2 and 3 kPa CO_2 : 2 kPa O_2) are greener than those from storage conditions of 0 kPa CO_2 : 21 kPa O_2 at 0^{o}C, RH 90-92% (Erkan et al., 2004). 'Pink Lady' apples from CA storage (1^{o}C and 92-93% RH, 2 kPa O_2 : 2 kPa CO_2) retain a greener background colour than those from NA storage (1^{o}C and 92-93% RH, 21 kPa O_2 : 0.03 kPa CO_2). One day after removal from 14

weeks of storage, 'Pink Lady' apples from ultra-low oxygen (ULO) storage (1^{o}C and 92-93% RH, 1 kPa O_2 : 1 kPa CO_2) retain higher pink surface colour values than those from NA storage. When the shelf-life period is extended to seven days, LO stored fruit exhibits superior maintenance of quality with greener colour on the shaded sides compared with those from NA storage. After a longer storage period of 25 weeks followed by 1 day at 20^{o}C, the apples from NA and ULO (1^{o}C and 92-93% RH, 1 kPa O_2 : 1kPa CO_2) had the highest degree of pink surface colouring (Lopez et al., 2007).

Ethylene

There are complex interactions between ethylene and quality attributes of horticultural produces (Watkins, 2002). Besides its roles in degreening and stimulation of ripening in apple fruit, ethylene also promotes red colour development. However, excessive application of ethylene to the fruit can adversely affect quality attributes. For example, it stimulates chlorophyll loss, excessive softening and acceleration of senescence (Saltveit, 1999).

2.6.2.3 Packing conditions

Packaging conditions also affect colour development in apple. The hue angle (*h*) of 'Starkrimson' apples stored unpackaged at 2^{o}C declines significantly after seven weeks of storage, while the *h* of the shrink-wrapped apples increases during the same period. This indicated that the unpackaged apples were more reddish or less yellowish than the shrink-wrapped apples due to lower intensity of yellowness (Lin et al., 1989).

2.7 Optimal conditions for apple storage

As mentioned previously, the principal factors of storage, which affect fresh produce quality, are storage temperature, gas ratio, RH and ethylene concentration. Generally, a combination of low storage temperature, low O_2 : CO_2 ratio, high RH and low ethylene concentration in storage best maintains the quality of the fresh fruit.

A decrease in O_2 level to 1 kPa can maintain apple firmness, delay colour change and reduce losses in acidity, vitamin C and flavour (Bohling and Hansen, 1985). A previous study revealed that a LO-CA storage can increase the accumulation of amino acids and reduce the production of fatty acids and aromatic compounds in apples (Choi and Kim, 1999). A combination of LO (1 kPa) with high CO_2 (3 kPa) in CA can significantly reduce loss in quality, while aroma production can be partially restored under NA (Brackmann and Saquet, 1995; Brackmann et al., 1993) after a period of CA storage. Moreover, CA storage with a LO level and a high CO_2 level can also solve problems with fruit quality such as core browning, scald or core flush. Core browning in apple can be eliminated in susceptible apple cultivars if they are preserved in CA storage (1.5 kPa O_2 : 1 kPa CO_2) (Nardin and Casera, 1988). ULO atmosphere storage is associated with reduced incidence of scald and core flush, delay in flesh softening and loss of green colour, and preserve of eating quality of apple (Chapon and Bony, 1991). ULO conditions are beneficial for 'Gala' apple quality in terms of high firmness and low acid loss after up to 210 days of storage (Ben, 2002).

However, an unsuitable gas composition can cause problems in fruit. A gas composition with insufficient O_2 and excessive CO_2 can lead to a higher level of flesh breakdown or physiological disorder in fruit (Bortoluzzi and Brackmann, 1999). The

mode of action by which elevated CO_2 and lowered O_2 concentrations inhibit ethylene production is complex, and may differ with the physiological age of the tissue (Gorny and Kader, 1996). Reduced O_2 atmospheres may inhibit ethylene biosynthesis by impeding the binding of ethlyene to the receptor responsible for triggering autocatalytic ethylene biosynthesis (Burg and Burg, 1967). When O_2 levels decrease below 8 kPa, ethylene biosynthesis in fresh fruit and vegetables is significantly inhibited (Kader, 1986). Specifically, 'Golden Delicious' apple fruit held in 2.5 kPa O_2 demonstrates to have a suppressed IEC and ACC accumulation (Lau et al., 1984). Most fruit produce ethanol when exposed to anaerobic (1-3 kPa O_2) (Kader, 1986) or hypoxic conditions (Dixon and Hewett, 2000). Under anaerobic conditions, conversion of ACC to ethylene can be entirely inhibited in apple fruit because O_2 is a substrate for the oxidation of ACC to ethylene by ACC-O (Yang, 1985). Elevated CO_2 also reduces ethylene production by inhibiting ACC synthase (ACS) and/or ACC-O activities (Bufler and Bangerth, 1981; Watkins, 2002).

According to a previous study, excessive CO_2 combined with inadequate O_2 during CA storage causes a breakdown in the respiratory metabolism of apples (Bachmann, 1983). A high concentration of CO_2 creates a high a level of succinic acid while an absence of O_2 leads to high levels of ethanol and acetaldehyde. After removal from extreme CA storage conditions into the NA, fruit react immediately with a rapid increase in CO_2 production leading to rapid deterioration of the fruit. Although ULO (0.7 kPa O_2) in CA storage can reduce scald incidence in apple, it also increases flesh breakdown and decay of the fruit (Bortoluzzi and Brackmann, 1999). Excessively low respiration due to inadequate O_2 can disturb ethylene production, which leads to a reduction in fatty acid

synthesis and low volatile production in the apple (Bangerth and Streif, 1998). The best CA conditions for 'Gala' apple are 1.2 kPa O_2 with 2 and 3 kPa CO_2 which give the highest titratable acidity and lowest incidence of rot and physiological disorders (Brackmann et al., 2001). The best CA storage conditions for 'Royal Gala' apples are reported to be an atmosphere of 1 kPa O_2 and 3 kPa CO_2 with ethylene absorption (Lima et al., 2002).

LE during CA storage can effectively maintain the quality and inhibit the ethylene production of apples (Liu and Samelson, 1986). Ethylene concentrations of $< 2\mu l.l^{-1}$ are necessary to retard softening and superficial scald on apples (Knee and Hatfield, 1981). This is confirmed by a report that after a storage period of eight months with removal of ethylene, 'Gala' apples maintained their flesh firmness, green peel colour and juiciness much better than the apples in conditions without removal of ethylene (Brackmann and Ceretta, 1999).

A good combination of all the aforementioned factors should be sought in order to achieve optimal fruit quality. A combination of CA and cold storage will give better apple colour, firmness and flavour than either method alone (Blank, 1982). In general, CA storage with 1.5 kPa O_2 and up to 3 kPa CO_2 at a storage temperature of 3^oC may be optimal for many apple cultivars (Dilley et al., 1989). This can delay ripening and minimise physiological disorders of the fruit. However, each type of fresh produce has an optimum combination. For examples, 'Gala' apple is reported to have much higher firmness, soluble solids and titratable acidity in CA storage than in NA cold storage. The optimum conditions in CA storage for 'Gala' apple are 1.1 kPa O_2 : 3.2 kPa CO_2 and storage temperature of 0-1^oC (Bender, 1988). The incidence of flesh breakdown after

eight months of CA storage decreases in 'Fuji' apple when the fruit is stored during the first month at higher temperature (2^{o}C) or lower RH (92%), and high temperature and low RH (Bortoluzzi-Maag and Brackmann, 2001). Another study showed that the optimum CA conditions for 'Cripps Pink' apples are 1.5-2 kPa O_2 and 1 kPa CO_2 at 0^{o}C (Watkins, 2003).

2.8 Prediction of changes of food quality during storage

2.8.1 Modelling and role of prediction of food quality

Modelling attempts to translate and convert theories or concepts of transforming mechanisms into mathematical equations that can be used to obtain quantitative analysis of the processes of interest. It has been utilised widely to quantitatively characterise a range of processes across different disciplines. For example, modelling has been used to obtain accurate predictions that can assist in decision-making to optimise management in different sectors of nature, society and business. In the field of crop production, the ability to accurately predict crop yield and quality helps farmers best manage their crop and cater to their target markets. In the sector of food storage, it is critical for exporters and distributors to accurately predict the shelf-life of their produces in order to determine the best market, set appropriate prices, and minimise the produce and financial losses.

2.8.2 Modelling approaches for prediction of food quality

2.8.2.1 Fundamental-deductive approach

Fundamental-deductive modelling is the conversion of theories and concepts into mathematical and computer formulations. The deductive approach can be used to build

mechanistic models for processes if the underlying physical principles of the processes to be modelled are well understood. Such modelling often begins with a hypothesis about the underlying processes. This hypothesis, based on a basic understanding of the processes, is translated into formulations that map the hypothesised mechanisms with model elements. The mathematical models are often formulated from experimental data sets or collected data sets, and are based on the fundamental theories as well as the laws of physics, chemistry, biochemistry and physiology that describe the food processes or post-harvest storage sectors of interest. Parameters in the models are often used to represent quantitative aspects of the hypothesised processes (e.g. reaction rates in food processes or post-harvest storage) (Tijskens et al., 2004a). The actual values of the model parameters are determined from theories or laboratory experiments. The structure and parameters of fundamental-deductive models can be used for the same matters in different situations. For instance, the structure and parameters of the model predicting changes in firmness or stiffness of 'Cripps Pink' apples grown in Australia could be used to predict changes in firmness or stiffness of other apple cultivars in different regions of the world.

In recent years, the deductive modelling approach has been applied widely in a range of fields including economics, industrial production, agricultural production, food processing, post-harvest storage of foods, fresh fruit and vegetables. For instance, in the field of agricultural production, based on input data such as certain conditions of natural environment (soil type, temperature, RH and light intensity), seed, irrigation pattern, nutrition supply (fertilizers) and harvesting time; the output including the yield and the quality of the crop can be predicted precisely by mathematical models. Another example

in the field of food storage, in order to predict the shelf-life and the keeping quality of food materials; causes and mechanisms of changes in food materials during storage have to be analyzed and estimated quantitatively based on fundamental theories during establishment of predicting models. Changes in food materials, which are mainly caused by chemical, biochemical reactions; or micro-organisms within the food materials or from the surroundings, lead to losses in both quality and quantity. For example, CO_2, water and heat will be generated by the reaction of starch within the food material with O_2 from the surrounding (respiration process). These could result in changes in the food properties such as temperature, water content, pH, concentrations of carbon and nitrogen sources; and in the surrounding properties such as temperature, O_2 and concentrations CO_2. Besides, under different conditions, these changes also have different rates. The parameters of kinetic models can be used to describe the rate of the reactions occurring within the food materials under certain conditions of temperature, water activity and pH. Moreover, kinetic models help us achieve a better understanding of the fundamental mechanisms of reactions occurring within foods.

Advantages and disadvantages of fundamental-deductive modeling

Fundamental-deductive models are aimed to realistically reflect the processes occurring within food materials, and are thus typically consistent and widely applicable. The major advantage of fundamental-deductive models compared to empirical-inductive models is that the structure and parameters of a given fundamental-deductive model can be applied to the same products in different contexts. Fundamental-deductive models can also be used to explore fundamental science matters as well as the relationships between

changes occurring in food. In addition, they can help modelers to easily recognise and eliminate nonsensical data from experiments by fitting the data to the models.

The fundamental-deductive method also has some disadvantages. Modelling becomes very complicated if the model includes many different parameters that must be determined by simultaneously solving a set of many equations. This will increase calculation time as well as reduce the universality and usefulness of the model. Conversely, over-simplifying the model to avoid complications during modelling due to numerical instabilities can reduce the realism of the model for practical applications. Besides, unavoidable limitations of current knowledge in certain fields associated with the processes being modeled can also reduce the applicability of the model.

2.8.2.2 Empirical-inductive approach

Limitations to our knowledge make it impossible to perfectly understand the real mechanisms of processes occurring within food materials. This causes great difficulty in translating and converting a process into mathematical formulations with parameters. In such situations, empirical-inductive modelling method becomes necessary and useful. Empirical-inductive modelling is an approach that uses as much collected data as possible for conversion into mathematical formulations, and disregards the theoretical knowledge or fundamental laws associated with matters or processes being modelled. The empirical-inductive approach has been applied widely in many sectors, particularly in the field of food storage (Tijskens et al., 2004a). It has also been applied widely in the prediction of changes the quality of fresh produce.

Advantages and disadvantages of the empirical-inductive approach

The main advantage of the empirical-inductive method is that models can be constructed quickly and give good predictions without fundamental knowledge associated with the processes. The main shortcoming of the method is that, because of its empirical nature, models cannot be applied outside the range of the experimental data. In addition, the models will not help to increase understanding or generate new knowledge of the underlying mechanisms being modelled (Tijskens et al., 2004a).

2.8.2.3 'Grey box' approach

'Grey box' modelling is a modelling method that combines the advantages from both fundamental-deductive and empirical-inductive modelling methods. It converts collected data or experimental data into mathematical formations, while it still takes into account relevant fundamental laws and rules and basic knowledge of the mechanisms of the principal processes being modelled. Owing to rapid development in all scientific fields, recent knowledge can help to better understand (though not with perfect accuracy) the phenomena and mechanisms of real processes occurring in food. Therefore, the 'Grey box' approach has become increasingly popular in a range of the sectors.

2.8.3 Prediction of changes in quality of fruit and vegetables

2.8.3.1 Keeping quality of fresh produce and apples

The acceptability of produce to a consumer is dependent not only on the produce behaviour but also the consumer's attitude. 'Keeping quality' is a concept which combines both these aspects. There are several recognised definitions of keeping quality:

'Keeping quality is an acceptance limit for intrinsic properties of the produce that are combined into a generally applicable and simplified index of quality' (Tijskens and Polderdijk, 1996); or 'Keeping quality is the minimal quality necessary for a consumer to accept the produce' (Tijskens and Polderdijk, 1994). Another generally accepted definition is that 'Keeping quality is the time until a commodity becomes unacceptable' (Beek et al., 1985; Fu and Labuza, 1993). Keeping quality of food materials is dependent on the initial quality and quality limits of the food materials (Brockmeulen, 2004). Depending on the type of produce, the quality attribute limiting produce acceptance can be predefined. It can be firmness for some types of fruit, for example tomato (Polderdijk et al., 1993), or colour for others, for example cucumber (Schouten et al., 1997). However, the limiting quality attribute may vary depending on circumstances.

For apple, the minimum acceptable quality for consumers is dependent not only on optimum quality, cultivar, season and storage atmosphere, but also the consumer demographic and testing panel (Konopacka et al., 2003). However, the consumer acceptability of apple fruit can generally be predicted by instrumental measurements of firmness, soluble solids content and titratable acidity (Hoehn et al., 2003). A study indicated that the optimum firmness values (MT method; lower and upper limits in brackets) for overall texture sensory preferences for 'Elstar', 'Jonagold' and 'Gloster' apples are 52 N (30-73 N), 52 N (31-75 N) and 54 N (46-62 N), respectively (Plocharski and Konopacka, 1999). A separate study suggested a more narrow range of optimal firmness, which corresponds with sensory preference (hardness, crispness and juiciness); that is, 50-60 N, 50-60 N and 50-70 N for 'Elstar', 'Jonagold' and 'Gloster' apples in NA cold storage, respectively (Konopacka et al., 2003). On the other hand, 55 N is an

unacceptable level of apple firmness according to industry standards (Watkins et al., 2000). Therefore, generally, the minimum acceptable level of apple firmness, which corresponds to the optimum and acceptable values of overall sensory preference, is in a range from 50 N to 60 N.

The keeping quality of fresh produce is also dependent on post-harvest conditions, particularly temperature, and can therefore be predicted by a dynamic model which is a function of temperature, initial quality and quality acceptance limits (Tijskens and Polderdijk, 1996). The effects of changes in post-harvest conditions on keeping quality can be predicted with simulation models that incorporate the models characterising dynamic quality change (Brockmeulen, 2004).

2.8.3.2 Prediction of post-harvest changes in quality of fruit and vegetables

As defined, the keeping quality of food materials in storage may be regarded as a quality point on a curve of quality reduction, for example, a curve of firmness or stiffness reduction (see 2.8.3.1). Therefore, in order to predict keeping quality, it is necessary to predict changes in quality of the food materials.

The prediction of post-harvest quality of agricultural produces is a new field. Research in this field began about 20 years ago (Tijskens, 2004). For vegetables, several papers have been published on the prediction of post-harvest quality including the prediction of the keeping quality of tomatoes (Polderdijk et al., 1993), modelling of tomato colour (Tijskens and Evelo, 1994), prediction of the keeping quality of vegetables during storage and distribution (Tijskens and Polderdijk, 1996), modelling of the

respiration rate of cauliflower (Ratti et al., 1996), shelf-life modelling for fresh cut-vegetables (Guerzoni et al., 1996), prediction of keeping quality for cucumber (Schouten et al., 1997; Schouten et al., 2002), modelling of tomato firmness (Schotte et al., 1999), modelling of the respiration rate of some fresh fruit and vegetables in MA packaging (Fonseca et al., 2002), prediction of post-harvest quality evolution of mushrooms (Lukasse and Polderdijk, 2003), modelling of the dependence of produce respiration on temperature and time of storage (Uchino et al., 2004), and modelling of quality deterioration in onion during drying and storage (Kaymak-Ertekin and Gedik, 2005). For fruit, published studies include modelling of peach firmness (Tijskens et al., 1998), prediction of the keeping quality of strawberry stored under MA conditions (Hertog et al., 1999), and empirical modelling of post-harvest changes in kiwifruit firmness (Benge et al., 2000).

2.8.3.3 Prediction of post-harvest changes in apple quality

There is some literature on the prediction of changes in post-harvest quality in apples under different storage conditions such as modified atmosphere (MA) or CA, modelling of apple respiration during storage, prediction of flesh browning of apples caused by CA storage (Volz et al., 1998), modelling of the effects of MA storage on apple firmness (Hertog et al., 2001), and modelling of the firmness of 'Elstar' apples during storage and transport (Tijskens et al., 2004b). However, there are not yet studies available on the prediction of changes in quality, especially firmness and stiffness, in 'Cripps Pink' apples stored under different conditions. 'Cripps Pink' apple is one of the most important apple cultivars in the world. Prediction of post-harvest changes of critical quality attributes can

help industry stakeholders to determine appropriate markets and prices for the apple, enhance competitiveness, and thereby benefit of exporters. Therefore, the determination of mathematical models for the prediction of changes in apple firmness, stiffness and keeping quality of the apple in a wide range of storage temperatures is necessary and valuable. It can help to have better understanding of the changes in firmness, stiffness and minimise losses in the texture and overall quality of the apple during storage.

2.9 General aims of the study

In comparison with NA storage, generally CA storage can better maintain fruit quality in terms of firmness, colour, soluble solid content, flavour and consequently, the shelf-life of apple (Brackmann et al., 2002b; Chapon and Bony, 1991; Meberg et al., 2000). There have been previous studies on effects of storage atmosphere on respiration and ethylene production of 'Cox's Orange Pippin' and 'Granny Smith' apples (Dadzie et al., 1996), effect of storage atmosphere on keeping quality of 'Idared' apple (Stow, 1995), effect of MA on the rate of firmness change in 'Braeburn' apple (Hertog et al., 2001). It appeared that flesh softness and core flush incidence in apples stored in LO atmosphere (1-1.5 kPa O_2 : 0-1.5 kPa CO_2) can be reduced in comparison with standard CA (3 kPa O_2 : 1.5–5 kPa CO_2) (Blanpied et al., 1987). Despite this, under the same storage conditions, apple cultivar can also affect change in the quality. For example, in the same cold storage conditions of 0±0.5^{o}C and 90-95% RH, loss in flesh firmness of 'Granny Smith' cultivars is less than that of 'Elstar' or 'Jonagold' cultivars (Ertur et al., 2003). Besides, each apple cultivar also has its own optimal storage temperature. For example, 'Gala' apple should be stored at 0^{o}C. At this temperature, the fruit has higher

titratable acidity after storage and higher pulp firmness, lower mealiness and lower internal breakdown after seven days shelf-life (Brackmann et al., 2001). There have also been many studies on the effect of gas composition of CA storage on the quality of different apple cultivars. In a range from 0 to 5 kPa of O_2 and CO_2 proportions in CA storage, both firmness and juice acidity of 'Fuji' apple can be maintained much more effectively in lower O_2 levels than in higher CO_2 levels (Argenta et al., 1994). It also showed that the 'Golden delicious' apple has highest quality and lowest physiological disorder at storage temperature of 0.5^{o}C with 3 kPa CO_2 and 0.75-1 kPa O_2 (Argenta and Brackmann, 1996). However, there has been little study on quality change of 'Cripps Pink' apple during storage. Therefore, we set out to understand the quality changes occurring in 'Cripps Pink' apple and relationships between these changes under different storage conditions.

Although from 0 to 3^{o}C is recommended as the optimum range of temperatures to slow down quality deterioration in most of apple cultivars, it is very difficult practically to maintain consistent optimal temperatures throughout the entire post-harvest handling chain. Apples can be exposed accidentally to sub-optimal conditions due to malfunctions of cool storage, containers or incorrect operations of the facilities. Therefore, determining changes in the quality attributes and the other physiological properties of 'Cripps Pink' apple in a wider range of temperature is very necessary. It can help to improve and develop the cooling systems as well as the supply chains in order to minimize quality loss of the fresh fruit.

Furthermore, effects of a combination between a CA or a low temperature NA in the first storage stage with a NA in the second storage stage on changes of the apple qualities should be studied.

The general aims of this research are to study changes in weight loss, firmness, stiffness, background colour, ethylene and CO_2 production of 'Cripps Pink' apples stored in single stage of NA at temperatures ranging from 0^oC to 30^oC or in combination between the first stage of CA (2 kPa O_2 : 1kPa CO_2, at 0^oC) with the second stage of NA at the temperatures; and to establish mathematical models using the 'Grey box' approach for characterising and predicting firmness, stiffness and keeping quality of the apple during storage.

CHAPTER III

CHANGE IN QUALITY OF 'CRIPPS PINK' APPLES UNDER DIFFERENT STORAGE CONDITIONS

3.1 Introduction

For climacteric fruit, there is an optimal harvest period to achieve the best eating quality for the consumer. However, because of commercial considerations, such as the time taken to reach market, the fruit are harvested earlier at the mature stage of development. This allows the fruit to be transported before softening process have begun as well as have a longer shelf-life. Thus, fruit often ripen after harvest or during storage. Ripening of apples involves many physiological and biochemical changes, such as increases in ethylene and CO_2 production, flesh softening associated with metabolic processes and weight loss, loss of chlorophyll, loss of acidity, conversion of starch to sugars, formation of cuticular waxes and synthesis of aromatic compounds (Hulme et al., 1969). Post-harvest ripening of apple is a complex process which may be influenced by many different factors such as cultivar, seasonal influences, as well as pre-harvest and post-harvest conditions. An understanding of the influence of storage conditions after harvest and how they affect the major quality attributes of the fruit is important information that can assist in applying appropriate technologies that prolong shelf-life and maintain quality. Although there were works addressed for the major apple cultivars, there is a gap in the knowledge related to 'Cripps Pink' apples.

The aim of this section of the thesis is to investigate changes in weight loss, firmness, stiffness, background colour, ethylene and CO_2 production of 'Cripps Pink' apples stored

in NA conditions at temperatures ranging from 0°C to 30°C with high air RH (≥ 90%), and in combination between the first storage stage of CA (2 kPa O_2 : 1 kPa CO_2, 0°C) with the second storage stage of NA at the same temperatures. In addition, we also investigate the relationships among these changes to better understand the biological properties of the fruit.

3.2 Materials and methods

3.2.1 Experimental design

'Cripps Pink' apples were harvested late in the evening at commercial maturity from a commercial orchard in Batlow region of New South Wales, Australia on 5 May, 2005. The apples, which have not any treatment before and after harvest, were transported to Sydney Post-harvest Laboratory at North Ryde in Sydney and temporarily stored overnight at 5°C. The next morning, 2,775 apples were selected for homogeneity in maturity, size, appearance and freedom from defects and divided randomly into three batches of 900 apples, plus three extra net bags of 25 apples each to be used for destructive firmness measurement at time zero.

The first batch of nine hundred apples (Lot I) was divided into 36 net bags of 25 apples. Six bags were randomly placed into each of six 65 Litres (L) plastic barrels, at different storage temperatures of 0°C, 2.5°C, 5°C, 10°C, 20°C and 30°C under NA. The apples from one bag from each barrel were numbered and three circles marked on the non-red region of the skin for periodic background colour measurement during the storage. During the storage period, and depending on the storage temperature, changes in firmness, stiffness, weight loss and background colour were measured and recorded at the time intervals shown in Table 3.1.

Respiration rate (CO_2 emission over time) and ethylene production of the apples from the six temperature treatments were measured at the same time interval of two weeks during storage time. The first measurement was taken at the beginning of the storage (day 1) when all apples from the six temperature treatments were equilibrated. One net bag of 25 apples was used for measuring firmness (MT method) of the apples at 20°C on day 0.

Table 3.1 Storage time and time intervals for measurements of firmness, stiffness, weight loss and background colour of the apple stored at different storage temperatures

	Storage temperature (°C)					
	0	2.5	5	10	20	30
Time interval for measurements (day)	30	25	20	15	10	5
Length of storage time (day)	180	150	120	90	60	30

The second batch of nine hundred apples (Lot II) was divided into six barrels as for Lot I, however, the treatment consisted of two different consecutive storage stages. The first stage consisted of the six barrels being held for 61 days at 0°C, supplied with NA at a flow rate of 18–24 $L.hr^{-1}$. In the second stage, the six barrels were placed into six different storage temperatures of NA as described for Lot I.

The third batch of nine hundred apples (Lot III) was divided into six barrels as for Lot II, and again treatment consisted of two different consecutive storage stages. The first stage consisted of 102 days in CA conditions (2 kPa O_2 : 1 kPa CO_2) at 0°C. In the second stage, the six barrels of apples were placed in six different storage temperatures of NA as for Lots I and II. For Lots II and III, net bags of 25 apples each were set aside for measuring firmness (MT method) of the apples at 20°C on day 0 of the second storage stage.

At the beginning of the second storage stage of Lots II and III, stiffness, weight loss and background colour of 25 numbered apples from each barrel were measured twice, once after the apples were removed from the first storage phase and reached the standard temperature of 20^{o}C and again 24 hours after putting in the second storage stage and reached the different temperatures. During the experiment, stiffness, weight loss and background colour of the apples were measured on the same 25 apples from each barrel for each temperature treatment, while CO_2 and ethylene production of the apples were measured for all remaining apples in the barrels for each temperature treatment. The time intervals for measurements of firmness, stiffness, weight loss and background colour of the apples at each temperature treatment were the same for all the three lots of apples stored in NA conditions. The measurements of ethylene and CO_2 production of the apples stored at all temperature treatments in the second storage phase of Lot II and II were similar to those of Lot I.

In order to minimise moisture loss, which may influence stiffness of the apples, all barrels of apples from Lot I and from the second storage phases of Lot II and III were closed, sealed with vaseline and supplied with NA after passing through distilled water barrels, at a flow rate of about 18-24 L.hr^{-1}.

3.2.2 Wvpd control

Change in stiffness of apples may result from the change in strength of the cell wall caused by biochemical reactions and/or water loss from the apple due to wvpd (Hertog et al., 2004). Therefore, to study change in stiffness of the apples stored at different temperatures caused by the change in cell wall strength independently of water loss of the apples, the water loss rate of the apples at all temperatures needs to be controlled and

equalised. This was achieved by maintaining a similar wvpd, for all six temperature treatments, together with controlling the air flow velocity. The rate of water evaporating from surface of the apples to the air surroundings is calculated using the formula:

$m' = k*A*(p_s - p_a) = k'*A*(\hat{H}_s - \hat{H}_a)$ (Lewis, 1987)

Where: m' is the mass (mostly water) transfer rate from surface of the apples to the surroundings, $kg.s^{-1}$;

k and k' are the mass transfer coefficients, $s.m^{-1}$ and $kg.m^{-2}.s^{-1}$;

$\hat{H}_s$ is the absolute humidity of the air at the apple surface, $kg.kg^{-1}$;

$\hat{H}_a$ is the absolute humidity of the air flowing around the apples, $kg.kg^{-1}$;

p_s is water vapour pressure at the point on the surface of the apples, $N.m^{-2}$;

p_a is water vapour pressure of the air flowing around the apples, $N.m^{-2}$.

The same wvpd for each temperature treatment was obtained by varying RH of the air flows at different storage temperatures. By means of the psychrometric chart, the RH of the air flows at storage temperatures of 30^oC, 20^oC, 10^oC, 5^oC, 2.5^oC and 0^oC were maintained consistently at 98%, 95.5%, 93.5%, 92%, 91% and 90% respectively. $\hat{H}_s - \hat{H}_a$ and $p_s - p_a$ at all temperature treatments were also determined to be 0.4 (g $water.kg^{-1}$ dry air) and 0.4575 mm of Mercury (approx. 61 $N.m^{-2}$), respectively. At high RH of air, $\hat{H}_a$ approximates to $\hat{H}_s$ and p_a is close to p_s. The dry bulb temperature of the air flow also closes to the temperature on the surface of the apples and approximates to the wet-bulb temperature. Under this state, the surface of the apples were covered with a free moisture film which restricts moving out of the moisture from the apple centre, leading to a very low rate of the apple water loss (m').

RH of air provided to the barrels was regulated by the total opening area of the regular at the end of plastic pipes in the water barrels and the passage by which the air get through the water. The dry-bulb temperature and RH of the air at each storage temperature, which were used to control wvpd, ($p_s - p_a$), were detected and recorded at a 10-minute time intervals during the experiment using two Dual channel temperature/RH Tinytag Extras^{TM} (TGX-3580, Gemini Data Loggers Limited, UK). These instruments operate within the ranges of - 30^{o}C to + 80^{o}C and 0% to 100% RH.

3.2.3 Measurement methods

3.2.3.1 Firmness measurement

The HortPlus Quick Measure Penetrometer based on the MT method was used to determine the firmness (N.cm^{-2}) of the fruit. Before carrying out the destructive puncture test, apple samples were removed from storage and left to reach to the standard temperature of 20^{o}C. A thin layer of apple skin at the measurement position was removed. A stainless round probe of 11mm was used to penetrate into the fruit flesh to a depth of 8 mm at a speed of 240mm.min^{-1}. Maximum force known as firmness value was recorded. Firmness of each apple was the average value of firmness values obtained at two opposite positions on the equator of the fruit. The firmness value of apples at each temperature treatment was the mean of the values obtained from 25 apples in the treatment.

3.2.3.2 Stiffness measurement

A commercial AFS-AWETA was used to non-destructively measure stiffness of apples. To determine stiffness of an intact apple, the apple was placed on cushioning

material to ensure the equator of the fruit was vertical. A small plastic plunger attached to the AWETA moved upward from beneath to strike the fruit, creating a sound wave. The sound was captured by a small microphone located close to the impact point and used to calculate the resonance frequency of the signal. Stiffness index (S) is calculated by the formula: $S = f^2W^{2/3}$ (unit: $Hz^2.kg^{2/3}$) (see 2.4.1). The apples having higher stiffness give higher resonance frequency (Nicolai et al., 2003).

In order to assure accuracy in measurements, the instrument was calibrated at every time of measurements after the instrument was kept in the storage and reached to the storage temperatures (or the standard temperature of 20^{o}C), before conducting stiffness measurements of the apples. Stiffness index of each apple was the average value of three measurements at equal distances on the equator of the apple. Stiffness index of the apples from each temperature treatment was the mean of the stiffness indexes obtained from 25 apples representing for the treatment.

3.2.3.3 Weight loss measurement

The weight of the same 25 apples from the allocated net bag for each barrel was recorded at each time interval during storage using an electronic scale (Mettler TOLEDO, Model PR-5003). Weight loss of each apple was calculated using the following formula:

$$w = \frac{(W_i - W_t)}{W_i}.100$$

The remaining weight, Re of the apple was computed using the formula:

$$Re = 100\% - w$$

Where:

w is the weight loss of the apple, %;

Re is the remaining weight of the apple, %;

W_i is the weight of the apple at the beginning of storage, g;

W_t is the weight of the apple at the storage time - t, g.

Weight loss of the apples from each temperature treatment at each time interval was the mean of weight loss values from 25 apples for the treatment.

3.2.3.4 Ethylene and CO_2 measurements

Gas sampling for each measurement was carried out twice, once at the beginning and once at the end of the sampling time. During the sampling time, the rotten apples were taken out of the barrels; the barrels were closed, stopped with supplied air and entirely sealed with Vaseline. At each time, three replicate syringes of 1ml and another three of 0.5 ml for CO_2 and ethylene, respectively, were taken from the headspace of the treatment barrels.

Ethylene production of the apples remaining in the closed barrel for each temperature treatment was measured using the gas chromatograph (PHOTOVAC 10S50, USA). The delivery pressure and flow rate of the carrier gas (O_2) of the PHOTOVAC were preset and maintained at 206.85 kPa and 0.2ml.sec^{-1}, respectively. The detection limit of the instrument was 0.5 milliliters. Ethylene production (µl.kg^{-1}.hr^{-1}) was calculated based on the ethylene concentration of the standard sample, the difference in ethylene concentrations between the end and the beginning of the sampling time, the time period between the beginning and the end of sampling time, and the weight of the apples in the

closed barrel. Ethylene production of the apples from each temperature treatment at each time interval was the average value of the three replicates.

A GOW-MAC gas chromatograph (Series 580, GOW-MAC Instrument Ltd. Co., Japan) was used to measure CO_2 production of the apples in the barrels. The instrument was set with a constant flow rate of the carrier gas (Helium) at 45 $ml.min^{-1}$, a column temperature of 75^oC, the detector temperature of 120^oC, the injector temperature 50 ^oC., current of the GM of 175mA. The calculation for CO_2 production ($ml.kg^{-1}.hr^{-1}$) of the apples for each temperature treatment was based on the CO_2 concentration of the standard sample, the difference in CO_2 concentrations between the end and the beginning of the sampling time, the sampling time and the weight of the apples in the closed barrel. CO_2 production of the apples from each temperature treatment at each time interval was the mean of the three replicates.

3.2.3.5 Background colour measurement

The background colour of the apple was measured using a colorimeter (Minolta CR-400, Minolta Co., Ltd, Japan). The background colour values at the three individually marked positions on the shoulder of 25 apples for each temperature treatment were measured and recorded individually during the experimental period. Background colour value of each apple was the mean of the background colour values at the same three positions on the same apple, and background colour value of the apples at each temperature treatment was the mean of the background colour values of the same 25 apples for the treatment. Only background colour values in green areas of the apples at the beginning of storage were used when analysing change in background colour of the apples at each treatment during storage.

3.2.4 Statistical analyses

All analysis was performed with the statistical package GraphPad Prism 4.03, 2005 (GraphPad Software, Inc, San Diego California, USA). One-way and two-way ANOVA were used to determine effects of each individual factor as well as combined effects between storage temperature and storage time on changes in firmness, stiffness, background colour, CO_2 and ethylene production of 'Cripps Pink' apple during storage. Mean comparisons were also performed to examine if differences in the quality attribute value between time intervals within the same treatments, and between the temperature treatments were significant at level 5% of confidence interval.

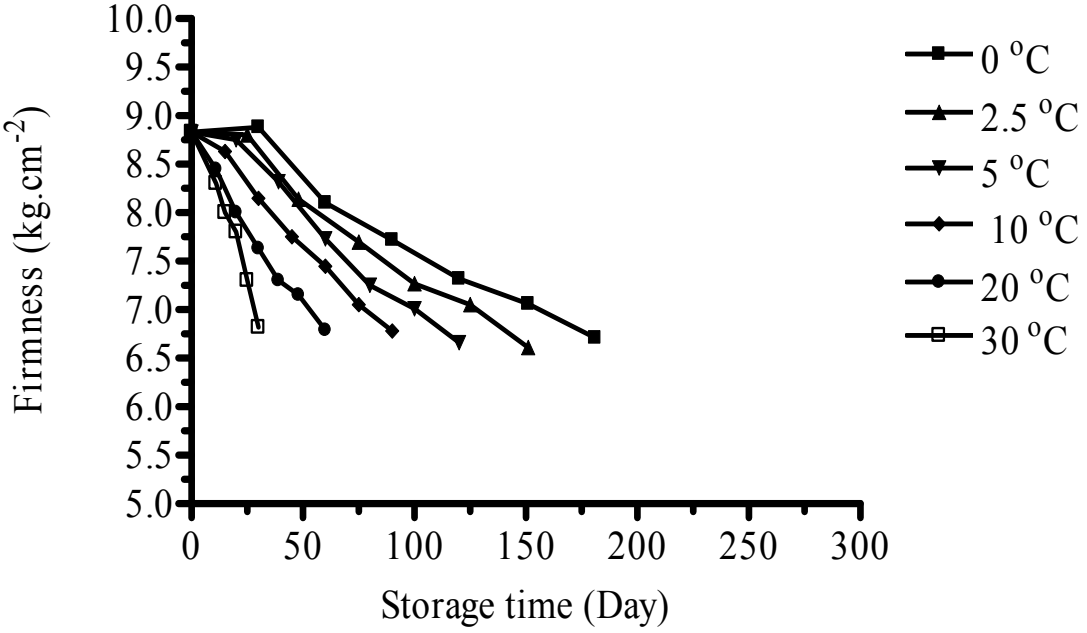
3.3 Results

3.3.1 Change in firmness of 'Cripps Pink' apples

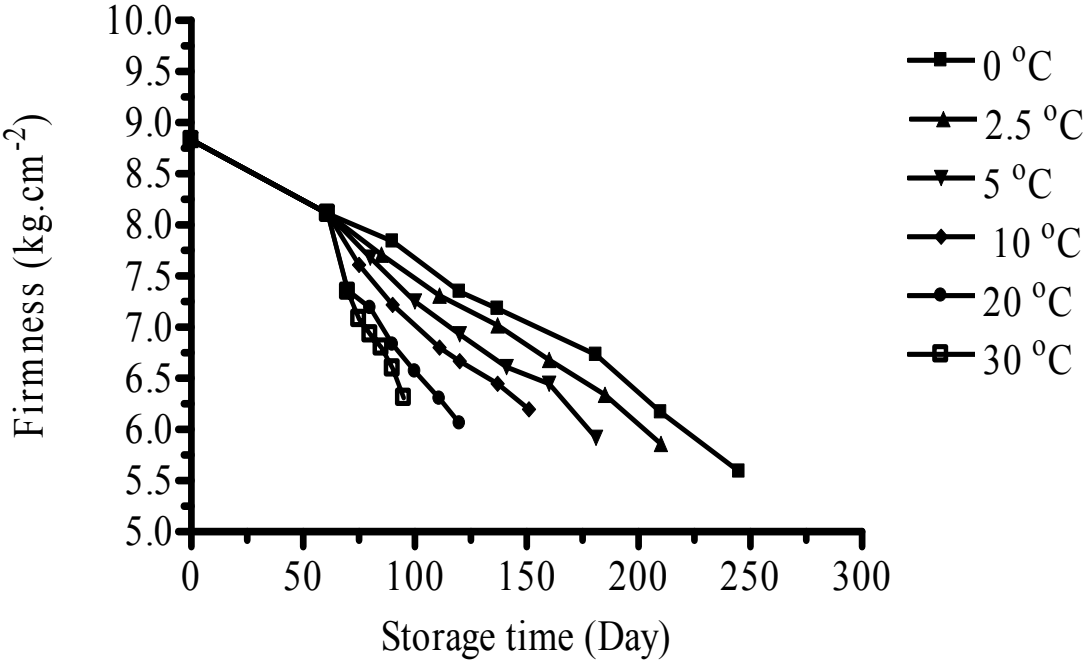
Firmness of the apples declined over time for all treatments, however the rate of change was significantly influenced by the temperature at which the apples were stored (Figs. 3.1a, b and c). The decline in firmness occurred at a greater rate at higher temperatures, whereas at lower temperatures the rate of softening was slower.

The change in firmness of the apples stored in the NA storage (Fig. 3.1a) showed that there were two softening patterns. For apples stored at the lower temperatures of 0^oC, 2.5^oC and 5^oC, the softening followed a typical tri–phasic pattern of initial low softening rate, followed by a rapid softening rate and a final low softening rate. The initial phase of low softening rate occurred within the first 30, 25 and 20 days for the apples stored at 0^oC, 2.5^oC and 5^oC, respectively. For treatments at 5^oC and 2.5^oC, firmness of apples stored in NA was reduced by $2.17 kg.cm^{-2}$ and $1.73 kg.cm^{-2}$ within 120 days, respectively.

The results also showed that within the tri-phasic softening rate, the higher the storage temperature, the higher the softening rate occurring in the second phase.



a



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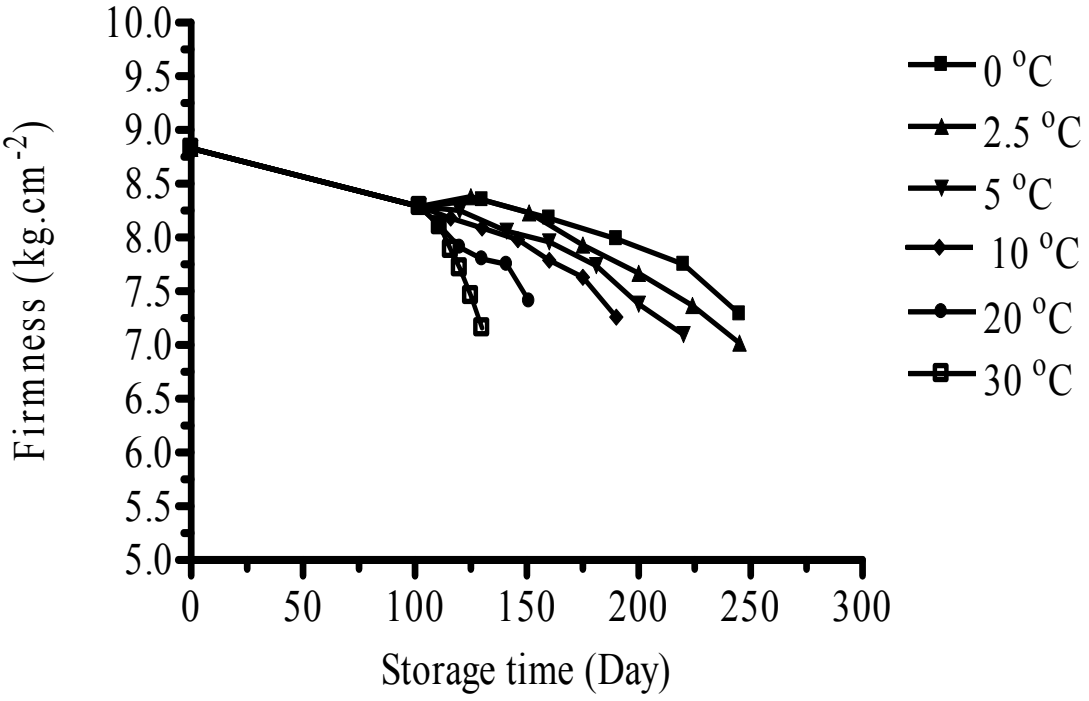


c

Figure 3.1 Change in mean firmness of 'Cripps Pink' apples stored under the following conditions: **a)** Lot I: Single stage storage in NA at six temperatures ranging from 0°C to 30°C, **b)** Lot II: Two-stage storage, firstly in NA at 0°C for 61 days followed by NA at six temperatures ranging from 0°C to 30°C. **c)** Lot III: Two-stage storage, firstly in CA (2 kPa O_2 : 1 kPaCO_2) at 0°C for 102 days followed by NA at six temperatures ranging from 0°C to 30°C. (n = 25).

In contrast to the apples stored at lower temperatures, there was no initial phase of slow softening for apples stored at higher temperatures of 10°C, 20°C and 30°C (Fig. 3.1a). The rate of softening increased with increasing temperature.

Apples stored at 0°C for the first stage of 61 days and subsequently moved to different temperature treatments under NA, firmness of the apples reduced in the same rate with that of the apples stored at 0°C from Lot I during the first 61 days. Therefore,

the apples from two-stage storage of Lot II considerably extended their storage life compared with apples from single stage storage of Lot I (Fig. 3b).

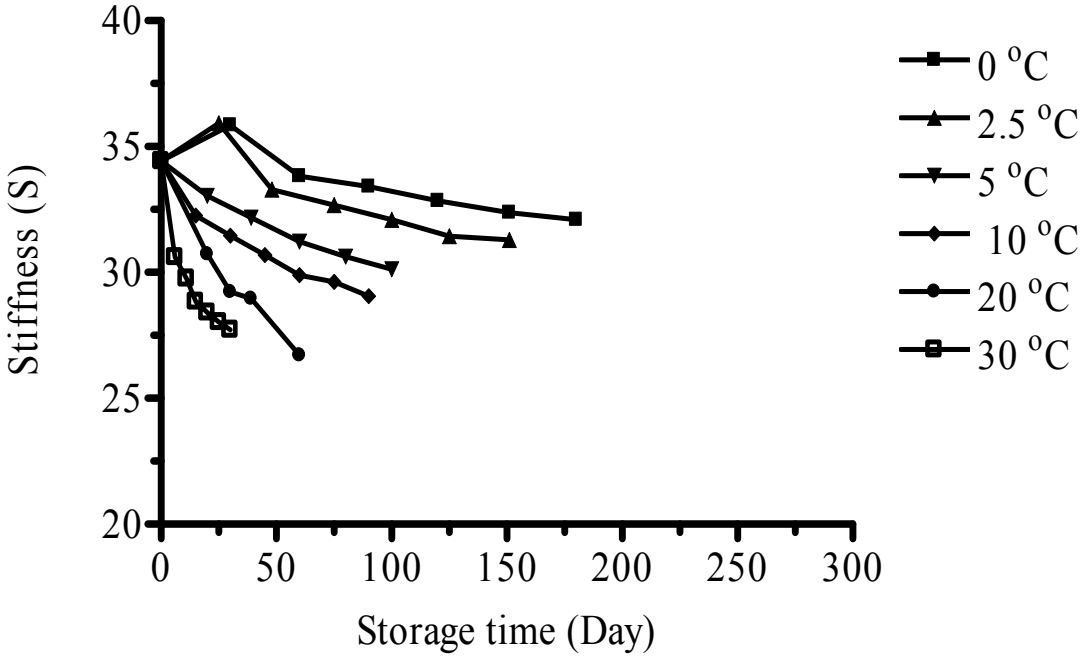
The application of CA slowed the softening rate of the apple compared with NA (Fig. 3c). Under CA firmness of the apples within 102 days was reduced by only 0.54 $kg.cm^{-2}$ compared with 1.23 $kg.cm^{-2}$ in the same period at 0^oC in NA storage (Figs. 3.1a & c). The CA storage treatment doubled the time taken to soften by 0.54 $kg.cm^{-2}$ compared with NA storage (102 days compared with 51 days) at the same storage temperature of 0^oC (Figure 3.1a & c).

The first stage storage of NA and CA reduced softening rate and extended the storage life of 'Cripps Pink' apples. However, in spite of the same storage temperature of 0^oC, because of low O_2 and high CO_2 concentrations in CA, softening rate of the apples in the latter storage reduced 4.149 times compared with that of the apples in the former storage, 0.005294 $kg.cm^{-2}.day^{-1}$ compared with 0.021967 $kg.cm^{-2}.day^{-1}$.

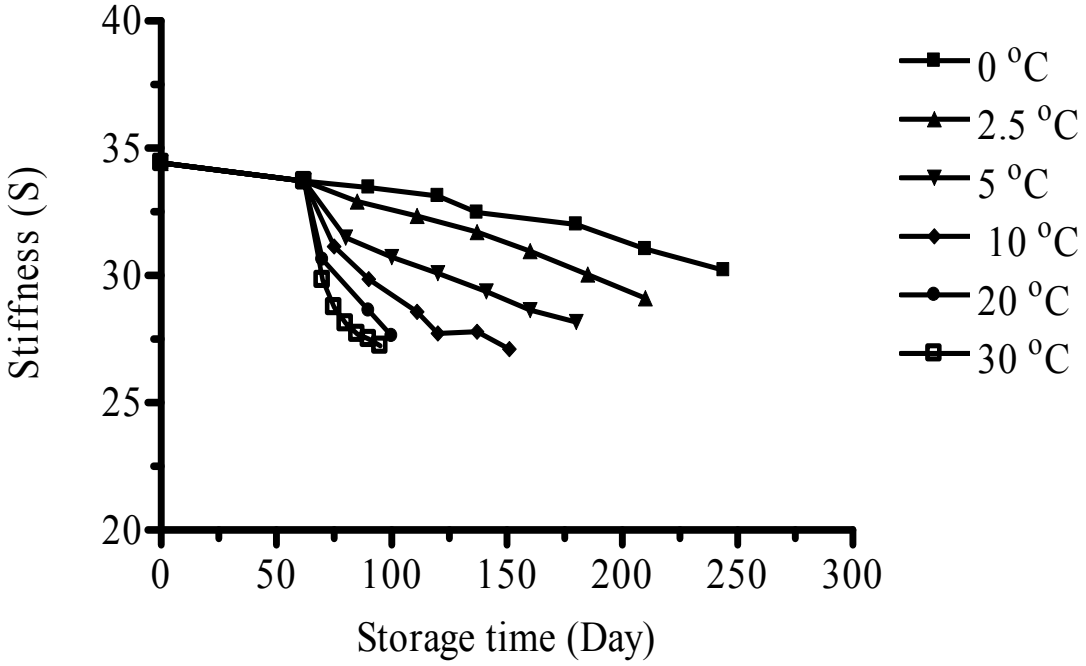
It was likely that the apples had also a tri-phasic pattern at 0^oC, 2.5^oC and 5^oC after being stored in CA for 102 days (Fig. 3.1c). However, the apples had only the two final phases after being stored at 0^oC in NA for 61 days (Fig. 3.1b).

3.3.2 Change in stiffness

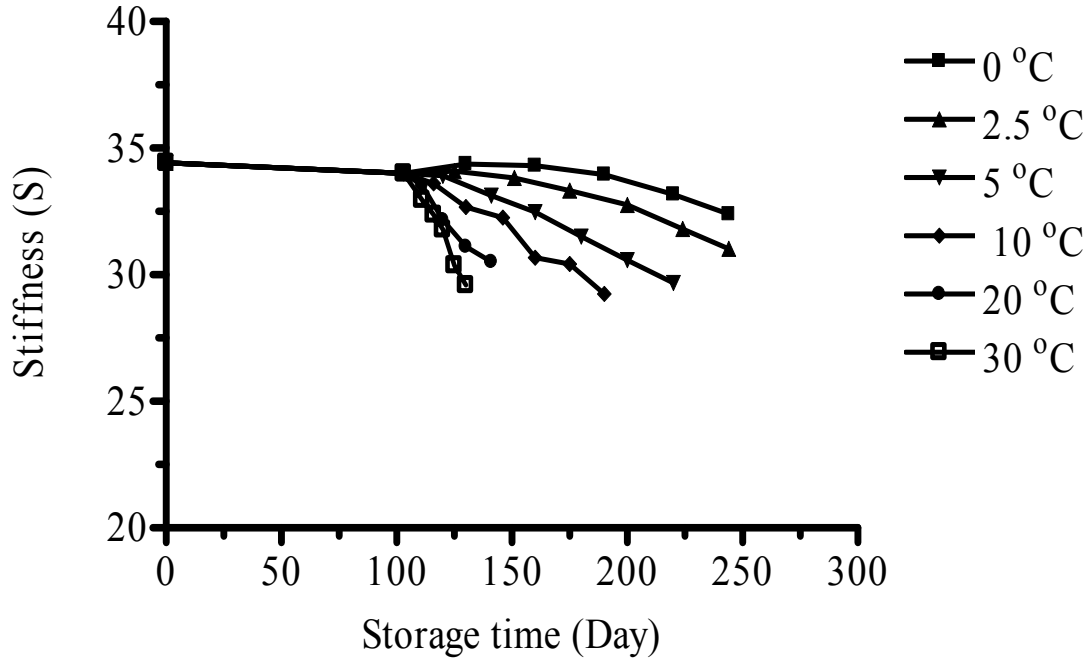
The change in stiffness for the single phase NA temperature treatments was similar to the changes observed for firmness (Figs. 3.2a & 3.1a), showing that stiffness of the apples stored reduced with time and increasing temperature. There were significant differences ($P < 0.05$) for change in stiffness between all storage temperature treatments (Fig. 3.2a) however, there was a strong interaction between storage temperature and


40
35
30
25
20
Stiffness (S)
0
50
100
150
200
250
300
Storage time (Day)
0 °C
2.5 °C
5 °C
10 °C
20 °C
30 °C

a


40
35
30
25
20
Stiffness (S)
0
50
100
150
200
250
300
Storage time (Day)
0 °C
2.5 °C
5 °C
10 °C
20 °C
30 °C

b



c

Figure 3.2 Change in mean stiffness of 'Cripps Pink' apples stored under the following conditions: **a)** Lot I: Single stage storage in NA at six temperatures ranging from 0°C to 30°C, **b)** Lot II: Two-stage storage, firstly in NA at 0°C for 61 days followed by NA at six temperatures ranging from 0°C to 30°C. **c)** Lot III: Two-stage storage, firstly in CA (2 kPa O_2 : 1 kPa CO_2) at 0°C for 102 days followed by NA at six temperatures ranging from 0°C to 30°C. (n = 25).

storage time. While storage temperature had the greatest affect on apple stiffness overall, there was no significant difference ($P > 0.05$) between corresponding stiffness values of apples stored at 0°C and 2.5°C during the first 80 days of storage. After that period however, significant differences ($P < 0.05$) in stiffness values became apparent. At lower storage temperatures higher stiffness values were maintained for longer periods of time. For example, at 0°C under NA storage, stiffness of 'Cripps Pink' apple was maintained almost unchanged during the first 60 days. Even at 5°C, stiffness of the apples decreased only slightly compared with those at higher storage temperatures, in the initial stage of storage (Fig. 3.2a).

Similar to firmness, stiffness had a typical tri-phasic pattern for apples stored at 0°C and 2.5°C in NA. However, it had only two final phases for apples placed at higher temperatures (Fig. 3.2a). Result showed that NA cold storage (0°C) and CA slowed reduction in stiffness of the apple leading to an extension of storage life. However, CA storage maintained stiffness of the apples better than NA storage (Figs. 3.2b & c).

3.3.3 Weight loss

Results of two-way analysis showed that there were no significant differences in weight loss of the apples between all six storage temperatures (Fig. 3.3). Under steady conditions of storage temperature (T) and RH, the rate of apple weight loss was constant, however, weight loss increased linearly with storage time (Weight loss = 0.008* Storage time, R^2 = 0.9878).

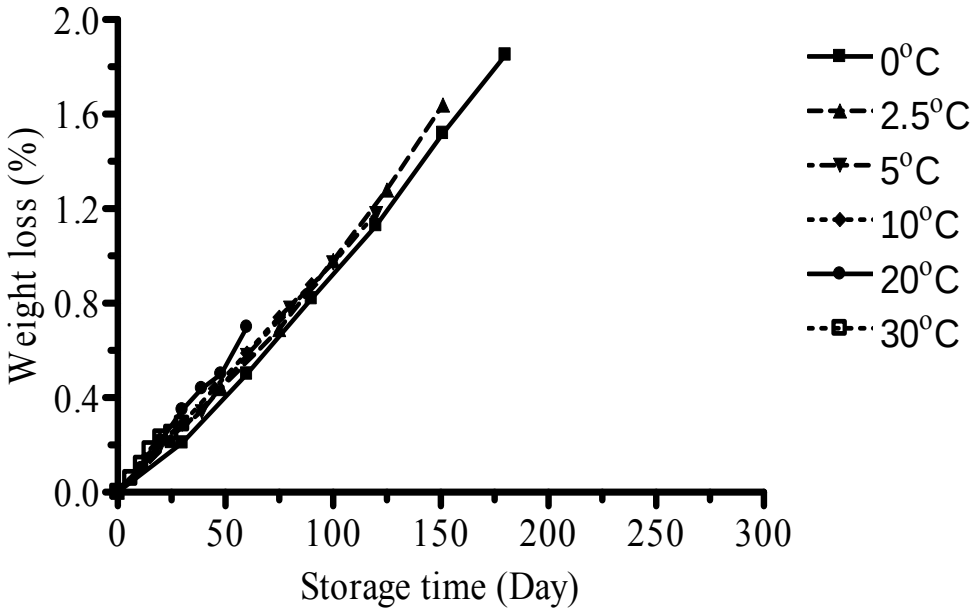


Figure 3.3 Mean weight loss of 'Cripps Pink' apples under NA storage at six temperatures ranging from 0°C to 30°C. (n = 25)

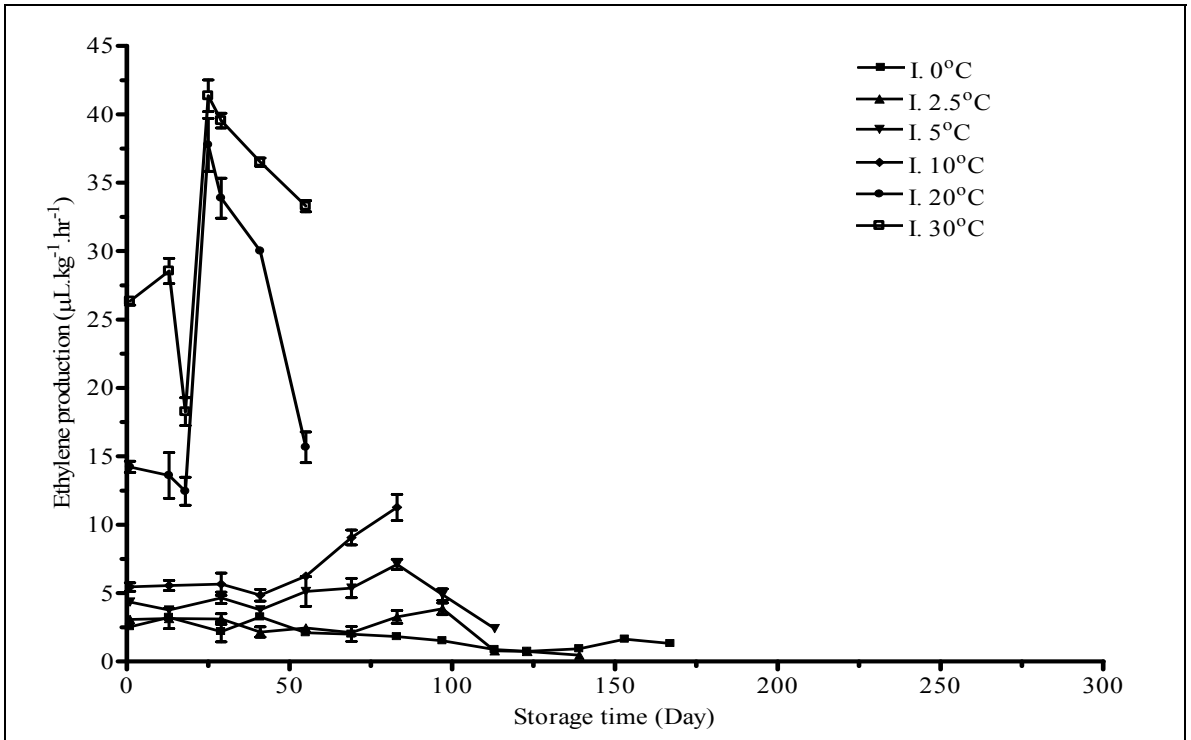
3.3.4 Ethylene and CO_2 production of 'Cripps Pink' apples

3.3.4.1 Ethylene and CO_2 production in NA storage

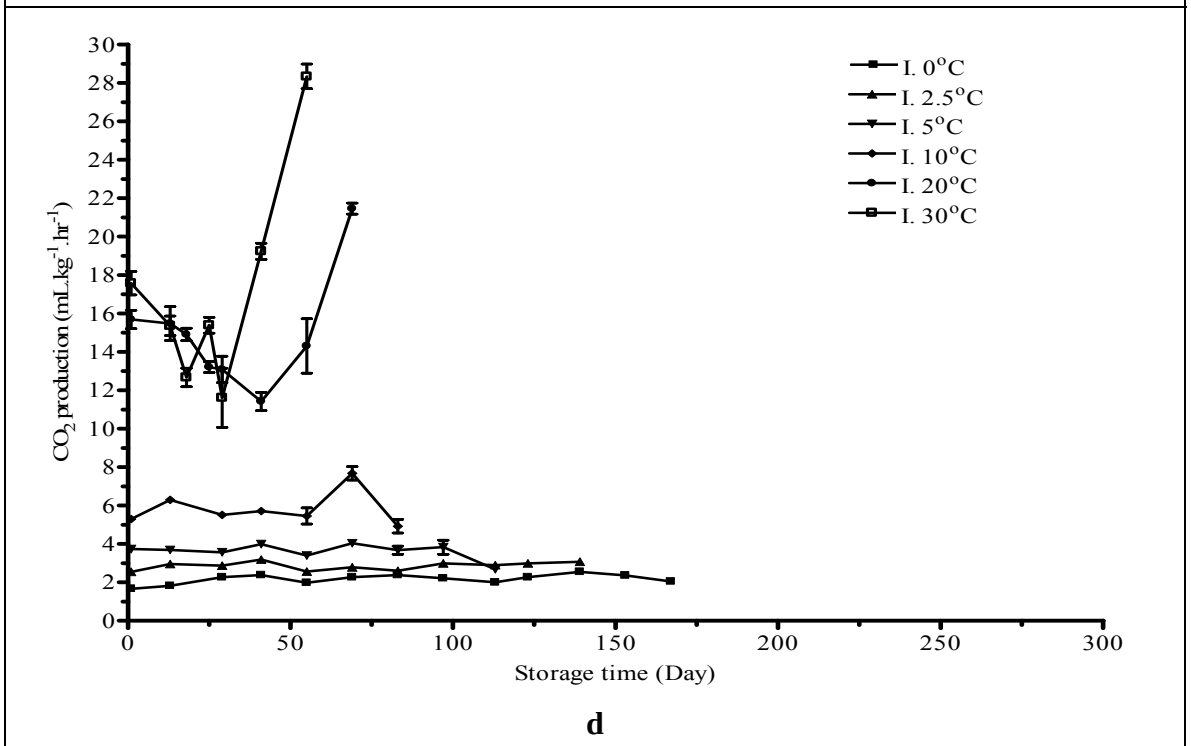
The analysis of results in Figure 3.4a indicated that ethylene production of the apples stored in NA at the lower temperatures of 0^oC, 2.5^oC, 5^oC and 10^oC did not change significantly (Appendix 3.15) in the first 55 days of storage. However, from day 69 to day 97, differences in ethylene production became significant ($P < 0.05$) between these lower storage temperatures of 0^oC, 2.5^oC and 5^oC.

At storage temperatures of 20^oC and 30^oC, however, there were significant increases in ethylene production for apples stored in NA after only 13 days of storage. In addition, there were significant differences (Appendix 3.15) in ethylene production between apples stored in NA at temperatures of 10^oC, 20^oC and 30^oC in the first 55 days of storage. There was an interaction between temperature and storage time influencing level of ethylene production of the apple.

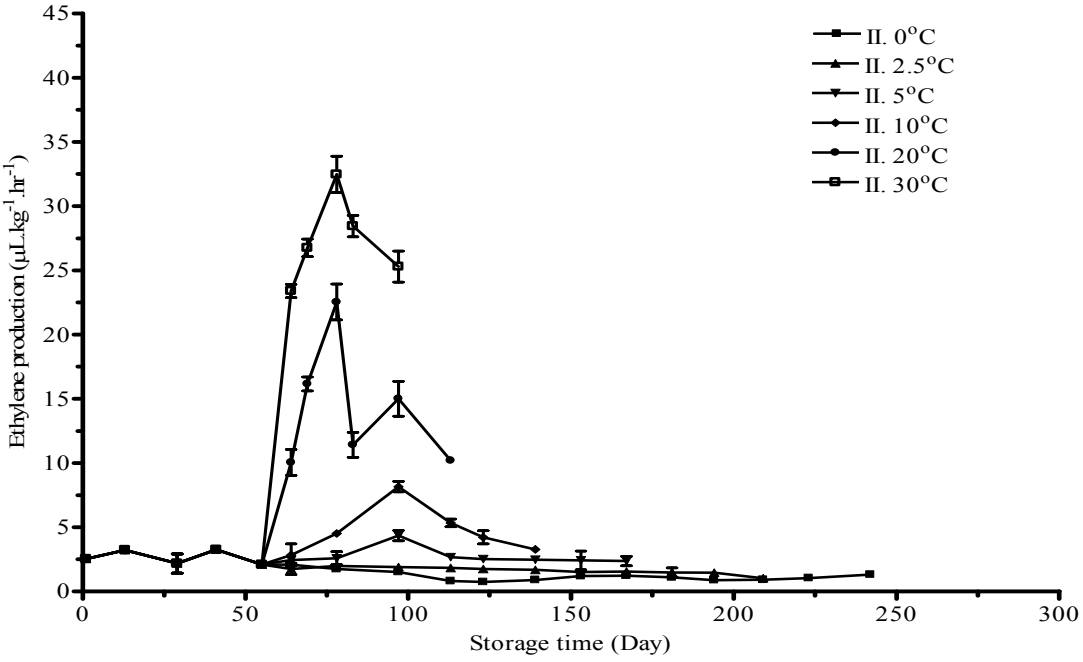
For CO_2 production (Fig. 3.4d), the results of the two-way ANOVA analysis (Appendix 3.16) indicated that there was no significant difference between the low storage temperatures treatments of 0^oC, 2.5^oC, 5^oC and 10^oC during the first 55 days of NA storage. The rate of CO_2 production, however, for temperatures treatments at 20^oC and 30^oC was twice to three times the rate at the lower temperatures. Furthermore, at 20^oC and 30^oC, CO_2 production began to increase significantly on the 41^{st} and 29^{th} day, respectively (Fig. 3.4d).


I. 0°C
I. 2.5°C
I. 5°C
I. 10°C
I. 20°C
I. 30°C
Ethylene production (µL.kg-1.hr-1)
Storage time (Day)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200
250
300

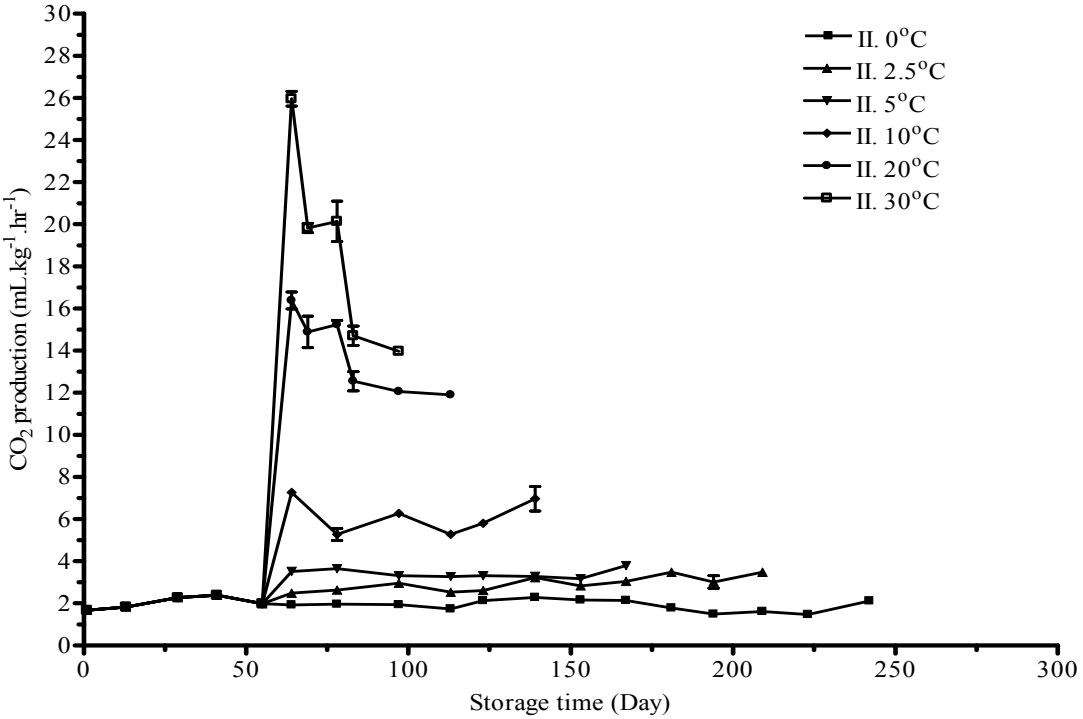
a


I. 0°C
I. 2.5°C
I. 5°C
I. 10°C
I. 20°C
I. 30°C
CO2 production (mL.kg-1.hr-1)
Storage time (Day)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
0
50
100
150
200
250
300

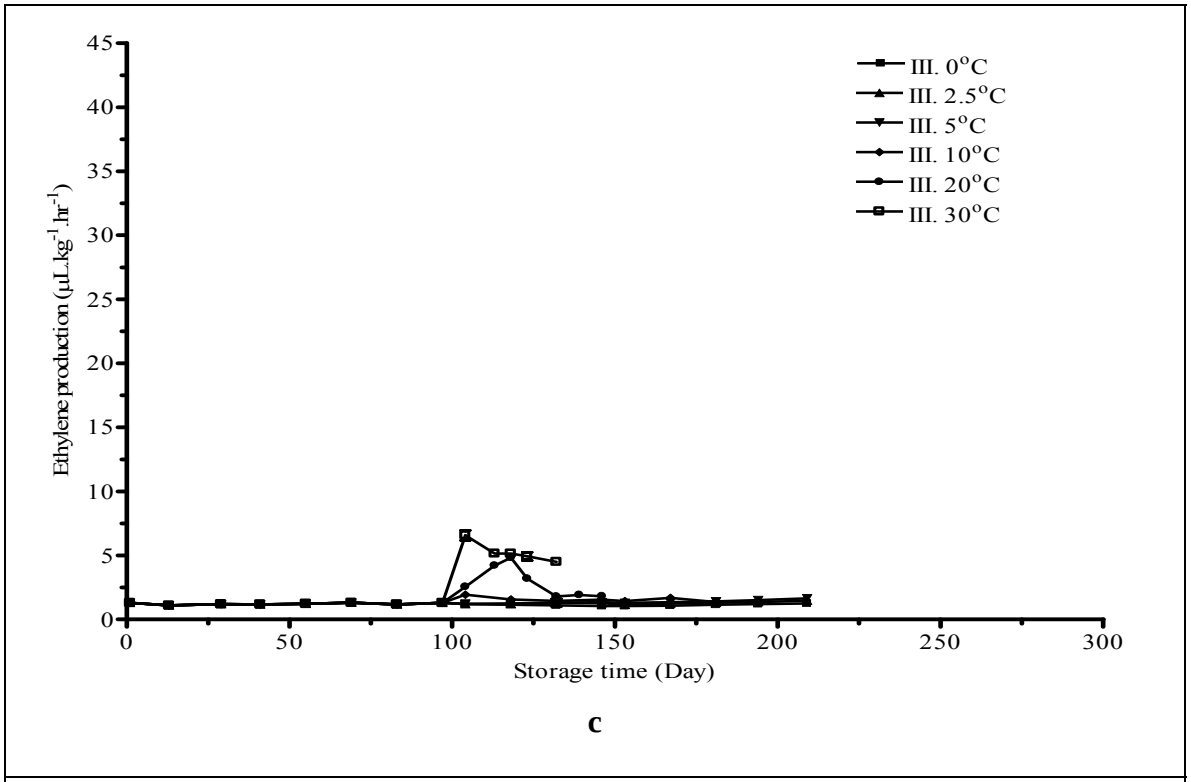
d


Ethylene production (µL.kg-1.hr-1)
Storage time (Day)
II. 0°C
II. 2.5°C
II. 5°C
II. 10°C
II. 20°C
II. 30°C

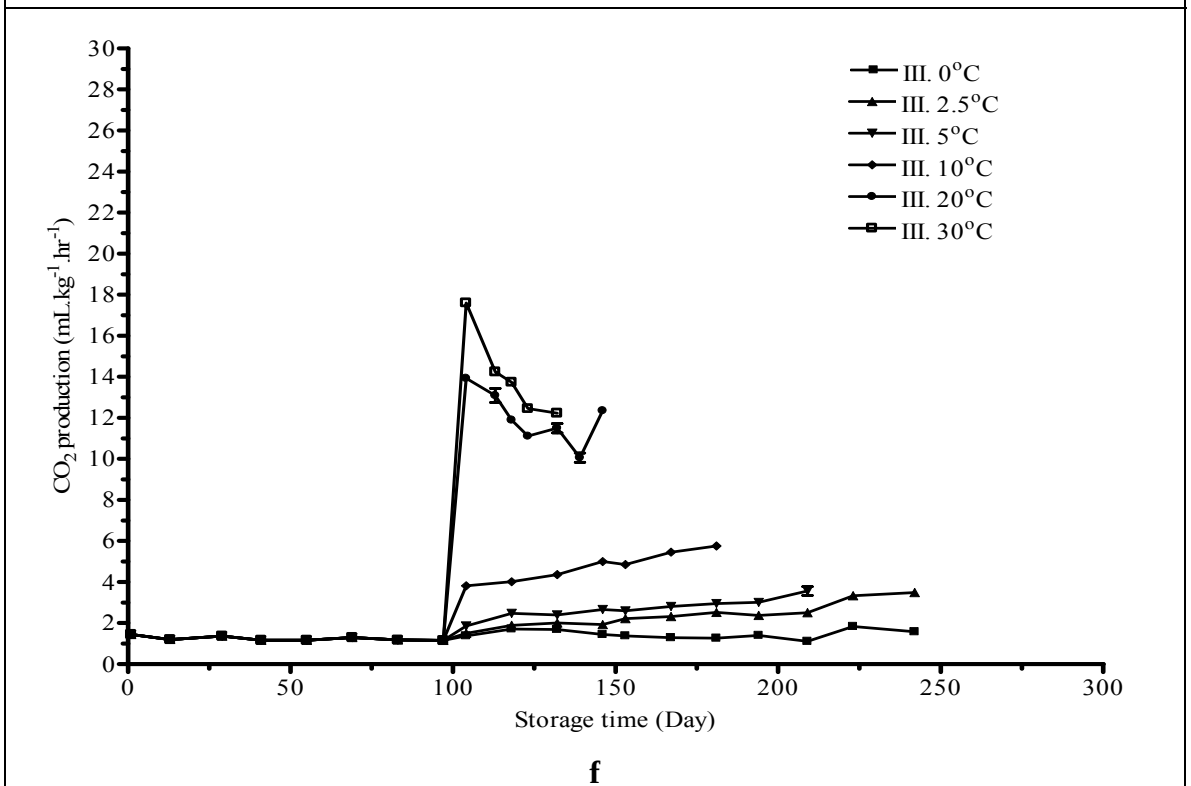
b


CO2 production (mL.kg-1.hr-1)
Storage time (Day)
II. 0°C
II. 2.5°C
II. 5°C
II. 10°C
II. 20°C
II. 30°C

e


Ethylene production (µL.kg-1.hr-1)
Storage time (Day)
III. 0°C
III. 2.5°C
III. 5°C
III. 10°C
III. 20°C
III. 30°C

c


CO2 production (mL.kg-1.hr-1)
Storage time (Day)
III. 0°C
III. 2.5°C
III. 5°C
III. 10°C
III. 20°C
III. 30°C

f

Figure 3.4 Change in ethylene and CO_2 productions of 'Cripps Pink' apples stored under the following conditions**: a) and d)** Lot I: Single stage storage in NA at six temperatures ranging from 0^oC to 30^oC, **b) and e)** Lot II: Two-stage storage, firstly in NA at 0^oC for 61 days followed by NA at six temperatures ranging from 0^oC to 30^oC, **c) and f)** Lot III: Two-stage storage, firstly in CA (2 kPa O_2 : 1 kPa CO_2) at 0^oC for 102 days followed by NA at six temperatures ranging from 0^oC to 30^oC. Symbols represent mean of measured data ± SE (n = 3).

For apples stored at 0^oC and 2.5^oC, climacteric ethylene and CO_2 production was almost entirely depressed (Figs. 3.4a & d). However, from 5^oC to 30^oC, the onset of climacteric ethylene and CO_2 production occurred earlier and at higher maximum production levels. The maximum ethylene and CO_2 production by apple stored at 10^oC, 20^oC and 30^oC was 11.26 $\mu l.kg^{-1}.h^{-1}$, 37.78 $\mu l.kg^{-1}.h^{-1}$, 39.55 $\mu l.kg^{-1}.h^{-1}$ and 7.68 $ml.kg^{-1}.h^{-1}$, 14.31 $ml.kg^{-1}.h^{-1}$ and 19.24 $ml.kg^{-1}.h^{-1}$, respectively. A sharp increase in ethylene production, reflecting the onset of the climacteric period of the apples stored at 20^oC and 30^oC, occurred after 18 days of storage. Similarly, sharp increases in CO_2 production for apples stored at 20^oC and 30^oC occurred at day 41 and day 29, respectively (Fig. 3.4d) while the onset of the climacteric period only began after 55 days of storage for apples stored at 10^oC (Fig. 3.4a).

3.3.4.2 Effect of NA cold storage

During the first phase of storage for 61 days at 0^oC under NA, production of ethylene and CO_2 of the apples was very low (Figs. 3.4b & e). During the second phase where apples were placed in different temperature treatments, ethylene and CO_2 production were significantly different. At 0^oC and 2.5^oC production of these gases continued to be depressed in the second stage of NA until the end of the experiment. However, for apples transferred to 5^oC and above, ethylene and CO_2 production increased immediately after

transfer to the higher temperatures. The higher storage temperatures caused the apples to produce higher ethylene and CO_2 production as well as a higher maximum production of the two gases. Therefore, temperatures ranging from 0^oC to 2.5^oC of NA storage could depress ethylene and CO_2 production of 'Cripps Pink' apples.

3.3.4.3 Effect of CA storage

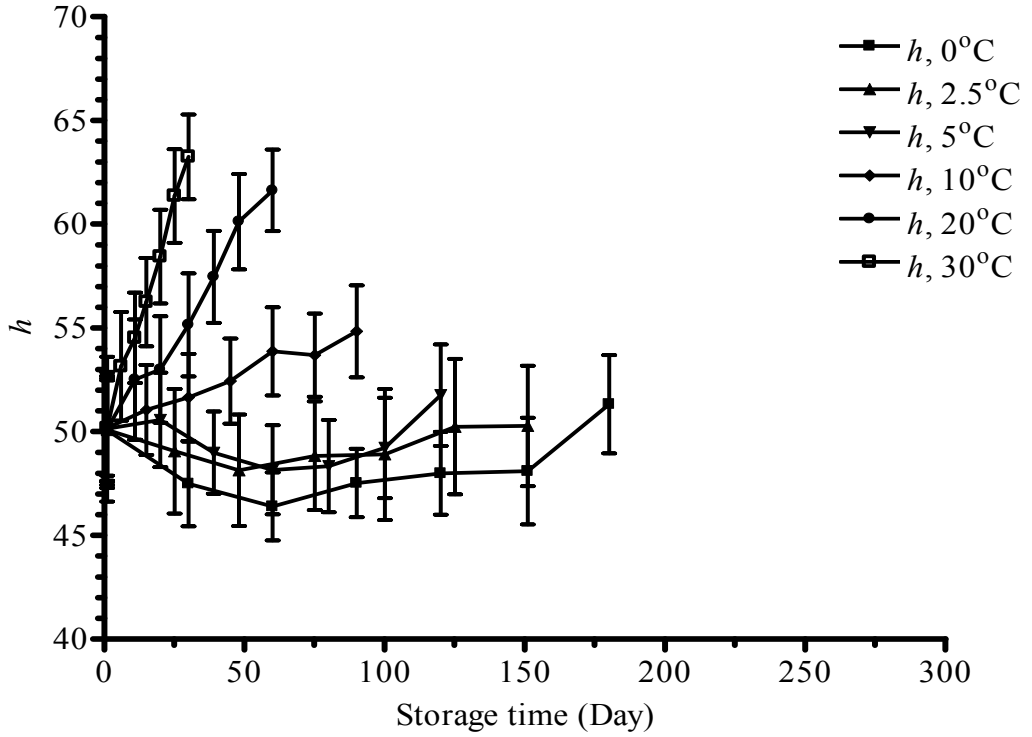
Under CA conditions for the first 55 days of storage, ethylene and CO_2 production of the apples were suppressed and did not change significantly over that period (Figs. 3c & f and Appendix 3.15 & 3.16). In spite of the same storage temperature of 0^oC, ethylene and CO_2 production of the apples stored in CA storage was significantly lower than those of the apples stored in NA (Figs 3.4b, c & e, f and Appendix 3.15 & 3.16). The average production of ethylene and CO_2 of the apples stored in CA storage was about half of that of the apples stored at 0^oC in NA (1.25 vs 2.35 µl ethylene.kg^{-1}.hr^{-1} and 0.93 vs 1.80 ml CO_2.kg^{-1}.hr^{-1}, respectively) (Figs 3.4b, c & e, f).

After 102 days in CA, the barrels of apples were moved to the second storage phase of NA. Ethylene and CO_2 production of the apples continued to be depressed at 0^oC and 2.5^oC until the end of the experiment (Figs 3.4c & f). In contrast, at temperature treatments of 10^oC and higher, the apples produced a climacteric peak of ethylene and CO_2 between days 102 and 110. However, these peaks were much lower than those of the apples in the single stage storage of NA.

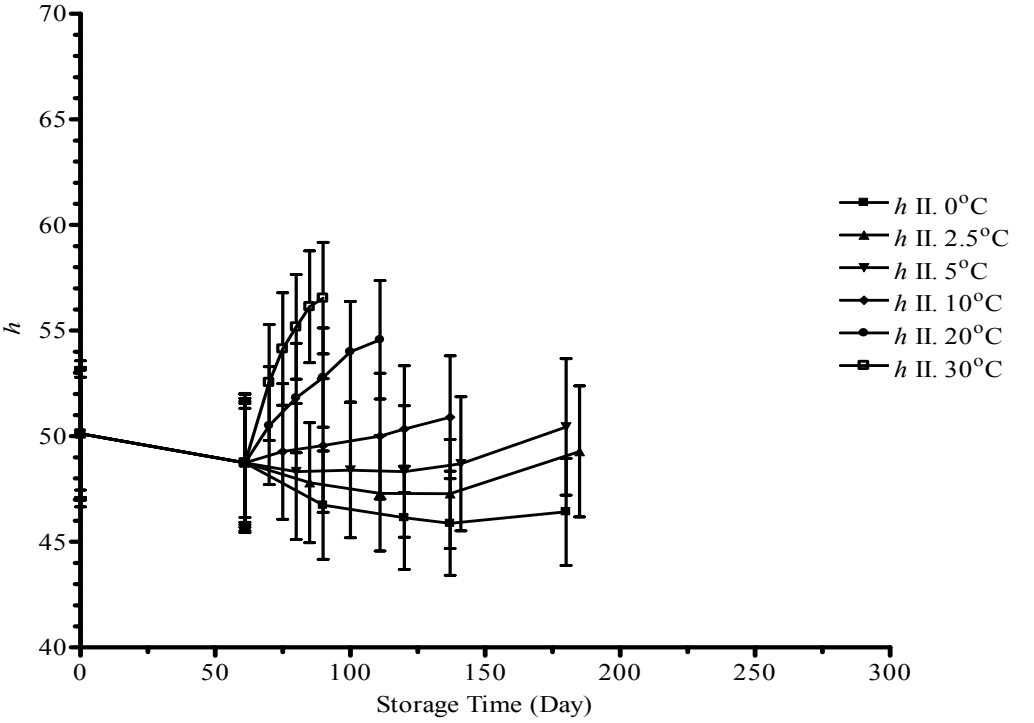
3.3.5 Change in background colour during storage

3.3.5.1 Change in background colour in NA storage

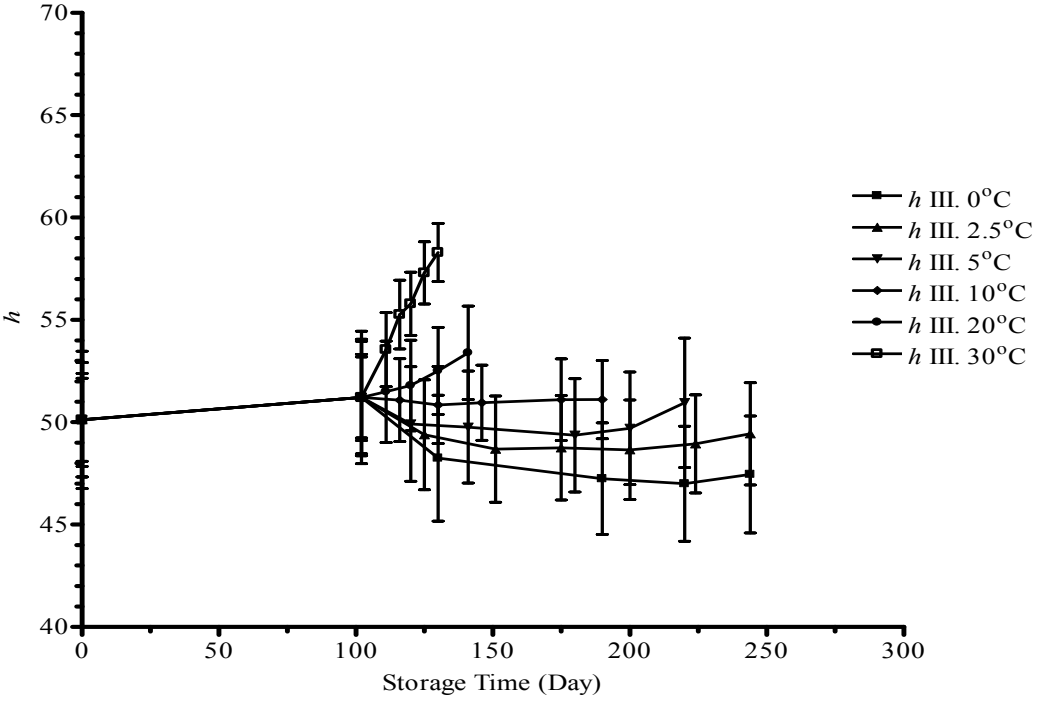
Although there were no significant differences ($P > 0.05$) in change of hue angle (h) of skin background colour between the apples stored at storage temperatures of 0°C, 2.5°C and 5°C, the h of the apples at these temperatures changed with time during the first 120 days in NA storage (Fig. 3.5a and Appendix 3.20). Also, for the treatments between 10°C and 30°C, there were no significant differences ($P > 0.05$) in the h of the apples between treatments during the first 15 days of storage. However, there were highly significant differences ($P < 0.001$) in h from day 25 onwards. Furthermore, the change in h of the apples within these temperature treatments over time was also highly significant ($P < 0.001$) in this period. Higher storage temperatures caused faster changes in h of the skin background colour.



a


70
65
60
55
50
45
40
h
0
50
100
150
200
250
300
Storage Time (Day)
h II. 0°C
h II. 2.5°C
h II. 5°C
h II. 10°C
h II. 20°C
h II. 30°C

b


70
65
60
55
50
45
40
h
0
50
100
150
200
250
300
Storage Time (Day)
h III. 0°C
h III. 2.5°C
h III. 5°C
h III. 10°C
h III. 20°C
h III. 30°C

c

Figure 3.5 Changes in *h* of 'Cripps Pink' apples stored under the following conditions: **a)** Lot I: Single stage storage in NA at six temperatures ranging from 0°C to 30°C, **b)** Lot II: Two-stage storage, firstly in NA at 0°C for 61 days followed by NA at six temperatures ranging from 0°C to 30°C, **c)** Lot III: Two-stage storage, firstly in CA (2 kPa O_2 : 1 kPa CO_2) at 0°C for 102 days followed by NA at six temperatures ranging from 0°C to 30°C. Symbols represent average values ±SE (n = 25).

For temperature treatments of 5°C and below, the skin background colour of the apples initially changed from green to red, followed by maintenance of the red colour in the second stage and finally turned into yellow. In contrast, for the apples stored at 10°C, 20°C and 30°C, *h* increased instantly at the beginning and continued throughout the storage period. These temperatures caused skin background colour of the apples to change dramatically from green to yellow. The rate of change in *h* increased with increasing storage temperature. Lower storage temperatures of 0°C, 2.5°C and 5°C maintained the initial *h* of the apple skin for longer, indicating that these temperatures retarded ripening in the apples.

3.3.5.2 Effect of NA cold storage

Although there was no significant decrease in *h* or lightness (L*) of 'Cripps Pink' apple during 61 days in NA storage at 0°C, the chroma (C*) increased significantly during this period (Fig. 3.5b and Appendices 3.21c & d). After the apples were placed in the second phase of storage at different temperature treatments in NA, the changes in *h* of the apples at the treatments were similar to those of the apples stored at temperatures in the single stage NA storage. However, there was a significant reduction in the maximum *h* values, particularly at the higher temperatures of 10°C, 20°C and 30°C (Figs. 3.5 a & b).

3.3.5.3 Effect of CA storage

There were no significant changes in *h*, C* and L* of the apple skin during 102 days of CA storage (Fig 3.5c and Appendices 3.21e & f) (P = 0.8153, 0.4384 and 0.8840, respectively). Again, after the fruit were removed from the first phase of CA storage and placed at six different temperatures in NA, the patterns of change in colour attributes were similar to those of the apples stored at the same temperatures in the single stage NA storage (Fig. 3.5a and Appendices 3.21a & b). However, the maximum *h* values of the apples stored at 10^{o}C, 20^{o}C and 30^{o}C were lower than those of the apples stored in the single stage NA storage (Figs. 3.5a & c).

3.4 Discussion

3.4.1 Effect of storage conditions on changes in firmness, stiffness and weight loss of the apples

Firmness and stiffness of 'Cripps Pink' apple declined over storage time, however the rate of softening was significantly influenced by storage temperature and cultivar. Under low temperatures in NA storage conditions, softening followed a tri–phasic pattern of an initial low softening rate, followed by a rapid softening rate and finally slowing down. A similar result was reported for 'Cox's Orange Pippin' apple (Johnston et al., 2003), except that in this experiment 'Cripps Pink' apples had a longer period of the initial slow softening rate and a lower rate of the rapid softening phase. For 'Cox's Orange Pippin' apple stored at 3^{o}C, the first phase of slow softening occurred within the first 13 days and the second phase of rapid softening occurred over the next 57 days (Johnston et al., 2003). Another study also reported that the first phase of low softening rate for 'Royal

Gala', 'Cox's Orange Pippin', and 'Granny Smith' and 'Pacific Rose' occurred between 0 to 25 days of storage at 0–5^{o}C, 0–2.5^{o}C and 0–12^{o}C, respectively (Johnston et al., 2001c). Transition points between the first and the second phases were also determined within 10, 20 and 30 days for 'Elstar' apples stored at 14^{o}C, 9^{o}C and 5^{o}C, respectively (Sman and Sanders, 2005). A similar pattern of softening was also observed for 'Royal Gala' apples stored at 12–35^{o}C (Johnston et al., 2001c). Results of the experiment showed that low temperature storage could slow down the rate of metabolism of apples, but even so, 'Cripps Pink' apples stored in NA softened slower than some apple cultivars.

In addition, softening could be reduced further by placing the apples in CA storage. The advantage of CA in slowing loss of firmness was also observed for 'Delicious' and 'Jonagold' apple (Drake, 1993), and 'Fiesta' apple (Colgan et al., 2006). Figures 3.1a & c showed that the application of CA at 0^{o}C reduced about a half the softening rate of 'Cripps Pink' apple within the first 102 days compared with NA storage at 0^{o}C. This result is in agreement with a previous study on 'Royal Gala' and 'Cox's Orange Pippin' in which the CA storage extended the life of the apples by 100-200% over NA storage at the same temperature (Johnston et al., 2002a). Besides, slowing softening rate and extending storage life of apples by CA is also influenced by a number of factors including apple cultivar (Dilley et al., 1989), gas concentrations of O_2 and CO_2 (Hertog et al., 2001; Kidd and West, 1933), exogenous ethylene concentration in CA storage (Liu, 1985), fruit maturity (Tu et al., 1997) and lag time of CA storage from harvest (Dilley et al., 1989).

Weight loss of 'Cripps Pink' apple during storage increased linearly with storage time, and weight loss rate was constant. Similar responses were also reported for pear

(Nerya et al., 2001) and tomato (Hertog et al., 2004). Because the weight (W) of the apples reduced with storage time, according the formula of stiffness, $S = f^2 * W^{2/3}$, stiffness of the apples also decreased with time. However, according to the wvpd control in the experiment, weight loss of the apples at all the storage temperatures was similar. In this instance weight loss of fresh fruit during storage caused by respiration is much less than that caused by water evaporation. Water evaporation is dependent on wvpd which is affected by both RH and temperature of storage air. Humidifying the supply air increased RH of air, reduced the wvpd and resulted in an important reduction in weight loss of apple during storage. These benefits were more evident at high storage temperatures. On the other hand, a normal and identical distribution of the apple populations and a small variation of measured apple weight values between the temperature treatments as well as within each treatment throughout the storage time also supported the homogeneity of initial status of maturity, size, etc. of the apples as well as accuracy in measurements of apple weight during the experiment.

3.4.2 Factors affecting ethylene and CO_2 production of the apples during storage

Apples follow a typical climacteric pattern of ethylene and CO_2 production, producing a peak at the onset of fruit ripening (Hulme et al., 1969). The extent and timing of the peak is cultivar dependant. In this experiment, an increase in ethylene production of 'Cripps Pink' apples stored at 20^{o}C in NA began after 18 days of storage and reached a maximum value on the 25th day. For the cultivar 'Golden Smoothee' apples, stored under the same conditions, ethylene production occurred much earlier after

only eight days of storage (Vilaplana et al., 2007). The size of the climacteric can vary with cultivar also. Here for 'Cripps Pink' the climacteric peak was very pronounced and similar to that observed for 'Gala' and 'Lady Williams' apples during ripening, but in 'Fuji' apples, ethylene production remains low (Jobling and McGlasson, 1995). At ambient temperature 'Lady Williams' and 'Gala' apples began a substantial increase in ethylene production on day 1 and day 4 after harvest, respectively and reached a maximum values 25 days after harvest (Jobling and McGlasson, 1995).

On the other hand, the result also showed that CO_2 production of 'Cripps Pink' apples increased with storage temperature. This was in agreement with the statement that over the physiological range of most crops, ie., 0 to 30^{o}C, increased temperatures caused an exponential rise in respiration and the Van't Hoff Rule states that the rate of a biological reaction increases 2 to 3-fold for every 10^{o}C rise in temperature. Results in Figures 3.4a & d indicated that increases in ethylene and CO_2 production of 'Cripps Pink' apples occurred within the initial 13 to 29 days of storage and an increase in CO_2 likely occurred after that of ethylene. Ethylene produced by fruit initiated the respiration climacteric.

In addition, CA storage inhibited the onset of climacteric period and depressed ethylene and CO_2 production compared with NA storage. Therefore, it appeared that the onset of climacteric period is dependent not only temperature, cultivar, maturity of the apple at harvest but also the gas concentrations of storage.

3.4.3 Effect of storage atmosphere on ethylene and CO_2 production

Within the same storage temperature of 0^oC, ethylene and CO_2 production of the apples stored in CA (2 kPa O_2 : 1 kPa CO_2) storage was significantly lower than that of the apples stored in NA. A lowered concentration of O_2 in CA storage is known to reduce activity of ACS (Colgan et al., 2006) leading a reduction in biosynthesis of ethylene in the apple tissue (Meigh and J. Reynolds, 1969; Murata and Minamide, 1970). Under anaerobic conditions, conversion of ACC to ethylene can be completely inhibited in apple fruit because O_2 is a substrate in the oxidation of ACC to ethylene by ACC-O (Yang, 1985). Reduced O_2 atmospheres may also inhibit ethylene biosynthesis in apple fruit by impeding the binding of ethylene to the receptor responsible for triggering autocatalytic ethylene biosynthesis (Burg and Burg, 1967). Both NA storage at 0^oC and CA storage maintaining low ethylene productions of $2.35\mu l.l^{-1}$ and $1.25\mu l.l^{-1}$, respectively delayed the initiation of the second rapid softening phase of the apples. Thus CA caused the fruit both a delay and a decrease in the production of ethylene compared with NA (Johnston et al., 2003). This result was in agreement with the previous studies showing that climacteric of apple was suppressed during refrigeration of the fruit below 5^oC (Fidler and North, 1967; Knee, 1971).

Respiration rate of the apples stored in CA (2 kPa O_2 : 1 kPa CO_2, 0^oC) was about half of that of the apples stored at 0^oC in NA storage. The reason is that a low O_2 concentration of CA storage restricted the respiration of the apples; and a high CO_2 concentration in CA suppressed respiration among other biochemical processes (Kader, 1986). Besides, a high CO_2 concentration of CA storage retarded the ethylene production

of apples (Meigh, 1960). Since CO_2 is a product of the respiratory process, it would be expected that respiration rate would decrease as CO_2 concentration in the atmosphere surrounding the plant tissues increases (Herner, 1987). However, elevated CO_2 can either slow down, stimulate or have no effect on respiratory rates depending on the sensitivity of the commodity, the concentration of the gas in the atmosphere, the physiological state of the produce, the temperature and the duration of exposure (Herner, 1987; Kader, 1986; Kubo et al., 1989). Therefore, the overall role of CO_2 in the regulation of respiratory metabolism is complex (Kays, 1991). In summary, exposing plant tissues to elevated CO_2 concentrations inhibits the activity of various enzymes involved in respiratory metabolism (Mathooko, 1996).

3.4.4 Effect of conditions in the first stage on ethylene and CO_2 production in the second storage stage

Conditions of the first storage stage affected ethylene and CO_2 production of 'Cripps Pink' apples in the next storage stage. External ethylene production was only 1.30$\mu l.L^{-1}$ on day 1 compared with the acceptable maximum level of 15 $\mu l.L^{-1}$ which can retard apple flesh softening (see 2.3.5.3 Storage conditions – Benefits of low ethylene in storage). After removal from the first stage of NA cold storage or CA storage into the second stage of NA storage, ethylene production and respiration of the apples stored at from 10^oC to 30^oC increased and reached a peak. On the other hand, softening rate of the apples stored in CA was about a half compared with that of the apples stored at 0^oC in NA conditions. However, the apple softening rate in CA will be the same as that in NA storage if fruit have already entered the rapid softening phase by the time they enter CA

(see 2.3.5.3 Storage conditions – Delay of CA establishment and initial cooling rate). This indicated that the apples are definitely pre-climacteric at the time of picking and ethylene biosynthesis was not impaired completely by conditions of the first stage of NA and CA as well as their storage times. A gas composition with O_2 partial pressure of 2 kPa of the first stage in CA storage could reduce respiration rate significantly but still permit aerobic respiration of the apples. The results from 'Cripps Pink' apples showed that ethylene production of the apples from CA storage (2 kPa O_2 : 1 kPa CO_2, 0^{o}C) recovered almost immediately at the beginning of transfer to 30^{o}C, 20^{o}C, 10^{o}C and 5^{o}C in NA. These results were in an agreement with the previous study. It stated that apples can ripen very rapidly after removal from CA storage (Watkins, 2002). Ripening is a process of physical, metabolic and biochemical changes, which is initiated by ethylene. Subsequent recovery of ripening is also dependent on ethylene (Watkins, 2002). Recovery of ethylene production of the apple at high temperatures after long term CA storage indicated that the storage did not entirely inhibit the ripening of the apple. This was in agreement with the report that ethylene production by 'Gala' apples from all CA (1, 1.9, 2.8 or 3.7 kPa O_2 and 2 kPa CO_2, at 1^{o}C) storage recovered during a 7-day post-storage ripening period at 20^{o}C (Mattheis et al., 1998). ACO appears to be crucial to this. This enzyme accumulates during cold storage. Immediately after transfer to 20^{o}C, the activity increases and is responsible for the high ethylene production (Larrigaudiere and Vendrell, 1993). The activation of this enzyme is more evident in less mature fruits (Larrigaudiere and Vendrell, 1993).

However, the production of ethylene continued to be suppressed for the apples stored at 0°C and 2.5°C in the second stage of NA storage. The result also showed that ethylene and CO_2 production of the apples in the second stage of NA storage at 5°C, 10°C, 20°C and 30°C, which were stored under CA (2 kPa O_2 : 1 kPa CO_2, 0°C) in the first stage, had the lowest values compared with those of the apples stored in the first stage in NA at 0°C and those of the apples stored in single stage storage of NA at the same temperatures. As explained previously, LO and high CO_2 concentration in CA storage and low temperature in long term NA storage might be the causes of these.

3.4.5 Effect of conditions in the first stage on maximum production of ethylene and CO_2 in the second stage storage

Conditions of the first stage storage also affected the maximum production of ethylene and CO_2 of the apples in the next stage storage. Rapid increases of ethylene and CO_2 production of 'Cripps Pink' apples stored at 5°C, 10°C, 20°C and 30°C after CA storage (2 kPa O_2 : 1 kPa CO_2, 0°C) indicated that the fruit retained the ability to ripen. However, the production of ethylene and CO_2 were significantly lower than that of the apples from the first stage of NA storage or from the single stage NA storage. This can be explained by the partially residual effect of CA. For example, CA storage might impair biosynthesis of ethylene within the apples much more than the NA storage at 0°C. A similar result for 'Gala' apple was also reported. Ethylene production of 'Gala' apples was reduced following CA (1, 1.9, 2.8 or 3.7 kPa O_2 and 2 kPa CO_2, at 1°C) storage plus 1 day at 20°C compared with the apples stored in NA (Mattheis et al., 1998).

On the other hand, storage time of CA has also certain effects on ethylene production of fruit after the storage. A previous result stated that the longer the tomato exposed to CA storage the slower the tomato ripens after removing from CA storage into NA (Batu and Thompson, 1998). A longer first stage in CA (102 days) compared with that of the first stage in NA storage (62 days at 0^{o}C) might result in a lower production as well as a lower maximum production of ethylene from the apples in the second stage of NA storage. Storage duration can affect apple quality because during storage, fruit qualitative characteristics and total aroma concentration are reduced or diminished (Ferrandino et al., 2001; Tough and Hewett, 2001). Recovery of volatile production occurred after NA storage, especially after 18 and 26 weeks in NA, but not in CA (Tough and Hewett, 2001). The similar result was also found for 'Gala' apples. Sixteen-week storage of CA (1.2 kPa O_2 : 1 kPa CO_2) has cause a negative effect on ethylene production of 'Gala' apple (Scalzo et al., 2003).

Therefore, it could be concluded that the time and gas concentrations of CA storage partially impaired the biosynthesis of ethylene in the apples and also affected their ability to ripen compared with their ability immediately after harvest.

3.4.6 Colour change

Change in skin background colour of 'Cripps Pink' apples was affected by storage temperature. A gradual reduction in *h* of the 'Cripps Pink' apples stored at 0^{o}C, 2.5^{o}C and 5^{o}C in the first phase of the single stage NA storage indicated that the apples were more reddish or less yellowish than the others stored at higher temperatures in the same periods. Then, a gradual increase in *h* caused the apples to become less red due to

gradation of the peel pigment. The conversion of apple skin into red colour during initial days of storage at temperatures below 5^{o}C might be result of an anthocyanin synthesis on the apple skin. Because of a considerable reduction of metabolic activities resulting from a minimum respiration, these low storage temperatures could reduce the loss of carbohydrate, mainly sugar. On the other hand, the low temperatures might permit accumulation of photosynthates which could help to increase content of carbohydrates in the tissue leading to an increase in anthocyanin synthesis (Creasy, 1968). This synthesis played an important role in improving red colour of the apple. A similar change of skin background colour was also observed on 'Starkrimson' apples (Lin et al., 1989). On the other hand, C* of the apples increased slightly during the first 50 days. This indicated that although green areas of the apples stored at 0^{o}C, 2.5^{o}C and 5^{o}C changed gradually to red colour, the intensity of red areas also increased slightly in this period. Therefore, for the apples stored at 0^{o}C, 2.5^{o}C and 5^{o}C, in the initial phase of NA storage, besides changing green areas into red areas and expanding the red areas, the red colour areas on skin also became more brilliant. For apples stored at temperatures higher than 5^{o}C, higher storage temperatures caused higher rates of increases in *h* and C*. This indicated that the time needed for colour saturation was shorter.

A significant increase in C* of the apples in the first stage of 61 days in NA storage at 0^{o}C intensified the red colour of the apples leading to an improvement in appearance quality of the apples. Besides, this first stage of storage also helped to prolong other quality attributes of the apples.

Changing rates in *h* of 'Cripps Pink' apples stored in NA at high temperatures ranging from 10°C to 30°C were very high. This indicated that storage temperature was the most important factor affecting change in *h* of the apples.

Storage atmosphere influenced change in skin background colour of 'Cripps Pink' apples. Non-significant changes in *h*, C* and L* of the apples during 102 days of CA storage indicated that the CA storage delayed the loss of chlorophyll and yellowing of the apple skin. The apples maintained their initial background colour at harvest and did not have any improvement in colour quality. A similar result was found in the previous report that treatment with CO_2 inhibits an increase in anthocyanin concentration of post-harvest fruit (Gil et al., 1997; Holcroft et al., 1998) by affecting anthocyanin biosynthesis, degradation, or both (Holcroft and Kader, 1999). During 102 days in the CA storage, L* of the apples was almost unchanged. This result was in agreement with a recent study that high CO_2 concentration of CA storage can maintain cherry brightness and extend storage life (Remo'n et al., 2004).

In addition, duration of storage might have an effect on the change in skin background colour of 'Cripps Pink' apple. In comparison of changing rates in *h* of the apples from the three lots in temperatures ranging from 10°C to 30°C, the apples from lot I had the highest rate, then the apple from lot II and lot III. This indicated that long term low temperature storage, particularly CA storage affected seriously anthocyanin biosynthesis and normal change in background colour of the apples after being moved out to NA.

3.4.7 Weight loss

Although weight loss of the apple increased linearly with time at the same rate for the apples stored at all six temperatures, stiffness of the apples stored at 0^oC and 2.5^oC decreased in three phases, and stiffness of the apples stored at 5^oC, 10^oC, 20^oC and 30^oC reduced in only two phases. This indicated that besides water loss, other factors also caused a reduction in stiffness of the apples. Higher temperatures could increase the rate of biochemical reactions occurring within the fruit flesh.

Because ethylene production and CO_2 production of the apples stored in CA storage were about half of those from the apples stored at 0^oC in NA storage, reduction in firmness of the apples in CA storage was also lower compared with apples stored at 0^oC in NA storage during the same storage time of 60 days.

Ripening process coincides with loss of background green colour and softening of the apple tissues (Landfald, 1966; Wills et al., 1998). An initial increase in *h* of the apple stored at high temperatures ranging from 10^oC to 30^oC, particularly 20^oC and 30^oC of all three lots indicated that the ripening process and softening of the apples occurred immediately at these temperatures. This coincided with increases of ethylene production and rapid reduction of firmness and stiffness of the apples at these temperatures. This result was in agreement with the previous studies on apple (Johnston et al., 2001c; Stow et al., 2000). The loss in firmness of apples is concomitant with an increase in background colour (Landfald, 1966; Paull, 1999). In contrast, inhibiting an increase in *h* of the apples stored at 0^oC, 2.5^oC and 5^oC from all the three lots also happened in the same periods as suppressing ethylene production and delaying the softening process of the apples at these low temperatures. These processes coincided together at certain time

points. Besides, because respiration rate is an excellent indicator of metabolic activity of the tissue and also a useful guide to the potential storage life (Wills et al., 1998), a low respiration rate of apples at 0°C in NA and a much lower rate under CA storage indicated that the apples at this temperature and under the CA conditions had a longer storage life compared with the others.

3.5 Summary

Storage temperature had effect on softening pattern of 'Cripps Pink' apples. During NA storage, firmness and stiffness of 'Cripps Pink' apples reduced with increase in storage temperature and longer time. Higher storage temperatures caused shorter time of the first slow softening phase and higher rates of the second rapid softening phase. Firmness (and stiffness) of the apples stored at lower temperatures of 0°C, 2.5°C and 5°C (and 0°C, 2.5°C for stiffness) reduced in three phases: initial slow rate, rapid rate and final slow rate within the first 30 days, 25 days and 20 days respectively. Conversely, firmness (and stiffness) of the apples stored at higher temperatures of 10°C, 20°C and 30°C (5°C, 10°C, 20°C and 30°C for stiffness) decreased in only two phases: a rapid rate immediately at the beginning of storage and follwed by a final slow rate.

Storage temperature also affected ethylene and CO_2 production of 'Cripps Pink' apples. The rates of ethylene and CO_2 production increased with storage temperature. Storage temperatures of 0°C and 2.5°C entirely suppressed the climacteric period and improved background colour of the apples. In the temperature range from 5°C to 30°C, higher temperatures caused earlier onsets of the climacteric period, shorter phases of the first slow softening rate, higher rates of the second rapid softening rate and higher

maximum production of ethylene and CO_2. An increase in ethylene production occurred before an increase in CO_2 production in the climacteric period. An increase in ethylene production of the apples stored at 20°C and 30°C occurred within the first 18 days of storage. An increase in CO_2 production of the apples stored at those two temperatures occurred within the first 41 and 29 days, respectively.

Temperature also influenced change in background colour of 'Cripps Pink' apples. Background colour of the apples stored at from 0°C to 5°C changed gradually from green to red colour in the first 60 days, then remained unchanged in the next 40 days at 5°C and 90 days at 0°C before turning to yellow color. This might result from anthocyanin biosynthesis on the apple peel at low temperatures. In contrast, background colour of the apples stored at higher temperatures ranging from 10°C to 30°C, turned from green to yellow colour immediately in the initial days of storage. The rate of colour change also increased with temperature.

There was a coincidence of an increase in *h* in the first days of storage with a rapid increase in ethylene, and rapid reduction in firmness and stiffness of the apples stored at 10°C, 20°C and 30°C. Moreover, there was also a coincidence of a slight decrease in *h* in the initial days of storage with inhibition of the onset of climacteric period and delaying the second phase of rapid softening rate of the apples stored at 0°C, 2.5°C and 5°C.

NA at 0°C in the first stage storage decreased reduction in firmness and stiffness and improved colour quality of the apples. However, it also reduced rates and the maximum production of ethylene and CO_2 of the apples in the second stage storage in NA.

CA in the first stage of storage inhibited the climacteric onset, suppressed ethylene and CO_2 production, maintained better firmness and stiffness of the apples compared

with NA at the same storage temperature. It reduced by half of production rates of ethylene and CO_2 compared with those in NA storage at 0^oC. However, it did not improve the colour quality of the apples in this stage. CA storage did not impair entirely activities of ACC synthase, and therefore, ethylene biosynthesis was recovered partly in the apple tissues after the CA storage. However, the CA also affected normal change in background colour and reduced seriously the rates and the maximum production of ethylene and CO_2 of the apples in the second stage of NA storage.

CHAPTER IV

PREDICTION OF CHANGE IN FIRMNESS OF 'CRIPPS PINK' APPLES

4.1 Introduction

Like other perishable fruit, 'Cripps Pink' apple is a living entity and therefore continues metabolic activities after harvest (Wills et al., 1998). Some of these metabolic acivities can lead to a loss in quality such as softening in apples. Softening is a complex biological process of physical and physiological changes. It occurs in many fresh fruit and results in changes in physical properties such as cell viscosity and turgor pressure, and flesh firmness and stiffness. The process is influenced by several factors including pre-harvest conditions, harvest date and post-harvest handling (Johnston et al., 2002a; Tu et al., 1997).

Firmness is recognised as one of the most important quality attributes of apple. Firmer fruit are considered to have better quality characteristics than softer fruit (Harker et al., 1997). This is evident in international markets where failure to meet firmness specifications can result in shipment rejections (Johnston et al., 2001c), and therefore maintenance of firmness after harvest is a key goal of post-harvest technologists. Prediction of softening rates under known post-harvest conditions can assist in marketing fruit more effectively. For example, there have been some studies reported for modelling firmness of 'Elstar' apple (Tijskens et al., 1999) which can predict outturn quality of the apples. In contrast to the kiwifruit, which has two phases of softening during storage (Benge et al., 2000), recent studies suggest that depending on cultivar, models predicting apple firmness can have two or three phases of softening (Hertog, 2004). Consequently

three-parameter non-linear models for prediction of change in apple firmness have been suggested (Hertog, 2004; Johnston et al., 2001c). Rate of softening is largely influenced by environment but is also cultivar dependant (Colgan et al., 2006; Ertur et al., 2003; Johnston et al., 2001c). There have been no studies on change in firmness or prediction of changes in firmness of 'Cripps Pink' apples during storage.

In the previous chapter we investigated the change in firmness and other quality attributes of 'Cripps Pink' apple under a wide range of temperatures in NA. In this chapter the data from that experiment was used to develop and establish mathematical models which characterise the softening process occurring within the apple flesh, and predict the change in firmness and keeping quality of the apple during NA storage. The results obtained from the study will contribute to a better understanding of firmness change in the 'Cripp's Pink' apples and better market management.

4.2 Materials and methods

4.2.1 Principal requirements in establishment of models predicting changes in firmness (and stiffness)

To obtain models that accurately describe and predict change in firmness or stiffness of the apple, model establishment was based on the following criteria:

- Data was obtained from experiments using freshly harvested homogeneous fruit without pre-harvest and post-harvest treatments and free of defects.
- No transformations were applied to the data sets.
- The model patterns selected on the basis of compatibility with the theories on post-harvest softening of apple. Depending on the characteristics of particular cultivars, the

firmness models could have one or more reflection point. However, all firmness models must comply with the rule of firmness change; that is, fruit firmness during storage at different storage temperatures always decreases (Hertog, 2004).

- The established model should not be too complicated or too simple, but be effective enough for predictions of apple firmness or stiffness.
- The structure of models and their parameters should provide information associated with the apple and storage conditions.
- If possible, the developed model should be tested against new experimental data obtained under similar conditions. If passed successfully, the developed model should be tested against data obtained in real-life situations to generalise the model for wider applications.

4.2.2 General methods in establishment of predicting models

Determination of models predicting changes in firmness or stiffness of 'Cripps Pink' apple began with the first step of data collection and determination of important independent variables for the models. The data sets of firmness (or stiffness) were collected from six temperature treatments: 0^{o}C, 2.5^{o}C, 5^{o}C, 10^{o}C, 20^{o}C and 30^{o}C. In order to obtain predicting models with a high accuracy, outliers from the data sets were discarded. However, no transformation was applied to the data sets to avoid errors occurring during the estimation. The correct determination of important variables in the models plays a significant role. According to the literature and our experiments in Chapter 3, storage temperature and storage time are the main factors affecting softening (reductions in firmness and stiffness) of 'Cripps Pink' apple during NA storage.

Therefore, these two factors should be the main independent variables that provide the best biological interpretation within the models.

The second step was determination and selection of models. Model selection was based on general patterns of change in firmness (or stiffness) with storage temperature and time, together with the theories on post-harvest softening of apple. The 'best' model was selected based on the criteria of good scientific explanation, high degree of fit, good representation of parameters, simplification in model structure and applicable over a wide range of temperature and time.

The third step was model validation. Best fit values of the parameters and fitness of the selected model were estimated by fitting the data sets of firmness (or stiffness) of the apple to the selected model. The iterative non-linear regression procedure and Least-Squares method were used in the modelling process. This procedure also provided estimation of parameter statistics, such as standard error and confidence intervals of the estimates.

The fourth step was further development and refinement of the model. Based on the results obtained from modelling and the knowledge of post-harvest softening, as well as fundamental laws on biochemical and biological processes, the selected model was improved and developed to characterize more accurately the softening process. The developed model was then used to simulate the softening process of the apple under different storage temperatures and durations. Keeping quality of the apple stored at different temperatures was also determined.

The final step was testing the developed model with firmness data sets of 'Cripps Pink' apple grown in different locations in NSW, and Australia, apples harvested in

different seasons, and apples stored in commercial conditions of NA storage. The values of the parameters and fitness of the developed model were also calibrated and estimated.

4.2.3 Data collection and analyses

Six firmness data sets of 'Cripps Pink' apples at 0^oC, 2.5^oC, 5^oC, 10^oC, 20^oC and 30^oC in single stage NA storage (Chapter 3) were used for establishing mathematical models for prediction of change in firmness of the fruit during storage. Outliers of the data sets were discarded but no transformation was applied to the data sets. Modelling and simulation of change in firmness of the apples with time and temperature were conducted using the Least-Squares method with the procedures of iterative non-linear regression in combination of the statistical packages of Prism GraphPad 4.03 (version 4, release 3, 2005, GraphPad Software, Inc, San Diego California, USA) and DataFit 8.1 (version 8, release 1, 2005, Oakdale Engineering, USA).

4.2.4 Comparison and selection of models

The following equations from existing literature were identified to characterise the softening behavior of 'Cripps Pink' apples:

Lorentz equation (Ratkowsky, 1990): $y = \alpha + \beta / [1 + \gamma*(x - \delta)^2]$ (4.1)

Bragg and Packer (1962) (Ratkowsky, 1990): $y = \alpha + \beta*Exp[- \gamma*(x - \delta)^2]$ (4.2)

Michaelis – Menten (CMM) (Benge et al., 2000): $y = \alpha*[1-(x/(\beta+x))]$ (4.3)

Johnston et al., 2001 (Johnston et al., 2001c):

$$y = \alpha - (\alpha - \beta)*[1-\{1 +Exp((x+\gamma-1*\ln(31)-(5.2*\gamma-1.0168))/\gamma-1)\}^{-0.2}] \quad (4.4)$$

Exponential Decay (Benge et al., 2000; Motulsky and Christopoulos, 2005):

$$y = \alpha + \beta* Exp[-\gamma*x] \quad (4.5)$$

Where y and x are flesh firmness and storage time; Greek symbols are parameters in the models.

The best model was determined by fitting and comparing all five models above to the six firmness data sets using the procedure of iterative non-linear regression and Least-Squares method of the software GraphPad Prism.

In the comparing the models, the exponential decay model (Eq. 4.5) had parameters which had biological meaning to characterise the softening process in the apples. Furthermore, the exponential decay model (Eq. 4.5) was simpler with fewer parameters compared with the Lorentz (Eq. 4.1), and Bragg and Packer (Eq.4.2) models. Among the five models, Eqs. 4.4 & 4.5 had application to the widest temperature range, from 0^oC to higher 30^oC, while temperature range of application for Eqs. 4.1, 4.2 and 4.3 models was limited to only 0^oC, 0^oC to 10^oC and 5^oC to 30^oC, respectively. In spite of having the same number of parameters, the Johnston et al model (Eq. 4.4) was more complicated than the exponential decay model (Eq. 4.5) in terms of model construction (Tab. 4.1). Therefore, the exponential decay model was selected as the best model.

Table 4.1 Comparison of the models selected from the existing literature that may potentially describe the softening of 'Cripps Pink' apples during storage

	Criteria					
	Scientific explanation	Fitness		95% confidence intervals of parameters	Complication of model	Temperature range of application
		Ab.Sum of Squares of Residuals	R^2			
Lorentz equation	Fair	Bad	Bad	Inconsistent	Complicated (4 parameters)	Narrow (0^oC)
Bragg equation	Good	Good	Good	Narrow	Complicated (4 parameters)	Narrow (0 to 10^oC)
Michalis-Menten	Bad	Fair	Fair	Wide	Very simple (2 parameters)	Wide $T > 2.5^oC$
Johnston (Sigmoidal equation)	Good	Very good	Very good	Narrow	Complicated construction of the model	Very wide (0 to $>30^oC$)
Exponential decay	Very good	Very good	Very good	Narrow	Simple (3 parameters.)	Very wide (0 to $>30^oC$)

Based on the theory of fruit softening (see 2.3.3 and 2.3.4), the exponential decay model was rewritten as the following:

$$F(t) = F_{min} + \text{Span}*\text{Exp}[-k_s*t] \quad (4.6)$$

Where $F(t)$ was firmness of fruit at storage time t, ($kg.cm^{-2}$); F_{min} was firmness of fruit at plus infinite time, ($kg.cm^{-2}$); Span was the drop in firmness from the initial value to the minimum value, ($kg.cm^{-2}$); k_s was softening rate, (Day^{-1}) and t was storage time, (Day).

4.3 Results

4.3.1 Modelling change in firmness of 'Cripps Pink' apples during storage

The exponential decay model (Eq. 4.6) was fitted to the six firmness data sets using the global modelling procedure of the software GraphPad Prism. The parameters of F_{min}

and Span were assumed to share with all six firmness data sets. In contrast, k_s was assumed as a specific parameter representing softening rate of the apples at different storage temperatures. Results obtained from fitting the data are shown in Table 4.2 and Figure 4.1 (Appendix 4.1).

As shown in Figure 4.1, it is possible that the curves from 0°C to 5°C were underestimated in the first 30 days of storage. However, the curves better describe the softening process occurring in the middle and the last phases of storage. This is acceptable in practice because prediction is often applied for apples stored for longer than 30 days at temperatures lower than 5°C.

Table 4.2 Analysis of non-linear regression for the exponential decay model (Eq. 4.6) using the global modelling procedure with time as an independent variable.

	Storage temperature (°C)						
	0	2.5	5	10	20	30	Global (shared)
Best-fit values:							
- F_{min} (kg.cm^{-2}) [(a)]	1.6	1.6	1.6	1.6	1.6	1.6	
- Span (kg.cm^{-2})	7.36	7.36	7.36	7.36	7.36	7.36	7.36
- k_s (Day^{-1})	0.002	0.00248	0.003059	0.0039	0.0061	0.0101	
Std. Error:							
- Span (kg.cm^{-2})	0.0396	0.0396	0.0396	0.0396	0.0396	0.0396	0.0396
(%)[(b)]	(0.538%)	(0.538%)	(0.538%)	(0.538%)	(0.538%)	(0.538%)	(0.538%)
- k_s (Day^{-1})	0.000093	0.000112	0.000140	0.000185	0.000285	0.000534	
(%) [(b)]	(4.655%)	(4.552%)	(4.606%)	(4.751%)	(4.625%)	(5.294%)	
Goodness of fit:							
-Degrees of freedom							34
- R^2	0.9661	0.9758	0.9736	0.9923	0.9811	0.9541	0.9745
-Abs. Sum Squares of residuals	0.1454	0.1076	0.1178	0.02778	0.06087	0.1171	0.5766
- Sy.x							0.1302
Normality of residuals:							
- *P value*	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	*P*<0.0001	

(a) Fixed value, based on the overall experimental data, not estimated, so no standard error
(b) Relative standard error (Rel. S.E) was expressed as a percentage of the estimate values (Rel. S.E. = Standard error / parameter estimate)

In contrast, the model quite reasonably characterised all softening phases of the apples stored at temperatures higher than 5°C. The results of model fitting in Table 4.2 also showed that the standard errors were small and consistent across all temperatures, only 0.538% and from 4.552% to 5.294% compared with the best fit values of the parameters Span and k_s (Tab. 4.2), respectively. On the other hand, the deviations of data points along the curves were not significant. The curves explained up to 95.41–99.23% of the observed variance at different temperatures and the model also explained up to 97.45% of observed variation of all the firmness data sets (Tab.4.2). In addition, the data points were distributed randomly above and below the curves (Fig. 4.1). These indicated that the exponential decay model can be used to characterise the softening process and predict change in firmness of the apples during storage.

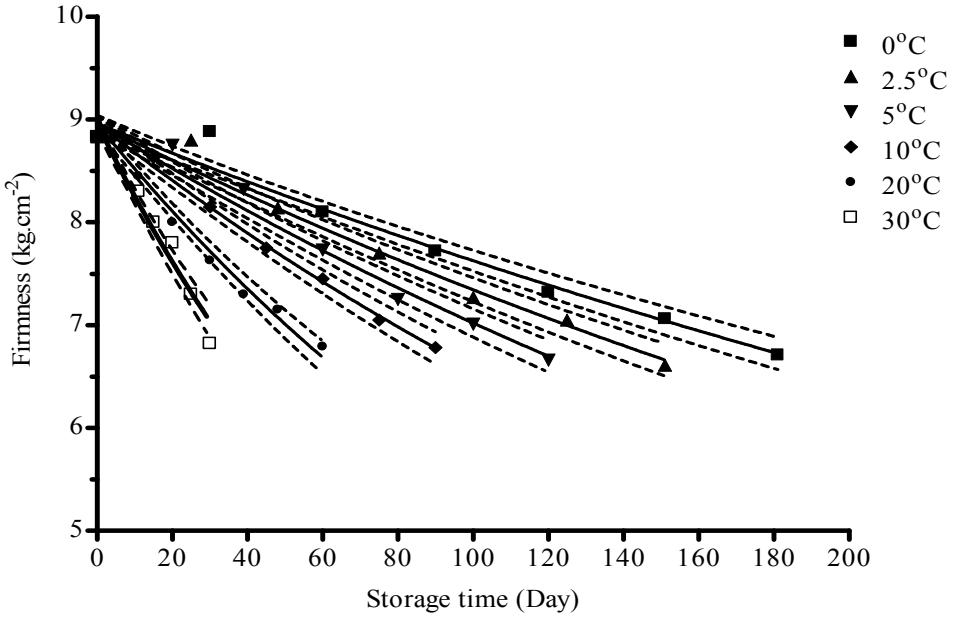


Figure 4.1 Result of fitting the exponential decay model (Eq. 4.6) to the firmness data sets of 'Cripps Pink' apples stored at six temperatures in single stage of NA. The symbols indicate the firmness mean of 25 apples. The continuous curves represent the model estimates describing responses of softening to different storage temperatures. The two broken curves, above and below of each continuous curve, show 95% confidence band.

4.3.2 Effect of storage temperature on softening rate of the apples

In order to study the effect of storage temperature on softening rate of 'Cripps Pink' apple, the Arrhenius equation (Eq. 4.7) was applied to the best fit values of the softening rate k_s from Table 4.2.

$$k_s = A*Exp[-E_a/(R*T)] \quad (4.7)$$

Where k_s was the softening rate of the apple flesh, (Day^{-1}); A was frequency factor, (Day^{-1}); E_a was activation energy, ($J.mol^{-1}$); R was the universal gas constant, R = 8.314 $J.mol^{-1}.K^{-1}$; and T was storage temperature, in Kelvin scale (K).

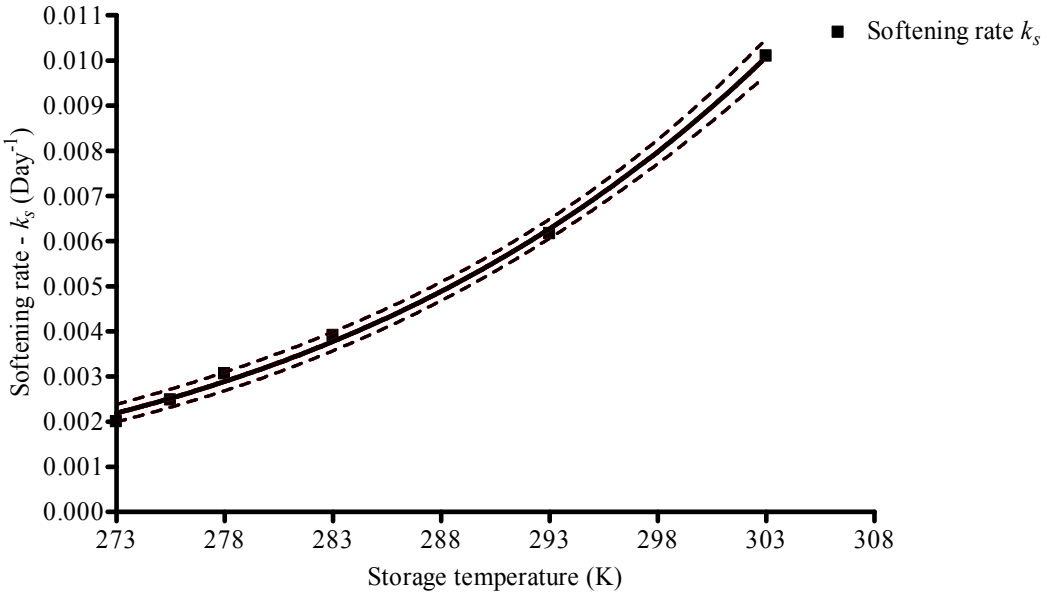


Figure 4.2 Change in softening rate (k_s) of 'Cripps Pink' apples with storage temperature using the Arrhenius equation $k_s = A*Exp[-E_a/(R*T)]$ (Eq. 4.7) and best fit values k_s from Table 4.2. The continuous curve represents the model estimate. The two broken curves, above and below of the curve, show 95% confidence band.

The results showed that the Arrhenius equation was capable of explaining up to 99.79% of the observed variance (Fig. 4.2) (Appendix 4.2). Furthermore the softening rate increased substantially with temperature and complied with the Arrhenius equation. Because the weight loss of the apples at all storage temperatures was restricted and

maintained almost the same by the wvpd control, the results indicated that the softening within the apple flesh was likely a biochemical process.

4.3.3 Development of the firmness predicting model

Based on results obtained from the exponential decay model and the Arrhenius equation above, the following three-dimensional compound model describing change in firmness with both storage temperature and storage time was also established:

$$F(t) = F_{min} + Span*Exp[-k_s*t] \quad (4.8)$$

$$k_s = A*Exp[-E_a/(8.314*T)] \quad (4.9)$$

Where $F(t)$ was firmness of apples stored in certain temperature and at certain time, ($kg.cm^{-2}$); T was storage temperature (K) and t was storage time (Day).

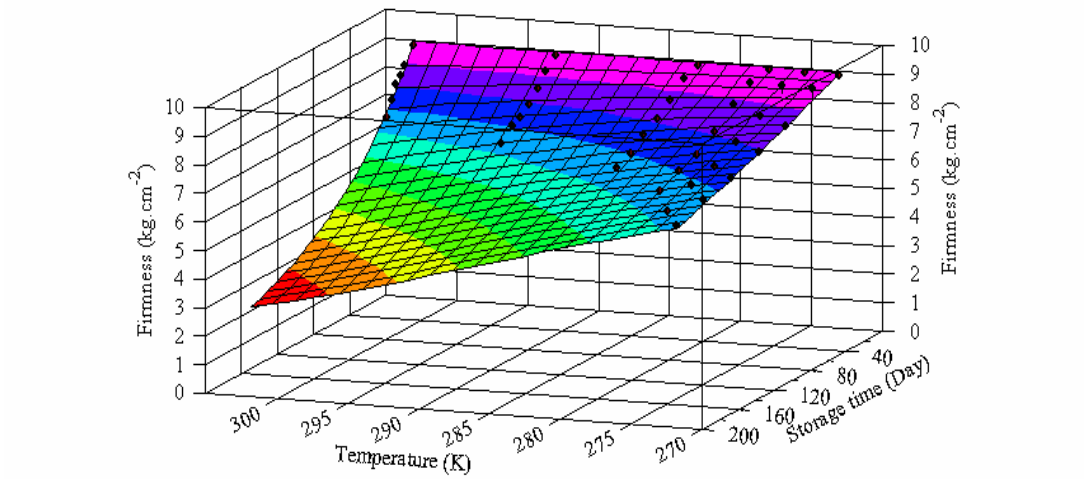


Figure 4.3 Fitting of the three-dimensional compound model of a 3D function of firmness changing with storage time $F(t) = F_{min} + Span*Exp[-k_s*t]$ (Eq. 4.8) and temperature $k_s = A*Exp[-E_a/(8.314*T)]$ (Eq. 4.9) to the firmness data sets of 'Cripps Pink' apples stored at six temperatures from 0^oC to 30^oC in the single stage of NA, using the Least-Squares method with the iterative procedure of the software package DataFit 8.1, 2005. Storage temperature (T) in K and storage time (t) in Day were independent variables of the firmness function, $F(t)$. The surface represents the prediction model while the symbols represent the firmness mean of 25 apples.

Statistical analyses were carried out using the iterative procedure for nonlinear regression analysis of the statistical package DataFit software (Version 8.1, 2005). A very high percentage of multiple variance accounted for by the model (R^2_{adj} = 96.89%) (Appendix 4.3) confirmed the reliability of the exponential decay model and the three-dimensional compound model (Fig. 4.3).

4.3.4 Simulation of change in firmness of 'Cripps Pink' apples at different storage temperatures

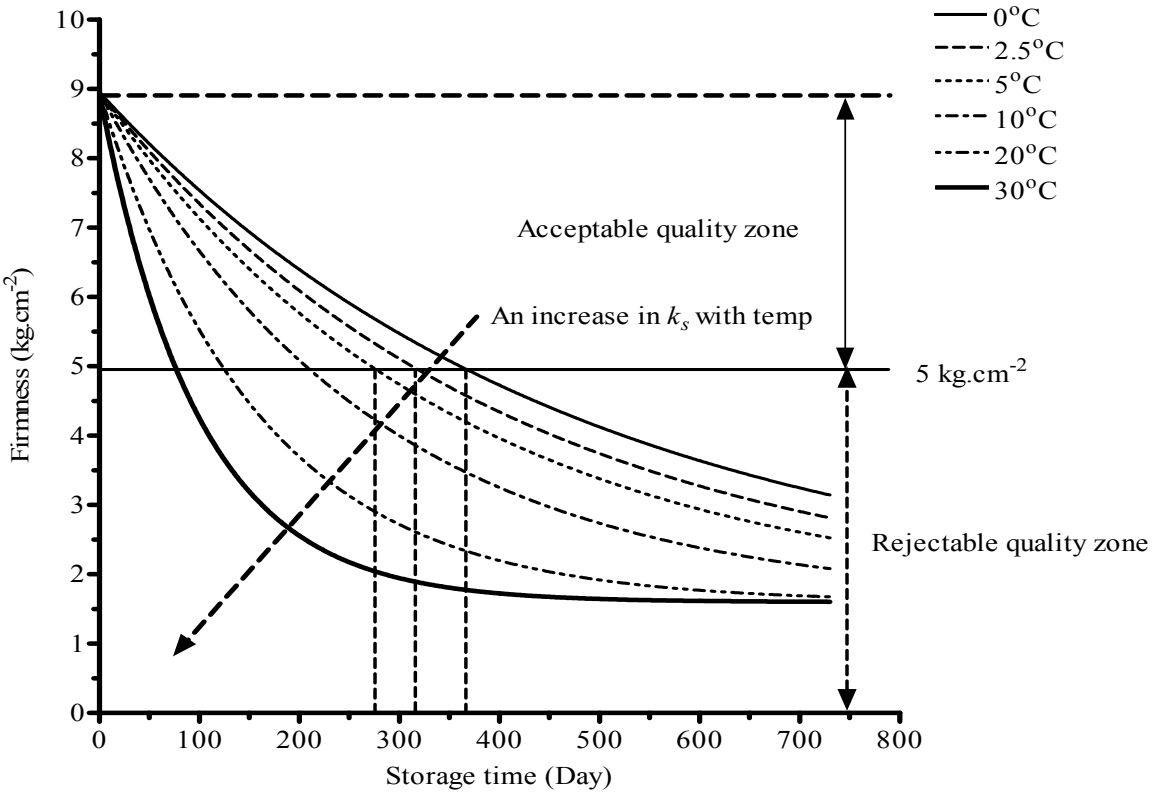


Figure 4.4 Simulation result of change in firmness of 'Cripps Pink' apples with softening rate at six temperatures and storage time, using Eq. 4.6 and best fit values of F_{min}, Span and k_s at six temperatures from Table 4.2

To simulate change in firmness of the apples with softening rate, the exponential decay model (Eq. 4.6) was used together with the best fit values of the common parameters (F_{min} and Span) and the specific parameter (k_s) representing for six storage temperatures (Tab. 4.2). As shown in Figure 4.4, if we assume that the apples will be rejected by consumers once their firmness reaches the limit for acceptance (F_{lim} = 5kg.cm^{-2}), the keeping quality, in term of firmness alone of 'Cripps Pink' apples stored at 0^{o}C, 2.5^{o}C and 5^{o}C, was around 365 days, 315 days and 275 days, respectively.

4.3.5 Model testing

The general applicability of the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eqs. 4.8 & 4.9) were examined by testing the models with the same apple cultivar. Two lots of apples, which were selected randomly from the same apple population harvested at another location of Batlow, New South Wales, Australia in May 2006, were stored at 0^{o}C and 3^{o}C in NA under commercial conditions (in carton boxes with air circulation but without controlling RH of the air). The Tinytags were also used to measure RH in the commercial storage. The apples from the two temperature treatments were stored within 30 days, 90 days, 150 days and 210 days. At the end of these periods, ten apples from each treatment were taken out of the storage. Firmness measurements of the apples using MT method were carried out after the fruit reached to 20^{o}C. Firmness data sets of ten apples from the two temperature treatments were used to calibrate values of the parameters: F_{min}, Span and k_s of the exponential decay model (Eq. 4.6) (Appendices 4.4 & 4.5); and parameters: A, Ea, F_{min} and Span of the three-dimensional compound model (Eqs. 4.8 & 4.9). The calibrated values of F_{min} and Span

were 1.60 kg.cm^{-2} and 7.95 kg.cm^{-2}, respectively, and calibrated values of k_s at 0^{o}C and 3^{o}C were 0.0021 day^{-1} and 0.0028 day^{-1}, respectively with a Global fitness: R^2, absolute sum of squares and Sy.x were 0.8996, 1.083 and 0.4249, respectively. Similarly, A, Ea, F_{min} and Span of the three-dimensional compound model was 2333348994 day^{-1}, 57678.45 J.mol^{-1}, 1.60 kg.cm^{-2} and 7.95 kg.cm^{-2}, respectively. The coefficient of multiple determination (R^2), residual sum of squares (Abs.), standard error of the estimates were 0.8995, 1.0832 and 0.4249, respectively (Appendices 4.6 & 4.7).

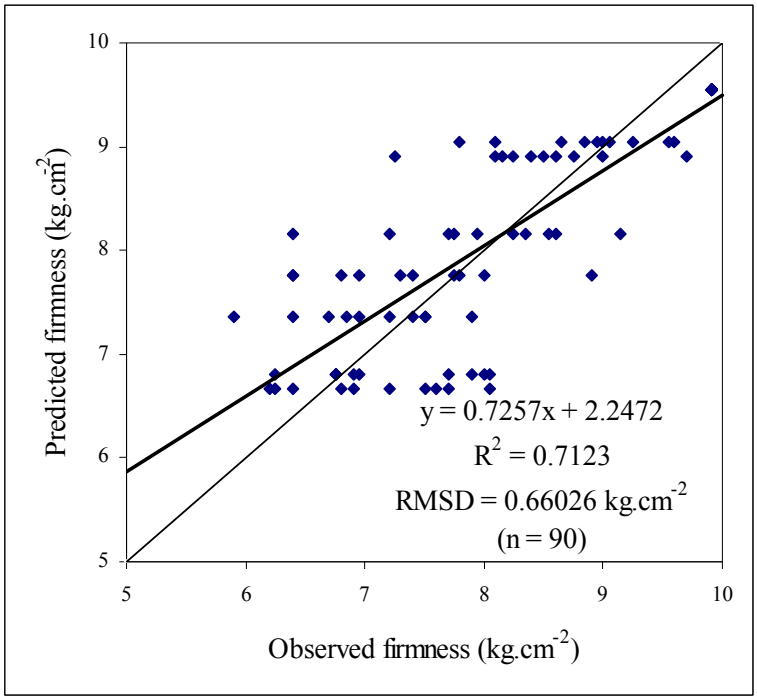


Figure 4.5 Correlation between the values for firmness predicted by the exponential decay model (Eq. 4.6) and the measured firmness values of 'Cripps Pink' apples harvested in Batlow, NSW, Australia in May 2006, and stored at 0^{o}C and 3^{o}C under commercial conditions.

Further firmness data sets of ten apples stored under the same conditions collected and together with calibrated values of the parameters were used to test the general applicability of the two models: the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eqs. 4.8 & 4.9). The results of the testing indicated that in

a range of storage temperatures from 0°C to 3°C, the correlation between the measured firmness values of 10 replicates and the firmness values predicted by the exponential decay model was strong, with Pearson coefficient (r) = 0.8439 and Root Mean Square Difference (RMSD) = 0.66026 kg.cm^{-2} (Fig. 4.5). On the other hand, the correlation between the measured firmness values and the firmness values predicted by the three-dimensional compound model was also strong, with Pearson coefficient (r) = 0.8437) and Root Mean Square Difference (RMSD) = 0.66075 kg.cm^{-2} (Fig. 4.6). This implied that the exponential decay and three-dimensional compound models were applicable to apples stored under commercial conditions in which the RH of the air was lower (65%) than that in the experiment. It also confirmed that MT firmness for 'Cripps Pink' apples was not affected by the wvpd in storage.

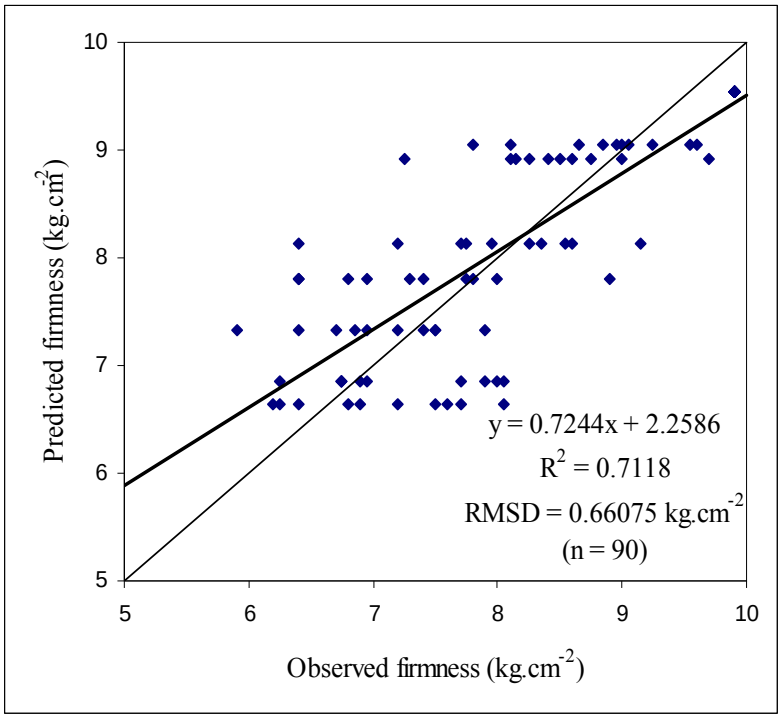


Figure 4.6 Correlation between the values of firmness predicted by the three-dimensional compound model (Eqs. 4.8 & 4.9) and the measured firmness of 'Cripps Pink' apples harvested in Batlow, NSW, Australia in May 2006, and stored at 0°C and 3°C under commercial conditions.

Although the 'Cripps Pink' apples for the model testing were harvested in a different location and a different season from the apples in the experiment, the value of Fmin was kept the same, 1.6kg.cm^{-2} (Table 4.2), and Span value was only calibrated a little, 7.95 kg.cm^{-2} compared with 7.36 kg.cm^{-2} (Table 4.2); the fitness of the model to the testing firmness data was also very high (R^2 = 0.8996). This small difference in Span could be caused by a difference in harvest date. Therefore, the two models can characterise accurately the softening of the apples harvested in different locations and different seasons in Australia. Therefore, when predicting changes in firmness of new batches of the same apple cultivar, values for F_{min} and k_s parameters may be kept constant, with only the Span parameter requiring calibration. Span should be dependent on harvesting date. The two models should be applicable to other apple cultivars, when values for the cultivar-specific parameters are calibrated.

4.4 Discussion

Firmness of the apples decreased exponentially with storage time, complying with the exponential decay model. Depending on storage temperature, the softening pattern of the apple underwent three or two phases. Apples stored at temperatures lower than 10^{o}C exhibited three phases of softening, while those stored at temperatures equal to or higher than 10^{o}C exhibited only two final phases of softening. The softening rate of 'Cripps Pink' apple was dependent on storage temperature. Softening rate increased substantially with storage temperature and complied with the Arrhenius equation, which implied that softening was a biochemical process. A high level of fit between the Arrhenius equation and the best fit values of softening rate (k_s) at different storage temperatures (Appendix

4.2) indicated that the Arrhenius equation could explain most of the variation in softening rate of apples stored at different temperatures. This finding agreed with previous reports that the Arrhenius equation alone could describe softening at different temperatures (Johnston et al., 2001c; Tijskens et al., 1999). Keeping quality of 'Cripps Pink' apples stored at 0°C, 2.5°C and 5°C were predicted by the exponential decay model to be 365 days, 315 days and 275 days, respectively. The exponential decay model and the three-dimensional compound model both had high fitness with the firmness data sets. The two models were validated with independent firmness data sets and gave good results. Therefore, the exponential decay model and the three-dimensional compound model were the best models for characterising softening in apple flesh and predicting changes in firmness and keeping quality during NA storage.

4.5 Summary

The results presented in Chapter 4 showed that the firmness of 'Cripps Pink' apples decreased exponentially with storage temperature, complying with the Arrhenius equation. The best model for prediction of changes in the firmness of 'Cripps Pink' apples had higher fitness at 2.5°C, 5°C, 10°C and 20°C and lower fitness at 0°C and 30°C (Tab. 4.2). Even so, at these temperatures, the model only underestimated softening within the first 30 days of storage and the fit improved thereafter. Moreover, the models would more likely be used for prediction of fruit softening at low temperatures during long term storage. The exponential decay model had a high global fitness of R^2 = 97.45% (Tab. 4.2) in a temperature range from 0°C to 30°C. Therefore, it was the best model for predicting changes in the firmness and keeping quality of apple during NA storage.

F_{min} and Span of the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eqs. 4.8 & 4.9) were shared parameters which were likely to be dependent on cultivar and pre-harvest factors, but not on post-harvest factors. Span was also affected by the harvest date of the fruit. Late harvested fruit may have lower values of Span compared to early harvested fruit. The softening rate k_s was a cultivar-specific parameter, which was influenced by post-harvest factors, particularly temperature. Softening rate k_s increased with storage temperature. The ratio between Span and F_{min} was appropriately 4.60.

CHAPTER V

PREDICTION OF CHANGE IN STIFFNESS OF 'CRIPPS PINK' APPLES

5.1 Introduction

Although MT method is an objective and instrumental test, it fails to measure all of the complex mechanical properties of fruit (Harker et al., 2006b), is destructive and expensive. Therefore, the non-destructive method using the AFS-AWETA for measuring texture is needed to estimate fruit quality in markets and to continuously monitor the fruit texture during experimentation. According to the characteristics of the AFS-AWETA (see 2.4.1), the instrument is considered to be a potential new standard for fruit texture evaluation, replacing the older destructive standard, due to its ability to measure firmness and detect changes in firmness in fruit having very different properties (De Ketelaere et al., 2006). AFS has been used in studies on changes in the stiffness of kiwifruit (McGlone et al., 1997) and tomato (Hertog et al., 2004) during storage. There have been several reports on changes in the stiffness of some apple cultivars during storage (De Ketelaere et al., 2006; Johnson and Dover, 2005). Cultivar also affects the pattern of quality change during storage (Dris, 1999; Jobling and McGlasson, 1995), particularly the softening processes of apples (Ertur et al., 2003; Johnston et al., 2001a; Johnston et al., 2001c). There have also been studies on the effects of storage humidity and temperature on tomato, pear and apple stiffness (Hertog et al., 2004). However, there have been no reports on change in the stiffness of 'Cripps Pink' apple associated with temperature

during storage, nor any development of mathematical models for predicting change in apple stiffness.

Many factors influence the decline in apple stiffness after harvest. Temperature has been recognised as the most important factor, particularly for long term storage (Johnston et al., 2002a). Maintaining optimum storage conditions for apple throughout the post-harvest period is difficult. Therefore, the ability to predict changes in the stiffness of apples over a wide range of temperatures would be valuable and beneficial.

The aims of this study were to investigate changes in the stiffness of 'Cripps Pink' apple in response to varying temperatures and durations of NA storage, and to use mathematical models to characterise the softening process and predict change in stiffness of apple, taking into account the effects of biochemical change and weight loss during storage. Another aim of this study was to use modelling to improve the understanding of AWETA measurement and to see whether stiffness is linked to properties of the fruit, and whether these properties are temperature-dependent.

5.2 Materials and methods

5.2.1 Data collection and analyses

Six stiffness data sets and six weight loss data sets of 'Cripps Pink' apples stored at 0^{o}C, 2.5^{o}C, 5^{o}C, 10^{o}C, 20^{o}C, and 30^{o}C in NA from the experiment on 'Cripps Pink' apple (Chapter 3) were used for the establishment of mathematical models for prediction of apple stiffness during storage. Outliers of the data sets were discarded. No transformation was applied to the data sets. Modelling was based on the Least-Squares method, using the iterative non-linear regression procedure of Prism GraphPad 4.03 (version 4, release 3,

2005, GraphPad Software, Inc, San Diego California, USA) and DataFit 8.1 (version 8, release 1, 2005, Oakdale Engineering, USA) software packages.

5.2.2 Selection of model

According to the theory and method of measuring apple stiffness as mentioned previously, the structure and properties of sound received and analysed by the AWETA microphone were affected by properties of the plunger, the surroundings and the apple itself. All stiffness measurements were conducted under uniform storage conditions (temperature, pressure and volume of the atmosphere). Besides, the properties of the plastic plunger were unchanged. Therefore, measured values of apple stiffness were a function of the apple properties only. Specifically, the properties of sound generated and scattered by the apple were influenced by the size, shape and surface characteristics of the apple. These factors could be neglected because the sample populations for all apple treatments were selected for homogeneity at the beginning of the experiment. The sound properties of the apple had two components, intrinsic and extrinsic sound attributes (Pedersini et al., 2000). The intrinsic attributes were the internal vibrational properties and the mechanical/fluidodynamical properties of the apple. The extrinsic properties included active attributes related to how the apple irradiated sound waves into the environment, and passive attributes related to how the apple reflected, diffused and absorbed incident sound waves (Pedersini et al., 2000). Internal properties of the apples changed during storage due to the effects of storage conditions. Properties of the apple included stiffness, elasticity, density and inertia, which affect the restoring forces by which the apple can recover from the deformation caused by a foreign impact. The apples

in this experiment had almost identical rates of water loss during storage, so differences in the sound generated from apples stored at different temperatures were dependent only on changes in the properties comprising the mechanical strength of the cell walls. These changes were caused mostly by biochemical reactions occurring within the fruit flesh. Changes in the properties of the apple have strong effects on the amplitude, frequency and damping of its vibration (Gaver, 1993) and irradiated sound. Therefore, the exponential decay model (Eq. 4.6), which reflected changes in apple texture caused by biological processes (see Chapter 4), was used to fit to the stiffness data sets.

To account for the theories of softening described previously, the exponential decay model (Eq. 4.6) was rewritten as follows:

$$S(t) = \text{Smin} + \text{Span}*\text{Exp}(-k_s*t) \qquad (5.1)$$

Where $S(t)$ was stiffness of the apples at certain storage time t (Stiffness index – S); Smin was stiffness of the apples at plus infinite time (S); Span was the drop in stiffness from the initial value to the minimum value (S); k_s was the rate of softening (Day^{-1}); t was the duration of storage (Day).

Smin and Span were assumed to be shared parameters, dependent on apple cultivars and independent of post-harvest factors. Span was also dependent on harvest date. The value of Span was lower if the apples were late harvested and higher if early harvested. In contrast, k_s was a specific parameter dependent on apple cultivar and storage conditions, especially temperature.

5.3 Results

5.3.1 Modelling change in stiffness of 'Cripps Pink' apples during storage

The results of fitting the exponential decay model (Eq. 5.1) to the six stiffness data sets are shown in Figure 5.1 and Table 5.1. These indicate that the model had higher fitness (R^2 and Abs. sum squares of residuals) in the middle of the temperature range (5°C, 10°C and 20°C) and lower fitness at the two ends of the range (0°C, 2.5°C and 30°C). The curves for 0°C and 2.5°C were likely to underestimate stiffness during the first 30 and 25 days of storage, respectively. The apples changed from being supplied with substances like water and nutrients by the apple trees at high temperature (10°C to 20°C) of the natural environment to being picked off the trees and put into the storage at much lower temperatures (0°C and 2.5°C). Because the apples were still living materials after harvested, they also have unusual responses to substantial changes of the environment. This large environmental variation may have caused an increase in the stiffness of apples when transferred from normal temperature (20°C) to very low temperatures (0°C and 2.5°C). This also led to high relative standard errors of k_s, 32.86% and 21.46%, at the two temperatures respectively. However, the two curves better described the softening process during the middle and final phases. The relative standard error of the parameter Span was very low (0.894%). Deviations of measured stiffness values of the apples from the curve for all temperature treatments were not significant and the model explained 89.82% (Appendix 5.1) of the variation in the stiffness data. This indicated that the

exponential decay model could be used to characterise the softening process and predict change in the stiffness of 'Cripps Pink' apples.

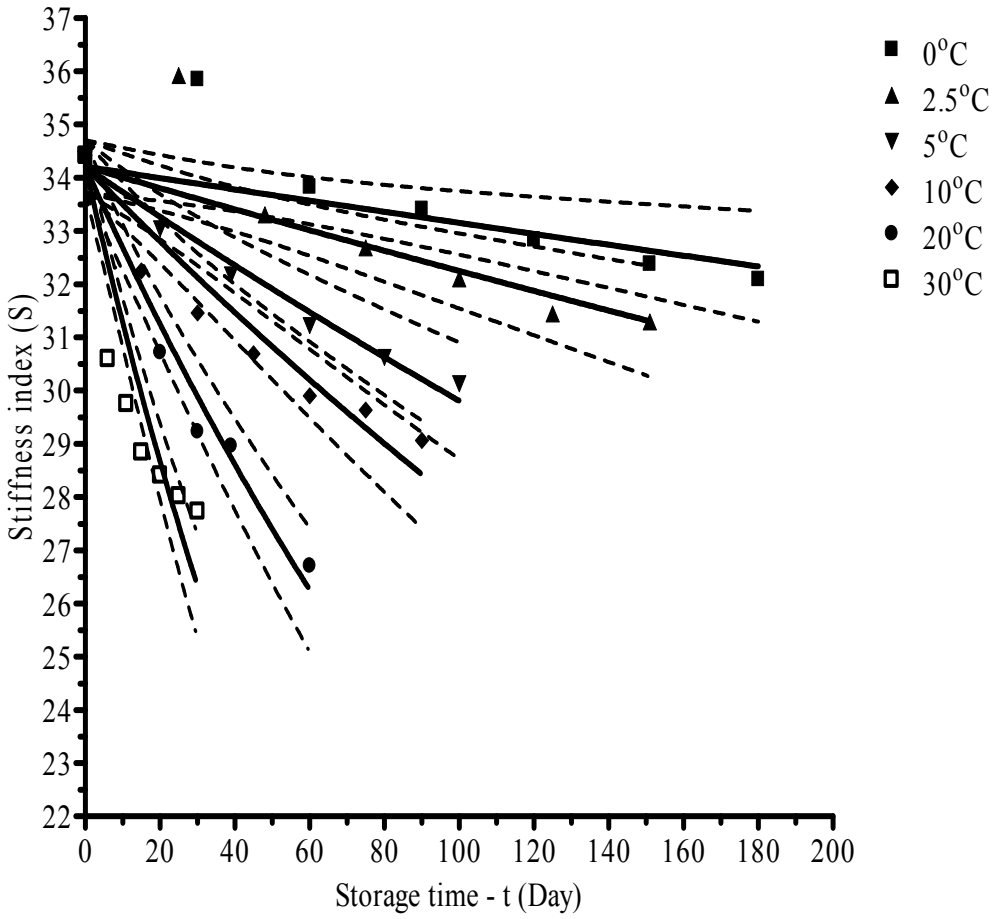


Figure 5.1 Result of fitting the exponential decay model (Eq. 5.1) to the six stiffness data sets together with 95% confidence bands. The symbols represent the stiffness mean of 25 apples. The continuous curves represent the model estimates describing responses of softening to different storage temperatures. The two broken curves, above and below of each curve, show 95% confidence band.

Table 5.1 Non-linear regression analysis of the exponential decay model (Eq. 5.1) using the global modelling procedure. The result is shown in Figure 5.1

	Storage temperature (°C)						
	0	2.5	5	10	20	30	Global (shared)
Best-fit values:							
- S_{min} (S) [(a)]	7	7	7	7	7	7	
- Span (S)	27.21	27.21	27.21	27.21	27.21	27.21	27.21
- k_s (Day^{-1})	0.000396	0.000747	0.001763	0.002652	0.005757	0.01139	
Std. Error:							
- Span (S) (%)[(b)]	0.2433 (0.894%)	0.2433 (0.894%)	0.2433 (0.894%)	0.2433 (0.894%)	0.2433 (0.894%)	0.2433 (0.894%)	0.2433 (0.894%)
- k_s (Day^{-1}) (%) [(b)]	0.000130 (32.86%)	0.000160 (21.46%)	0.000264 (14.99%)	0.000290 (10.94%)	0.000536 (9.31%)	0.000924 (8.11%)	
Goodness of fit:							
- Degrees of freedom							32
- R^2	0.593	0.698	0.9756	0.8971	0.9689	0.7387	0.8982
- Abs. Sum squares of residuals	4.131	5.103	0.3165	2.098	1.014	8.412	21.07
- Sy.x							0.8115
Deviation from Model	ns	ns	ns	ns	ns	ns	

(a) Fixed value, based on the experimental data, not estimated, so no standard error

(b) Relative standard error (Rel. S.E) was expressed as a percentage of the estimate values (Rel. S.E. = Standard error / parameter estimate).

ns: Non significant deviation from the model *(P > 0.05)*

5.3.2 Relationship between stiffness and weight loss of 'Cripps Pink' apples

The water potential and physical properties of cell walls are interdependent and affect the cell turgor of fresh fruit. These are significant factors forming and influencing the elasticity of the cell wall and fruit overall. Elasticity is the ability of materials to return to their original shape after being deformed (Landahl et al., 2003). Therefore, change in water content will affect turgor, and therefore elasticity, as well as the physical properties of the fresh fruit. Turgor decreases exponentially with water content (Wills et al., 1998), and exhibits linear correlation with stiffness (Nilsson et al., 1958). Therefore, stiffness relates exponentially to the water content of fresh fruit (Hertog et al., 2004). Water loss due to evaporation is the main cause of apple weight loss during storage, so apple stiffness can be expected to decrease exponentially with increase in weight loss.

Based on theories of fruit softening and an exponential reduction in stiffness with weight loss, an exponential model (Eq. 5.1) was developed and fitted to the six data sets of apple stiffness corresponding to weight loss at six temperatures.

$$S(w) = S_{min} + \text{Span}*\text{Exp}[-k_{s'}*w] \qquad (5.2)$$

Where $S(w)$ was stiffness of the apples at weight loss level w, (S); $k_{s'}$ was the rate of softening with weight loss (% $^{-1}$); and w was weight loss of the apples (%).

A very good global fitness (R^2 = 0.936), sum of squares of residuals (Abs) = 10.78 and Sy.x = 0.5995, and good fit at all six temperatures (Appendix 5.2) indicated that the stiffness of 'Cripps Pink' apples reduced exponentially with an increase in weight loss

(Fig. 5.2). The exponential model (Eq. 5.2) was able to characterise change in apple stiffness in response to an increase in weight loss at all storage temperatures from 0°C to 30°C.

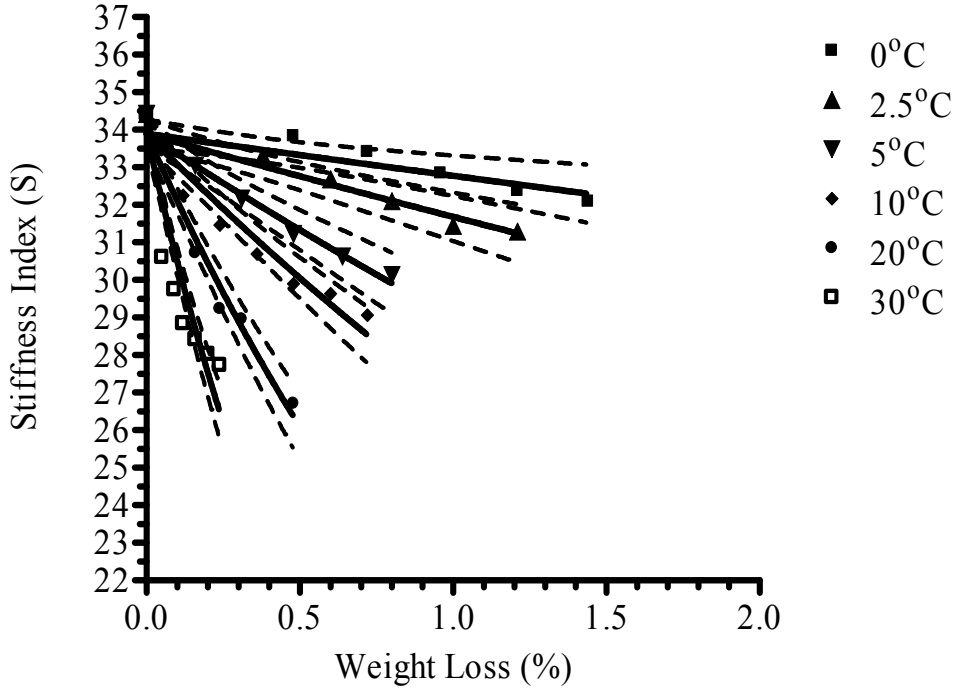


Figure 5.2 Result of fitting the exponential model (Eq. 5.2) to the six stiffness data sets, which were collected at different levels of weight loss values during storage. The symbols represent the stiffness mean of 25 apples. The continuous curves represent the model estimates describing the change in apple stiffness with weight loss at six temperatures. The two broken curves, above and below of each curve, show 95% confidence band.

5.3.3 Effect of storage temperature on stiffness reduction rate of 'Cripps Pink' apples

Although wvdp, ($p_s - p_a$) as well as weight loss was minimised and maintained equally for all treatments of apples, the stiffness of the apples decreased faster at higher storage temperatures, as shown in Figure 5.2. This implied that stiffness was affected by factors other than wvpd (or weight loss). Despite uniform weight loss, higher storage

temperatures led to greater loss of stiffness in the fruit flesh. This may have been caused by temperature-dependent biochemical reactions that resulted in degradation of the cell walls in the apple flesh. Previous studies suggest that other cellular factors also affect stiffness, and cause apples to soften during storage (Johnston et al., 2002a).

In order to investigate change in apple stiffness caused by biochemical reactions, the Arrhenius equation (Eq. 4.7) was fitted to best fit values of the parameter k_s at different storage temperatures from Table 5.1. The curve of fit is shown in Figure 5.3.

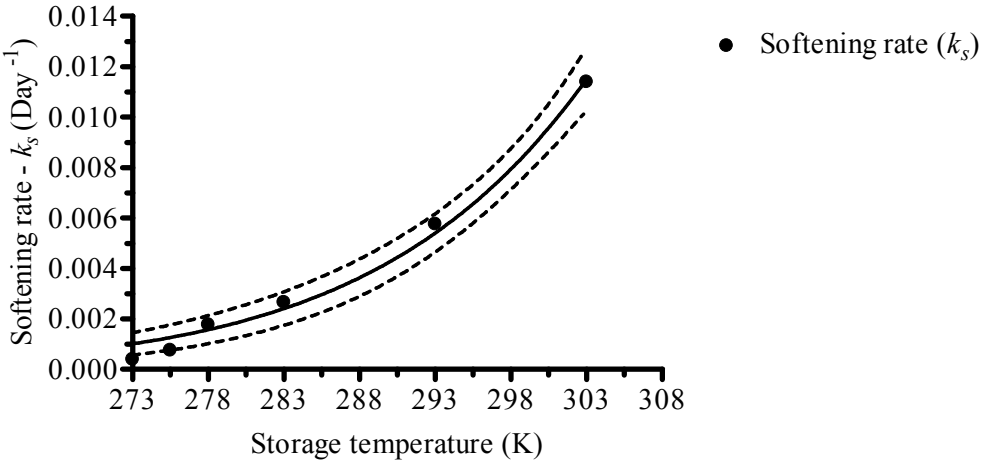


Figure 5.3 Change in softening rate (k_s) of 'Cripps Pink' apples with storage temperature using Equation 4.7 and best fit values of k_s from Table 5.1

The rate of apple softening in terms of stiffness (k_s) increased exponentially and substantially with storage temperature (T). The model of the Arrhenius equation had very good fit to the k_s' values, with a very high coefficient of determination (R^2 = 0.99). The sum squares of residuals (abs) and Sy.x were very low at 8.755e-007 and 0.0004679, respectively (Appendix 5.3).

5.3.4 Improvement of the exponential decay model

Although the exponential decay model (Eq. 5.1) explained 89.82% of variation in the stiffness data (Tab. 5.1), a single exponential decay model could not explain another 10.18% of variation in apple stiffness. As mentioned, weight loss also contributed to a reduction in stiffness of the fruit. Results from the experiment indicated that under consistent conditions of storage temperature - T and RH as determined, the rates of weight loss of apples from all temperature treatments were constant and uniform. Weight loss increased linearly with storage time (weight loss = 0.008*Storage time; R^2 = 0.9878). On the other hand, stiffness of apples decreased exponentially with weight loss (Eq. 5.2 & Fig. 5.2). Consequently, the stiffness component affected by weight loss (or wvpd) of the apples also decreased exponentially with storage time. As a result, total reduction in apple stiffness, incorporating both biochemical process and weight loss components, could be described by a new model that combined equations (5.1) and (5.2), whereby the variable *w* (weight loss) in the equation (5.2) was substituted by the variable *t* (storage time).

$$S(t) = \text{Smin} + (1/2)*\text{Span}*[(\text{Exp}(-k_{sbc}*t)) + (\text{Exp}(-k_{swl}*t))] \quad (5.3)$$

Where k_{sbc} was the softening rate caused by biochemical reactions (Day^{-1}) using Equation 4.7; and k_{swl} was softening rate caused by weight loss (Day^{-1}).

Table 5.2 Non-linear regression analysis of the improved exponential decay model (Eqs. 4.7 & 5.3) (Appendix 5.6) using the global modelling procedure. The result is shown in Figure 5.5.

	Storage temperature (°C)						
	0	2.5	5	10	20	30	Global (shared)
Best-fit values:							
- S_{min} (S) [(a)]	7	7	7	7	7	7	
- Span (S)	27.39	27.39	27.39	27.39	27.39	27.39	27.39
- k_{sbc} (Day $^{-1}$)	0.00092	0.00170	0.00406	0.00626	0.01417	0.02864	
- k_{swl} (Day $^{-1}$)	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001	0.0000001
Std. Error:							
- Span (S)	0.2652	0.2652	0.2652	0.2652	0.2652	0.2652	0.2652
- k_{sbc} (Day $^{-1}$)	0.00222	0.00240	0.00279	0.00315	0.00407	0.00491	
- k_{swl} (Day $^{-1}$)	0.00186	0.00186	0.00186	0.00186	0.00186	0.00186	0.00186
Goodness of fit:							
-Degrees of freedom							31
- R^2	0.6543	0.7314	0.9793	0.9119	0.9867	0.812	0.9198
- Abs. Sum squares of residuals	3.509	4.539	0.2683	1.797	0.4343	6.052	16.6
- Sy.x							0.7318
Deviation from model	ns	ns	ns	ns	ns	ns	

(a) Fixed value, based on the experimental data, not estimated, so no standard error
ns: Non significant deviation from the model *(P > 0.05)*.

The rate of weight loss was kept constant and equal for apples in all six temperature treatments, so the softening rate caused by weight loss (k_{swl}) was also constant (Tab. 5.2). Results from the global modelling using the equation (5.3) showed that the softening rate caused by the biochemical reactions (k_{sbc}) also increased exponentially with storage temperature - T (Tab. 5.2), thereby complying with the Arrhenius equation (Eq. 4.7). Fitting the model of Arrhenius equation (Eq. 4.7) to the best-fit values of the parameter k_{sbc} from Table 5.2 resulted in a very high level of fit (R^2 = 0.9913, abs. sum squares of

residuals = 4.892e-006 and Sy.x = 0.001106) (Appendix 5.7). The fitness of the Arrhenius equation (Eq. 4.7) to the best fit values of k_{sbc} was slightly improved (Fig. 5.4) (compare Appendix 5.3 with Appendix 5.7).

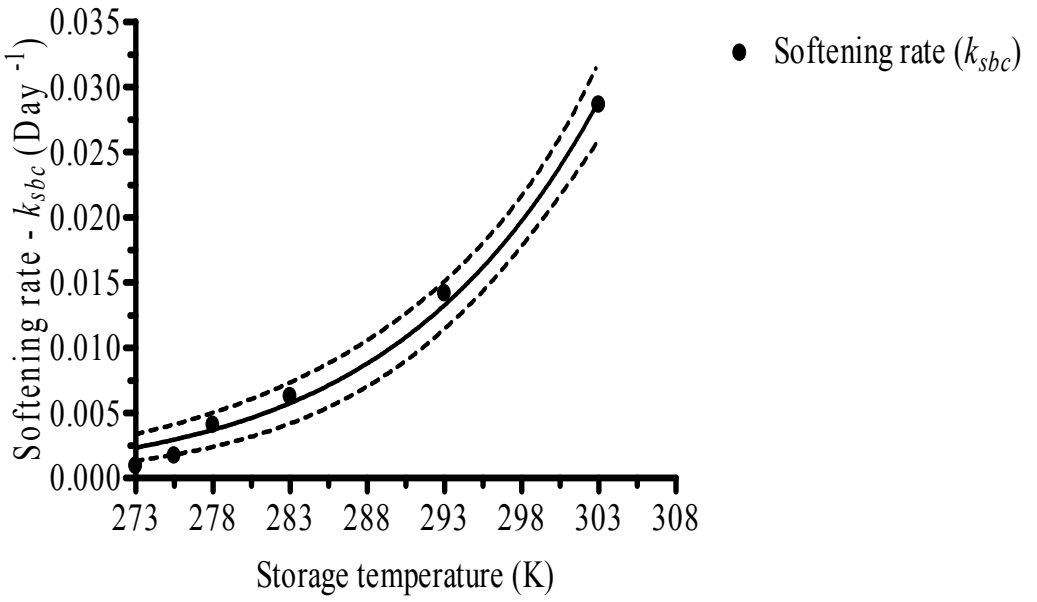


Figure 5.4 Change in the softening rate (k_{sbc}) of 'Cripps Pink' apples with storage temperature, using Equation 4.7 and the best fit values of k_{sbc} from Table 5.2.

In comparing the fitness (R^2, Abs. sum of squares of residuals and Sy.x) between the two models, the improved exponential decay model (Eq 5.3) & (Fig. 5.5) was slightly better than the exponential decay model (Eq. 5.1) & (Fig. 5.1), at both the global level (0.9198, 16.60 and 0.7318 compared with 0.8982, 21.07 and 0.8115, respectively) and at each temperature regime (Tabs. 5.1 & 5.2). This implied that under conditions in which RH of the NA was 90% to 98% in storage temperatures of 0^{o}C to 30^{o}C, the reduction in stiffness of 'Cripps Pink' apples measured using AFS–AWETA was caused almost entirely by biochemical processes and partly by the wvpd in the storage. The improved

exponential decay model (Eq. 5.3) was the better model compared with the exponential decay model (Eqs. 5.1). In addition, AFS-AWETA could be used to measure both the mechanical strength of the cell wall and the tissue turgor pressure of the apples.

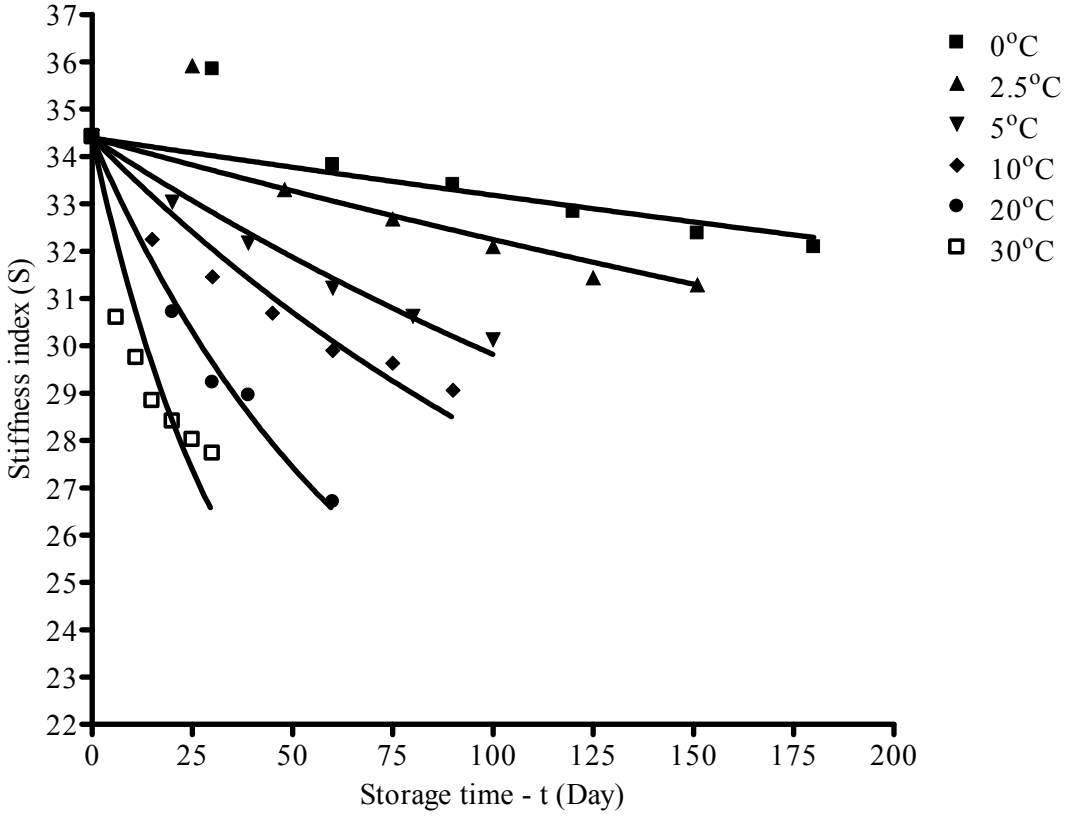


Figure 5.5 The improved exponential decay model (Eqs. 4.7 & 5.3) describing change in stiffness of 'Cripps Pink' apples with storage temperature and time. The model takes into account the biochemical processes and weight loss of the apples during storage. The symbols represent the stiffness mean of 25 apples. The continuous curves represent the model estimates describing the change in apple stiffness with time at six temperatures.

5.3.5 Development of the stiffness predicting model

Another new model to predict change in the stiffness of 'Cripps Pink' apples in response to storage temperature and duration was established that combined both equations (4.7) and (5.3). This accounted for the softening behavior due to both biochemical reactions and weight loss of the apples during storage. The result of fitting

the three-dimensional improved model (Eqs. 4.7 & 5.3) to the six stiffness data sets is shown in Figure 5.6. The fit of both the three-dimensional decay model (Eqns. 4.7 & 5.1) and the three-dimensional improved model (Eqns. 4.7 & 5.3) to the six stiffness data sets are shown in Table 5.3. While the two models had the same best fit values for the parameters Smin = 7 and Span = 27.199 (Appendices 5.4 & 5.8), the latter model had a better fit than the former. This indicated that the three-dimensional improved model was the better model for characterising the softening process and predicting the change in stiffness of 'Cripps Pink' apples during storage (Appendix 5.5 compared with Fig.5.6).

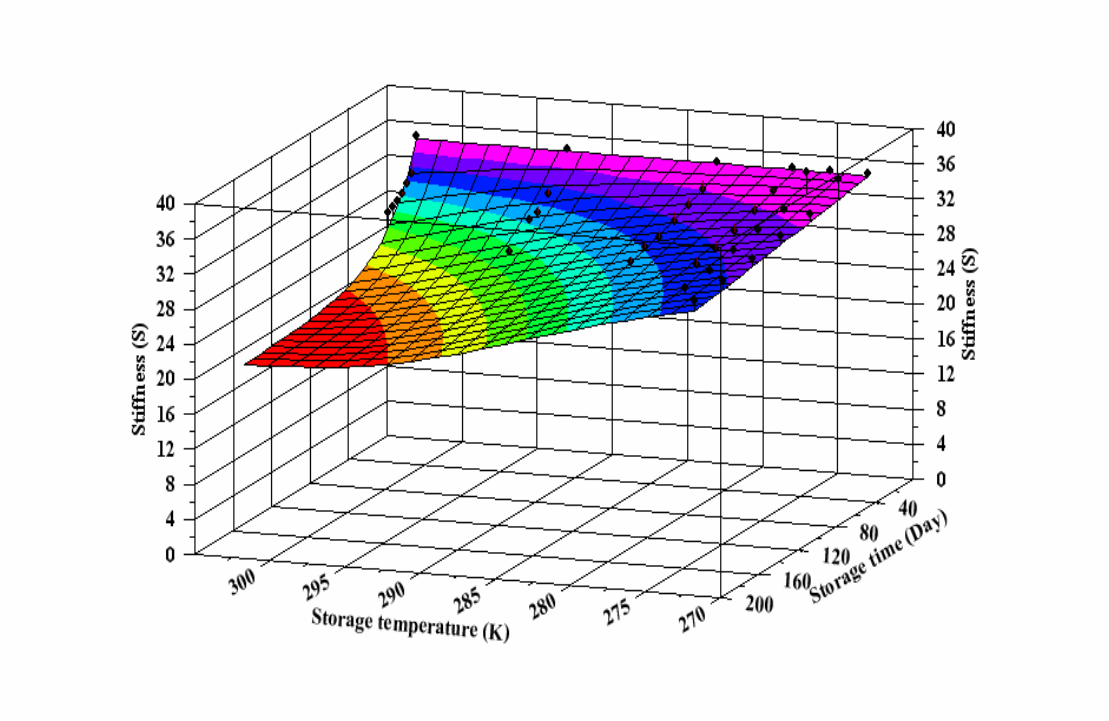


Figure 5.6 Fitting of the three-dimensional improved model of a 3D function of stiffness changing with storage temperature and time (Eqs. 4.7 & 5.3) to the stiffness data sets of 'Cripps Pink' apples stored at six temperatures from 0°C to 30°C in the single stage of NA, using the Least-Squares method with the iterative procedure of of non-linear regression analysis of the software package DataFit 8.1, 2005. Storage temperature (T) in K and storage time (*t*) in Day were independent variables of the stiffness function, *S(t)*. The model takes into account the biochemical processes and weight loss of the apple. The surface represents the prediction model while the symbols represent the stiffness mean of 25 apples.

Table 5.3 Comparison of the fitness of the three-dimensional decay model and the three-dimensional improved model

	Model and equations of model	
	The three-dimensional decay model (Appendix.5.4)	The three-dimensional improved model (Appendix.5.8)
	(4.7) & (5.1)	(4.7) & (5.3)
Residual sum of squares (Abs)	38.467761632	33.473527587
Standard error of the estimate	1.033706406	0.951151898
Coefficient of multiple determination (R^2)	0.814109725	0.838243688
Adjust coefficient of multiple determination (R_a^2)	0.803782488	0.833871896

5.4 Discussion

The fitting results indicated that the softening rate of apple stiffness, k_s of Equation 5.1 and k_{sbc} of Equation 5.3 increased exponentially and substantially with storage temperature (T) (Figs. 5.3 & 5.4). This confirmed an earlier recommendation that the non-destructive acoustic stiffness method is dependent not only on storage RH but also storage temperature (Hertog et al., 2004). Higher storage temperature can accelerate the rate of metabolism processes occurring in the fruit flesh (Watkins, 2003) and result in faster softening. This result was in agreement with earlier reports that short term texture changes under unfavorable storage conditions were mainly due to reduction in cell water potential. These changes were due to the decline in water volume while mechanical

properties of the cell wall remained the same (Herppich et al., 1999). Changes in fresh fruit texture during long term storage were totally independent of water potential and mostly related to alterations in the chemical composition of cell wall substances caused by metabolic processes (Herppich et al., 2003). In addition, a high fitness of the exponential decay model (Fig. 5.1 and Tab. 5.1) and a very high fitness of the model using the Arrhenius equation (Fig. 5.3) indicated that at high RH where weight loss was negligible, loss in stiffness of 'Cripps Pink' measured by acoustic response methods could be attributed almost entirely to metabolic activity. Similar results were found in pear (Nerya et al., 2001) and tomato (Hertog et al., 2004). This finding confirmed previous suggestions that softening rate is dependent mainly on biochemical reactions occurring within the apple flesh.

Softening in the apple flesh was a complex process caused mainly by biochemical processes and partly by weight loss during storage at high RH. The rate of softening caused by biological processes increased exponentially with storage temperature, complying with the Arrhenius equation. The rate of softening caused by weight loss was constant and independent of storage temperature, under conditions of uniform wvpd. Higher storage temperature caused a higher rate of flesh softening and resulted in fruit having shorter storage life. AFS-AWETA could be used widely for monitoring changes in apple stiffness due to changes in textural properties and weight loss during storage, especially in commercial supply chains. The three-dimensional improved model was the best model for characterising the softening process in apple flesh and predicting change in the stiffness of apples during storage.

5.5 Summary

In summary, the stiffness of 'Cripps Pink' apples decreased exponentially with storage time. On the other hand, softening rate k_s and k_{sbc} increased substantially and exponentially with storage temperature, complying with the Arrhenius equation. Both the exponential decay model (Eq. 5.1) and the improved exponential decay model (Eq. 5.3) had higher fitness at 5°C, 10°C and 20°C than at 0°C, 2.5°C and 30°C. However, for temperatures from 0°C to 2.5°C, the models only underestimated softening during the first 30 days of storage, and provided more accurate prediction thereafter. In contrast to firmness, decrease in apple stiffness was dependent on both biochemical processes caused by temperature and on weight loss generated by wvpd in storage. Therefore, the model for prediction of change in apple stiffness was a combination of the improved exponential decay model (Eq. 5.3) and the Arrhenius equation (4.7), which took both effects into account. Wvpd was kept uniform for 'Cripps Pink' apples in all temperature treatments, so decline in stiffness due to water loss of the apple was independent of temperature; and the softening rate k_{swl} was constant. In the case of low wvpd or high RH (90-98%) in storage, loss in stiffness as measured by AFS-AWETA may be caused mostly by biochemical processes occurring in the fruit flesh. The improved exponential decay model (Eq. 5.3) had a high global fitness of R^2 = 91.98% (Tab. 5.2) in a temperature range from 0°C to 30°C. The three-dimensional improved model also had high fitness (R^2 = 83.82%) (Tab.5.3). Therefore, the two models were the best models for prediction of change in the stiffness of apples during NA storage.

Smin and Span for both stiffness prediction models were shared parameters that were dependent on cultivar and pre-harvest factors but not on post-harvest factors. Span was also affected by the harvest date of the fruit. Late harvested fruit may have lower values of Span compared to early harvested fruit. On the other hand, the softening rate k_{sbc} was a cultivar-specific parameter influenced by post-harvest factors, particularly temperature. The softening rate k_{sbc} increased with storage temperature. The ratio of Span to S_{min} was appropriately 4.00.

Finally, AFS-AWETA had potential for use as a standard and objective tool for repeated measurement of overall apple texture in commercial supply chains because of its convenience and low cost relative to the MT puncture test.

CHAPTER VI

GENERAL DISCUSSION AND CONCLUSION

6.1 General discussion

This experiment was designed to characterise changes in the firmness, stiffness, background colour, ethylene and CO_2 production of 'Cripps Pink' apples stored under different conditions. As in previous studies of apple softening in other cultivars, the softening pattern of 'Cripps Pink' apples were dependent on storage temperature (Johnston et al., 2001c). The firmness of apples stored at 0^{o}C, 2.5^{o}C and 5^{o}C and the stiffness of apples stored at 0^{o}C and 2.5^{o}C, exhibited three typical phases of softening: a slow softening phase, followed by a rapid softening phase, and then a final slow softening phase. However, at higher temperatures, the firmness and stiffness of 'Cripps Pink' apples entering immediately into the phase of rapid softening, which was followed by the final slow softening phase.

Softening patterns of apple are affected by their polysaccharide structures, which is cultivar-specific (Colgan et al., 2006). According to this study, the initial slow softening phase of 'Cripps Pink' apple stored in NA at 2.5^{o}C and 5^{o}C had durations of 25 and 20 days, respectively. In contrast, this initial phase lasted only 13 days for 'Cox's Orange Pippin' apple stored at 3^{o}C (Johnston et al., 2003), was as long as 30 days for 'Elstar' apple stored at 5^{o}C (Sman and Sanders, 2005).

Softening of fruit flesh is mainly due to degradation of cell wall strength (Knee, 1973) caused by biochemical reactions occurring within the fruit flesh. CA with LO concentration may reduce softening and prolong the first phase of softening compared to

NA storage due to its influence on the oxygen-dependent reactions. NA storage significantly improved the red colour of the apples compared to CA, despite having the same storage temperature (0^oC). This indicated that besides low temperatures (Creasy, 1968; Faragher, 1983), a relatively high concentration of O_2 in air stimulated anthocyanin accumulation in the apple skin. Low temperatures of 0^oC to 2.5^oC in NA also suppressed production of ethylene and CO_2, inhibited the onset of climacteric period, retarded softening, improved the background colour of the apples, and lengthened storage life. On the other hand, at higher temperatures of 5^oC to 30^oC accelerated the onset of the climacteric, led to shorter initial slow softening phases, higher rates of the second rapid softening phase, and higher rates of ethylene and CO_2 production, leading to shorter storage life.

NA cold storage and CA storage also had some adverse effects on apple. Both methods affected apple ripening after removal from storage, even though they did not completely inhibit ethylene production during storage. They caused a significant reduction in the maximum productions of ethylene and CO_2 after removal from storage (Figs. 3.4 a, b, c, d, e & f). Apples transferred from the first stage of CA storage treatment had lower maximum production of ethylene at high temperatures compared with apples from the first stage of NA treatment. This may have resulted from longer storage duration (102 days) in CA compared to NA (61 days), but may also have been caused by a lower O_2 concentration in CA, which partly impaired ethylene biosynthesis in the apples. However, an immediate increase in ethylene production in apples transferred to NA at higher temperatures of 5^oC, 10^oC, 20^oC and 30^oC may have some benefits for apple marketing. On the other hand, increases in the production of ethylene and CO_2 coincided

with yellowing of the apple skin background colour and the beginning of the second phase of rapid softening for apples from all storage treatments. Softening occurred simultaneously and was proportional to the production of ethylene and CO_2 and changes in background colour from yellow-green to yellow.

The exponential decay model (Eq. 4.6) contained just three parameters, namely, Fmin, Span and k_s. Although simplistic in structure, the model satisfied the existing theories on softening in fruit (see 2.3.3 and 2.3.4) and had a high fitness to experimental data collected from a wide range of temperature conditions. The three parameters of the model represented the natural properties of the apple and basic characteristics of the softening process. According to the theories on softening in fruit, the first component of firmness was represented by the mathematical expression of Span*Exp[$-k_s*t$] in which Span was defined by the type and cultivar of fruit. It may also be influenced by harvest date. The expression of Span*Exp[$-k_s*t$] has maximum value at the time of harvest (t = 0 day). On the other hand, the second component of firmness was represented by the parameter, Fmin, and is constant during storage. Fmin was defined by the type and cultivar of fruit. Span and Fmin were likely to be independent of post-harvest and storage conditions. The parameter for softening rate, k_s was dependent on the interaction between the natural properties of the fruit and storage conditions such as temperature and gas concentration. The softening rate k_s increased exponentially with storage temperature, complying with the Arrhenius equation. It had lower values in CA storage compared with NA storage. The exponential decay model (Eq. 4.6) also complied with the theory that the firmness of the fruit stored at different temperatures always decreases during storage (Hertog, 2004). From Equation 4.6, we can see that apples had maximum firmness at the

beginning of storage, at t = 0 day. The value of the maximum firmness was Fmax = Fmin + Span. The apple had minimum firmness at the plus infinite time, $t = +\infty$ day. The value of the minimum firmness was Fmin = Fmin + 0. On the other hand, the mathematical expression of Span*Exp[-k_s*t] also reduced from Span (at t = 0 days) to approximately 0 (when t approximates to $+\infty$). The value of Span*[-k_s*t] was dependent on the natural properties of the apple and on storage conditions. The softening rate k_s increased with storage temperature because it was defined by the Arrhenius equation (Eq. 4.7). The parameters of the frequency factor A and the activation energy Ea of the Arrhenius equation were dependent on biochemical constituents in the fruit flesh, which were defined by the natural properties of apple cultivars. Generally, the mathematical expression of Span*Exp[-k_s*t] decreased with increase in storage temperature or duration. Therefore, the three-dimensional compound model, which consists of Equation 4.8 and Equation 4.9, including all parameters mentioned, was used to characterise the softening process of the apples during storage. The two models were also used to predict changes in firmness and keeping quality of the apples during storage. As described previously, the parameters of A, Ea, Fmin and Span were predefined by the cultivar of apple. The softening rate k_s was also predefined by cultivar in certain conditions of storage. From the exponential decay model (Eq. 4.6), it showed that if the value of the softening rate k_s is known and the storage time t is predefined, the firmness value of the apple at that the storage time can be determined. Similarly, for the three-dimensional compound model (Eqs 4.8 & 4.9), it indicated that if the storage temperature (T) and the storage time (t) were given, the firmness value of the apple at that storage time could also be determined. On the other hand, if the storage temperature (T) and the minimum

acceptable firmness value ($F(t)$) are predefined, the keeping quality of the apple (t), (in Day) could also be calcualated easily.

According to the theory on sound generation and transmittance relating to the physical properties of any objects, including fruit (see 2.4.1), similar to firmness, the rigidity of apple also declined during storage. The improved exponential decay model (Eq. 5.3), which combined the exponential decay model (Eq. 5.1) with the exponential model related to reduction in stiffness resulted from weight loss (Eq. 5.2), was used to predict change in both the rigidity and the turgor pressure of 'Cripps Pink' apple. Similar to the parameter for softening rate k_s in the exponential decay model for firmness prediction (Eq.4.6), the parameter of softening rate k_{sbc} of the improved exponential decay model (Eq. 5.3) was also dependent on apple cultivar and storage conditions. The wvpd was kept uniform for all temperature treatments, so the parameter for softening rate caused by weight loss k_{swl} was constant and independent of storage temperature and RH. The improved exponential decay model (Eq. 5.3) had slightly increased fitness for the same stiffness data sets as the exponential decay model (Eq. 5.1). The three-dimensional improved model (Eqs. 4.7 & 5.3) also had improved fitness compared to the three-dimensional decay model (Eqs. 4.7 & 5.1). This indicated that, although apple stiffness was affected mainly by biochemical reactions, particularly in long term storage, it was also influenced partly by weight loss.

6.2 Conclusion

The flesh firmness and stiffness of 'Cripps Pink' apples stored under NA conditions decreased with storage temperature and duration. The firmness of the apples stored at

0^{o}C, 2.5^{o}C and 5^{o}C, and the stiffness of the apples stored at 0^{o}C and 2.5^{o}C, exhibited three typical phases of softening, while the apples stored at higher temperatures underwent just two softening phases, that is, a rapid softening phase followed by a final slow softening phase. The durations of these softening phases varied with cultivar. The optimal temperature range for storage of 'Cripps Pink' apples was likely therefore 0^{o}C to 2.5^{o}C. Within this temperature range, the first softening phase was considerably extended, the softening rate of the second phase was reduced, and the apple background colour was also improved.

Apples in CA storage retained better firmness and stiffness than NA storage, but did not have improved background colour of the apple skin. On the other hand, CA had some adverse effects on ethylene production in the apples, particularly for long term stored apples. However, normal apple ripening could largely be restored by removal from CA storage.

Change in the background colour of 'Cripps Pink' apple skin may be considered as an indicator of apple softening and ripening under NA conditions since rapid softening and changes in background colour, ethylene and CO_2 production occurred simultaneously.

A combination of cold storage at 0^{o}C and CA (2 kPa O_2 : 1 kPa CO_2) during the first stage of apple storage was advantageous compared to NA cold storage alone, by reducing further production of ethylene and CO_2, and facilitating better maintenance of apple firmness, stiffness and background colour. This resulted in overall longer storage life.

Of the determined models, the exponential decay model (Eq. 4.6) gave best characterisation of decline in apple firmness and stiffness during NA storage. The model described apple firmness better than stiffness. Both apple firmness and stiffness

decreased exponentially with increases in storage temperature and time, complying with the Arrhenius equation. The softening curves describing firmness and stiffness displayed similar temperature-dependent patterns. The softening curves of apples stored at low temperatures (0^oC to 5^oC for firmness and 0^oC to 2.5^oC for stiffness) exhibited three typical softening phases, while those stored at higher temperatures only underwent the two final softening phases.

Although water status of the apple affected change in stiffness, the stiffness of apples stored at high RH (90%-98%) was mainly influenced by changes in the strength of the cell wall. Like firmness, apple stiffness also decreased with increase in temperature, due to acceleration of biochemical processes occurring within the fruit flesh. The softening rate k_s and k_{sbc} increased with temperature, complied with the Arrhenius equation. Similar to the case of firmness, the exponential decay model (Eq. 5.1) and the improved exponential decay model (Eq. 5.3) better characterised decline in apple stiffness in the middle of the temperature range than at the two ends of the temperature range. At lower temperatures of 0^oC-2.5^oC, the models underestimated softening in the first 30 days, but gave more accurate predictions thereafter. The models were therefore suitable for predicting the softness of apples placed into long-term low temperature storage, rather than short-term.

A combination of the exponential decay model (Eq. 4.6) and the improved exponential decay model (Eq. 5.1) with the Arrhenius equation (Eq. 4.7) was used to characterise and predict changes in the firmness, stiffness and keeping quality of 'Cripps Pink' apples stored at different temperatures during storage.

The Fmin and Span parameters of the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eq. 4.8); and the Smin and Span parameters of the exponential decay model (Eq. 5.1) and the improved exponential decay model (Eq. 5.3) were dependent on cultivar-specific properties, but were likely to be constant for a given cultivar harvested from different locations and in different seasons. However, the softening rate parameter k_s in the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eq. 4.8); and the parameter for softening rate caused by biochemical reactions k_s in the exponential decay model (Eq. 5.1) and k_{sbc} in the improved exponential decay model (Eq. 5.3) were likely to be dependent on both the cultivar-specific properties and the storage conditions. Validation of the exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eqs. 4.8 & 4.9) confirmed their usefulness for prediction of changes in the firmness and keeping quality of apples grown in different zones and harvested in different seasons.

In addition, because it is not practical to have repeated measures of fruit in a commercial supply chain, prediction of firmness as well as stiffness of the models becomes important in estimation of fruit quality and decision on target markets. Besides, because the AFS-AWETA may be useful for repeated evaluation of apple softening in research and for estimation of overall apple texture in commercial contexts, it is a convenient and relatively low cost instrument.

6.3 Future research

Temperatures of 0^oC to 3^oC are considered optimum for apple in NA storage. The exponential decay model (Eq. 4.6) and the three-dimensional compound model (Eqs. 4.8

& 4.9) were validated using two firmness data sets at 0^{o}C and 3^{o}C from the 'Cripps Pink' apple stored in conditions of commercial NA cold storage. However, for wider practical applications, the two models should be further validated using data sets covering over a greater temperature range, for example, from 0^{o}C to 30^{o}C. The exponential decay model (Eq. 4.6), the three-dimensional compound model (Eqs. 4.8 & 4.9) and the three-dimensional improved model (Eqs. 5.3 & 4.7) may be used to predict changes in the firmness and stiffness of 'Cripps Pink' apples during storage. Again, for wider practical application of the models, further validation using firmness and stiffness data sets from 'Cripps Pink' and other apple cultivars would be worthwhile. The three-dimensional improved model (Eqs. 5.3 & 4.7) should also be validated with stiffness data sets from apples stored at lower RH. In practice, apples may be subject to a series of different storage conditions before reaching consumers. Therefore, further development of the models using more varied data sets would add value by better reflecting the real-world commercial context of apple management.

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APPENDICES:

Appendix 1. Conferences attended and publications from this thesis

Appendix 1.1. Conferences attended and presented during the PhD candidature

24 November 2006	The Faculty Symposium of New Horizons in Agricultural Research Postgraduate Innovations at the University of Sydney, New South Wales, Australia.
1-5 July, 2007	The International Symposium on Modelling in Fruit Research and Orchard Management in Einsiedeln, Switzerland, organized by ISHS.
9 – 13 Sep, 2007	The 2007 Australasian Post-harvest Conference in Central Coast, New South Wales, Australia.

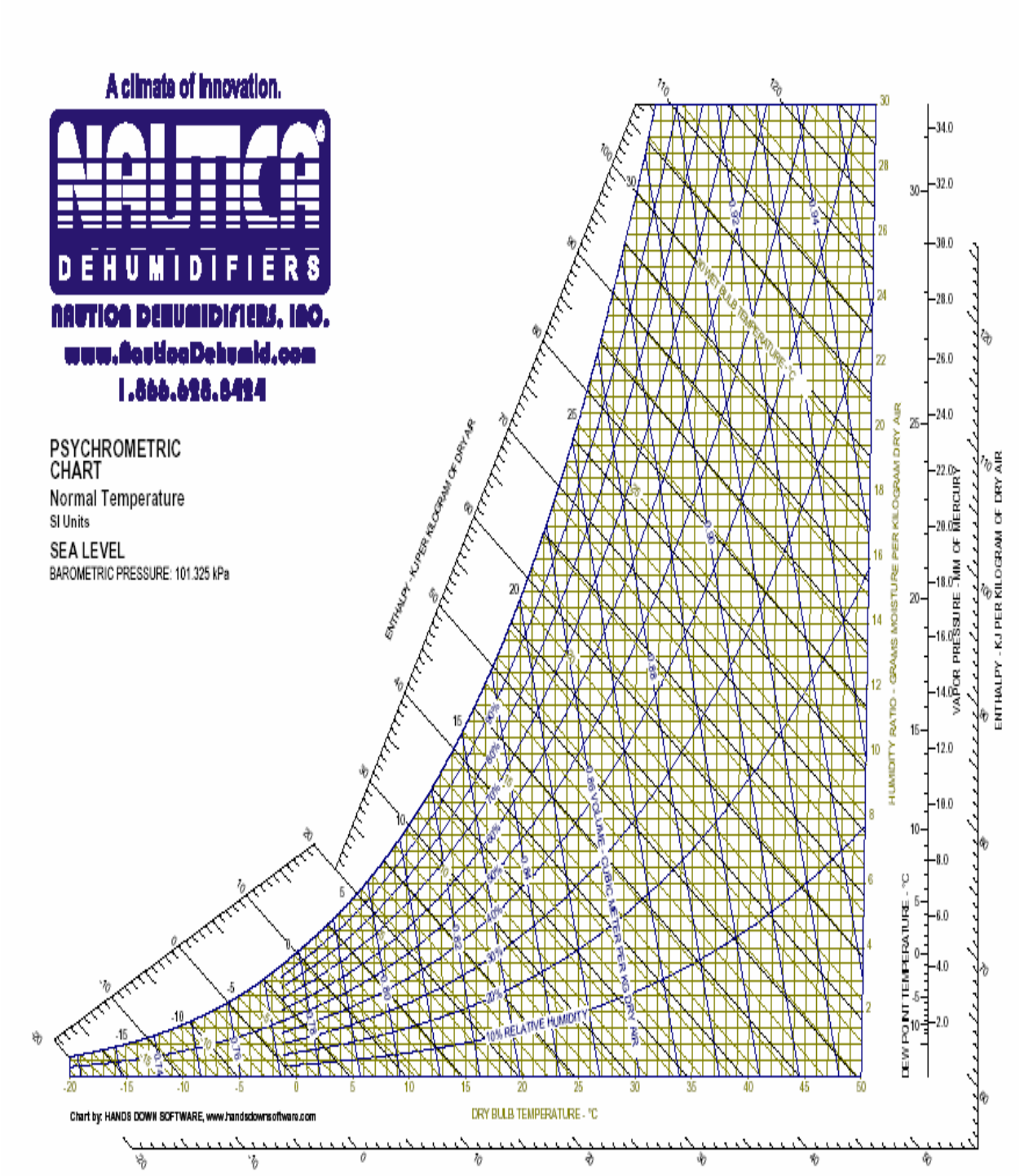
Appendix 1.2. Publications from this thesis:

1. Pham, V.T., R. McConchie, S. Morris, D. Tanner and R. Herbert. A mathematical model for predicting change in firmness of 'Pink Lady' ™ apple after harvest. *Proceedings of the Faculty Symposium of New Horizons in Agricultural Research Postgraduate Innovations,* 24 Nov., 2006, the University of Sydney, Australia.

2. Pham, V.T., R. McConchie, S. Morris, D. Tanner and R. Herbert. Prediction of firmness change in 'Cripps Pink'™ apples during storage. *Proceedings of the 8th International Symposium on Modelling in Fruit Research and Orchard Management,* 1 – 5 July, 2007 in Einsiedeln, Switzerland.

3. Pham, V.T., R. McConchie, S. Morris, D. Tanner and R. Herbert. Prediction of change in stiffness of 'Cripps Pink'™ apples during eegular air storage. *Proceedings of the 2007 Australasian Postharvest Conference,* 9 – 13 Sep., 2007 in Central Coast, New South Wales, Australia.

Appendix 2. Psychrometric chart (Literature)



Appendix 3 Statistical analysis of changes in quality attributes of 'Cripps Pink' apples stored in different conditions of storage

Appendix 3.15 Effect of storage conditions on ethylene production (µl.Liter^{-1}) of 'Cripps Pink' apple during storage

Storage Time (Day)	Storage temp. in CA 0°C	Storage temperature in the single phase NA storage (SNAS)					
		0°C	2.5°C	5°C	10°C	20°C	30°C
1	1.30 Aa	2.55 Ba	3.08 Ba	4.35 BCa	5.45 Ca	14.23 Da	26.35 Ea
13	1.10 Aa	3.22 Ba	3.14 Ba	3.76 BCa	5.56 Ca	13.60 Da	28.55 Ea
29	1.20 Aa	2.18 ABa	3.10 BCa	4.65 CDa	5.65 DEa	33.87 Fb	39.55 Gb
41	1.18 Aa	3.26 BCa	2.16 ABa	3.78 BCa	4.85 Ca	30.01 Dc	36.50 Ec
55	1.24 Aa	2.10 Aa	2.45 Aa	5.12 Ba	6.25 Ba	15.66 Ca	33.29 Dd

Means followed by the same letter within a row (capital letters) or a column (small letters) is not different at 5% level of significance according to Tukey test. Temperature, time and interaction account for 89.47%, 2.42% and 7.62% of the total variance, respectively.

Appendix 3.16 Effect of storage conditions on CO_2 production (ml.Liter^{-1}) of 'Cripps Pink' apple during storage

Storage Time (Day)	Storage temp. in CA 0°C	Storage temperature in the single phase NA storage (SNAS)						
		0°C	2.5°C	5°C	10°C	20°C	30°C	
1	1.45 Aa	1.67 B (*)a	2.55 Ca	3.74 Da	5.30 Ea	15.69 Fa	17.58 Fa	***
13	1.20 Aa	1.83 Ba	2.95 Ca	3.70 Da	6.30 Ea	15.48 Fa	15.36 Fb	***
29	1.36 Aa	2.28 Ba	2.88 Ca	3.57 Da	5.51 Ea	13.09 Fbc	11.61 Fc	***
41	1.17 Aa	2.39 Ba	3.19 Ca	3.99 Da	5.71 Ea	11.42 Fbd	19.24 Gd	***
55	1.17 Aa	1.98 Ba	2.57 Ca	3.40 Da	5.46 Ea	14.31 Fac	28.35 Ge	***

(*: significant difference at $P < 0.05$)

Means followed by the same letter within a row (capital letters) or a column (small letters) is not different at 5% level of significance according to Tukey test. Significance of F test for comparisons between storage times within a storage temperature (column) and between storage temperatures for each storage time (row) are also shown: 5% (*), 1% (**) and 0.1% (***).

Appendix 3.17 Maximum ethylene and CO_2 production of single stage of NA storage and the second stage of two-stage storage (NA storage at 0°C + NA storage at six temperatures ranging from 0°C to 30°C) of 'Cripps Pink' apples

Storage Tempe-rature (°C)	Maximum ethylene production (µl.kg^{-1}.hr^{-1})						Maximum CO_2 production (ml.kg^{-1}.hr^{-1})					
	Single stage	S.E.	Second stage	S.E.	*t*		Single stage	S.E.	Second stage	S.E.	*t*	
0	3.26	±0.2698	2.08	±0.1314	+3.931	*	2.55	±0.1239	1.97	±0.1235	+3.371	*
2.5	3.86	±0.4275	1.90	±0.1826	+4.215	*	3.08	±0.06173	3.48	±0.04217	+5.354	**
5	7.09	±0.3690	4.35	±0.4081	+4.980	**	4.04	±0.1124	3.80	±0.04097	+2.023	ns
10	11.26	±0.9647	8.16	±0.4067	+2.964	*	7.68	±0.3491	7.27	±0.01673	+1.182	ns
20	37.78	±1.940	22.54	±1.401	+6.366	**	21.46	±0.2839	16.39	±0.3984	+10.38	***
30	41.35	±1.157	32.47	±1.422	+4.844	**	28.35	±0.6410	25.96	±0.3510	+3.260	*

ns : Non significant difference ($P > 0.05$)

* : Significantly difference ($P < 0.05$).

**: Very significant different ($P < 0.01$)

***: Extremely significant difference ($P < 0.001$)

Values are means of three samples

Appendix 3.18 Maximum ethylene and CO_2 production of single stage of NA storage and the second stage of two-stage storage (CA + NA storage at six temperatures ranging from 0°C to 30°C) of 'Cripps Pink' apples

Storage Tempe-rature (°C)	Maximum ethylene production (µl.kg^{-1}.hr^{-1})						Maximum CO_2 production (ml.kg^{-1}.hr^{-1})					
	Single stage	S.E.	Second stage	S.E.	*t*		Single stage	S.E.	Second stage	S.E.	*t*	
0	3.26	±0.2698	1.25	±0.01535	+7.436	**	2.55	±0.1239	1.84	±0.1039	+4.429	*
2.5	3.86	±0.4275	1.47	±0.2598	+4.778	**	3.08	±0.06173	3.49	±0.1744	+2.206	ns
5	7.09	±0.3690	1.65	±0.1658	+13.45	***	4.04	±0.1124	3.57	±0.2189	+1.917	ns
10	11.26	±0.9647	1.68	±0.1087	+9.873	***	7.68	±0.3491	5.76	±0.05815	+5.421	**
20	37.78	±1.940	4.82	±0.1061	+1.6.96	***	21.46	±0.2839	13.93	±0.1364	+23.91	***
30	41.35	±1.157	6.57	±0.3869	+ 28.55	***	28.35	±0.6410	17.61	±0.1864	+16.09	***

Values are means of three samples

Appendix 3.19 Maximum ethylene and CO_2 production of second stage in NA storage between 'Cripps Pink' apples stored in the first stage in NA at 0^oC (*) and the apples stored in the first stage in CA (**).

Storage Tempe-rature (^oC)	Maximum ethylene production ($\mu l.kg^{-1}.hr^{-1}$)						Maximum CO_2 production ($ml.kg^{-1}.hr^{-1}$)					
	Seco-nd stage (*)	S.E.	Second stage (**)	S.E.	*t*		Seco-nd stage (*)	S.E.	Seco-nd stage (**)	S.E.	*t*	
0	2.08	±0.1314	1.25	±0.01535	+6.276	**	1.97	±0.1235	1.84	±0.1039	+0.7840	ns
2.5	1.90	±0.1826	1.47	±0.2598	+1.357	ns	3.48	±0.04217	3.49	±0.1744	+0.04421	ns
5	4.35	±0.4081	1.65	±0.1658	+6.129	**	3.80	±0.04097	3.57	±0.2189	+1.031	ns
10	8.16	±0.4067	1.68	±0.1087	+15.40	***	7.27	±0.01673	5.76	±0.05815	+24.88	***
20	22.54	±1.401	4.82	±0.1061	+12.61	***	16.39	±0.3984	13.93	±0.1364	+5.833	**
30	32.47	±14.22	6.57	±0.3869	+17.57	***	25.96	±0.3510	17.61	±0.1864	+21.02	***

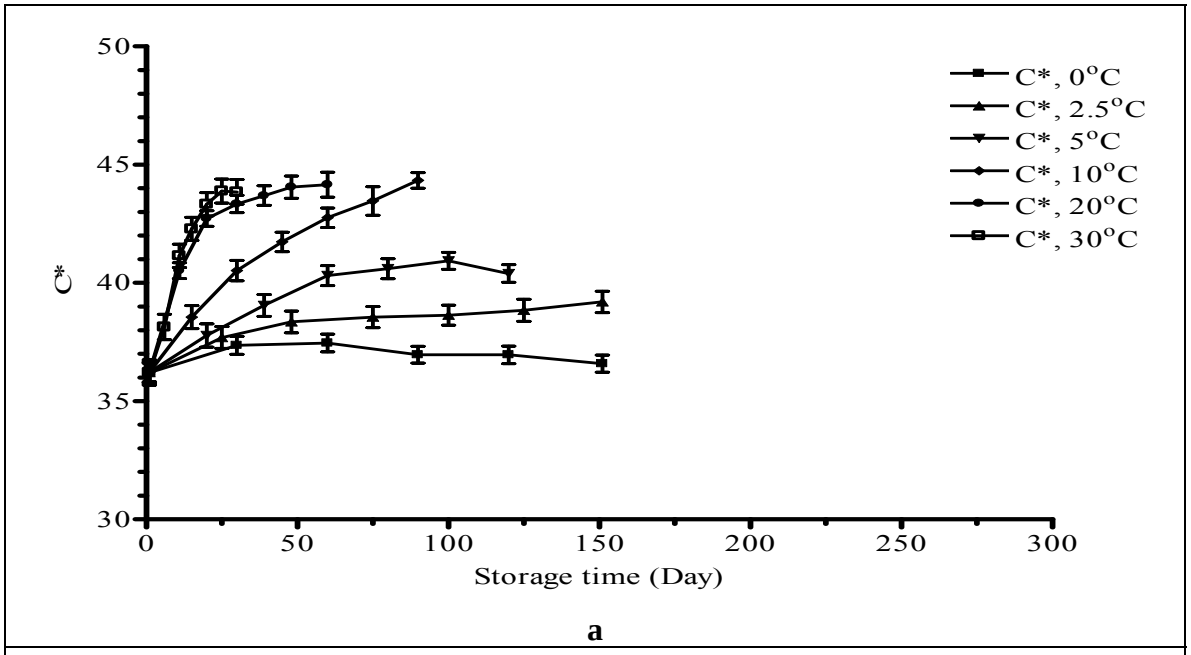
Values are means of three samples.

Appendix 3.20 Effect of storage temperature on change in *h* of skin background colour of 'Cripps Pink' apple (green apples) during NA storage

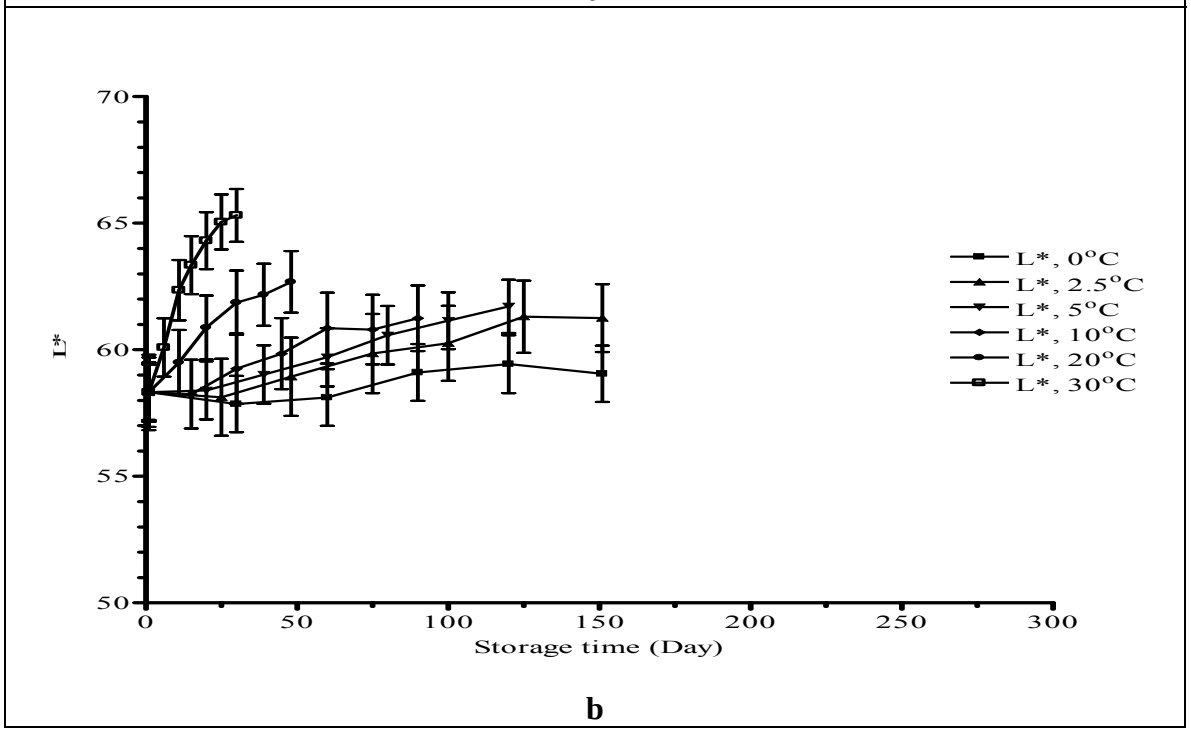
Storage time (Day)	Storage temperature in NA (°C)					
	0	2.5	5	10	20	30
1	50.07Aa	50.09Aa	50.14Aab	50.18Aa	50.12Aa	50.17Aa
11					52.51Ab	54.52Ab
15				51.04Aa		56.25Ac
20			50.57Aa		53.00Aab	58.44Ac
25		49.05Aa				61.36Bd
30	47.48Aab			51.65Aac	55.15ABc	63.24Bd
39			48.98Aab		57.46Ae	
48		48.14Aa			60.12Bd	
60	46.39Abc		48.16ABb	53.87BCbc	61.63Cd	
75		48.84Aa		53.69Ab		
90	47.53Aac			54.83Ab		
100		48.90Aa	49.22Aa			
120	47.99Aab		51.75Aa			
151	48.10Aa	50.27Aa				

Means followed by the same letter within a row (capital letters) or a column (small letters) is not different at 5% level of significance according to Tukey test. Significance of F test for comparisons between storage times within a storage temperature (column) and between storage temperatures for each storage time (row) are also shown: 5% (*), 1% (**) and 0.1% (***)

Appendix 3.21 Changes in C* and L* of 'Cripps Pink' apples stored under the following conditions: a) and b) Lot I: Single stage storage in NA at six temperatures ranging from 0°C to 30°C, c) and d) Lot II: Two-stage storage, firstly in NA at 0°C for 61 days followed by NA at six temperatures ranging from 0°C to 30°C, e) and f) Lot III: Two-stage storage, firstly in CA (2 kPa O_2 : 1 kPa CO_2) at 0°C for 102 days followed by NA at six temperatures ranging from 0°C to 30°C. Symbols represent average values ±SE (n = 25).



a



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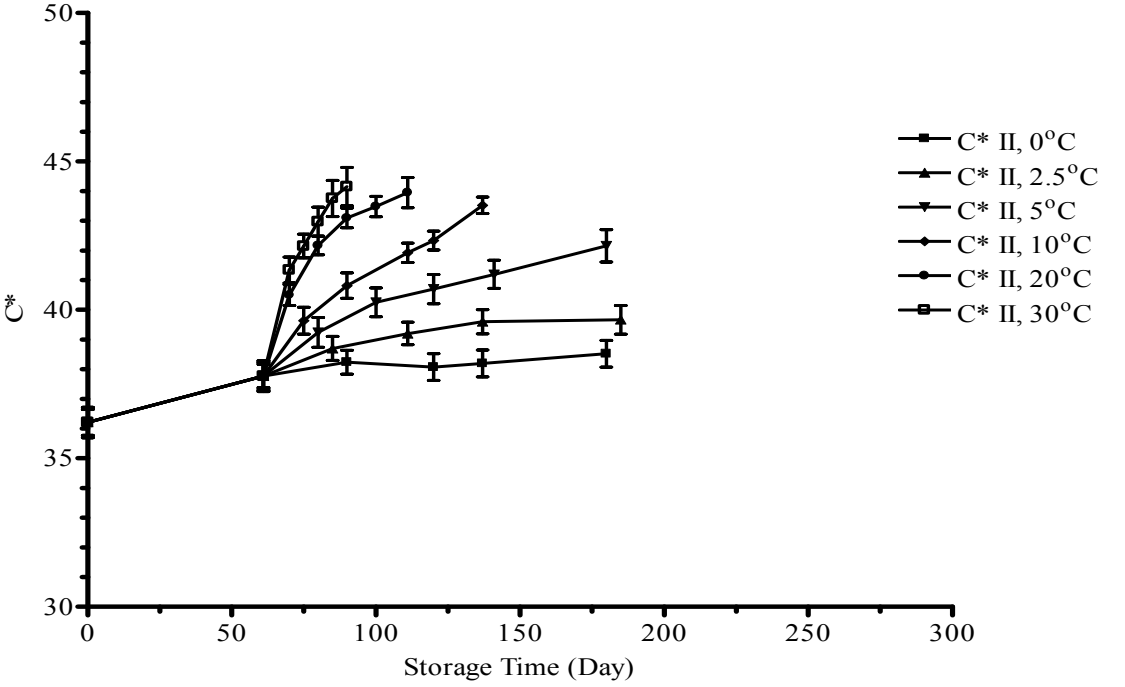
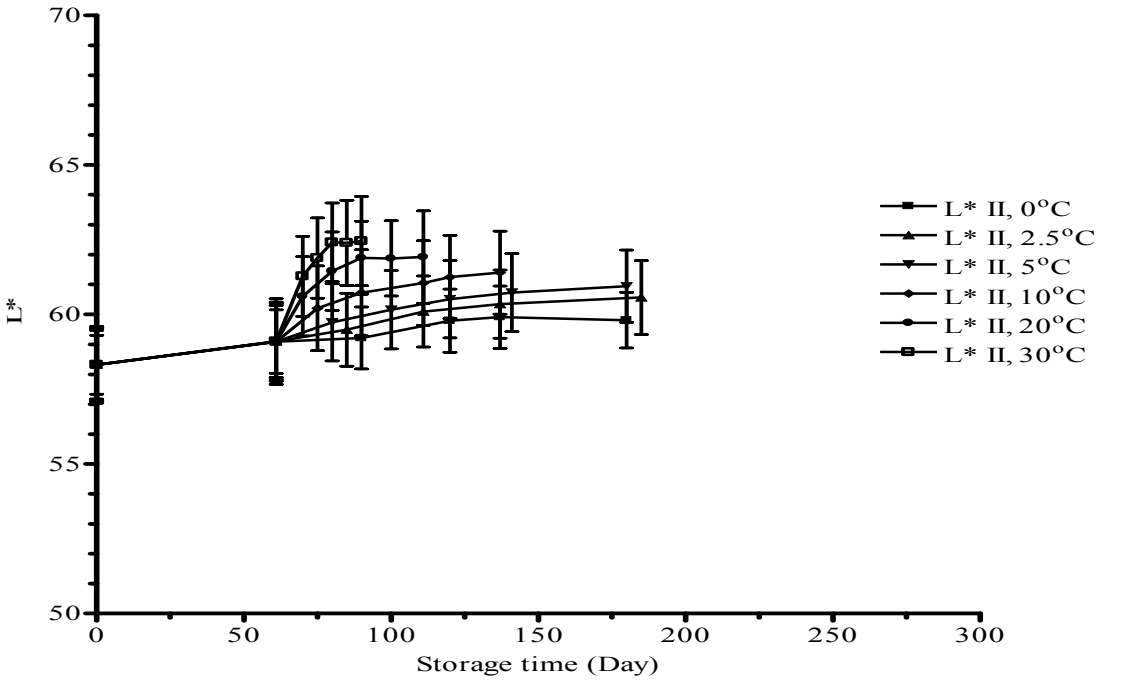
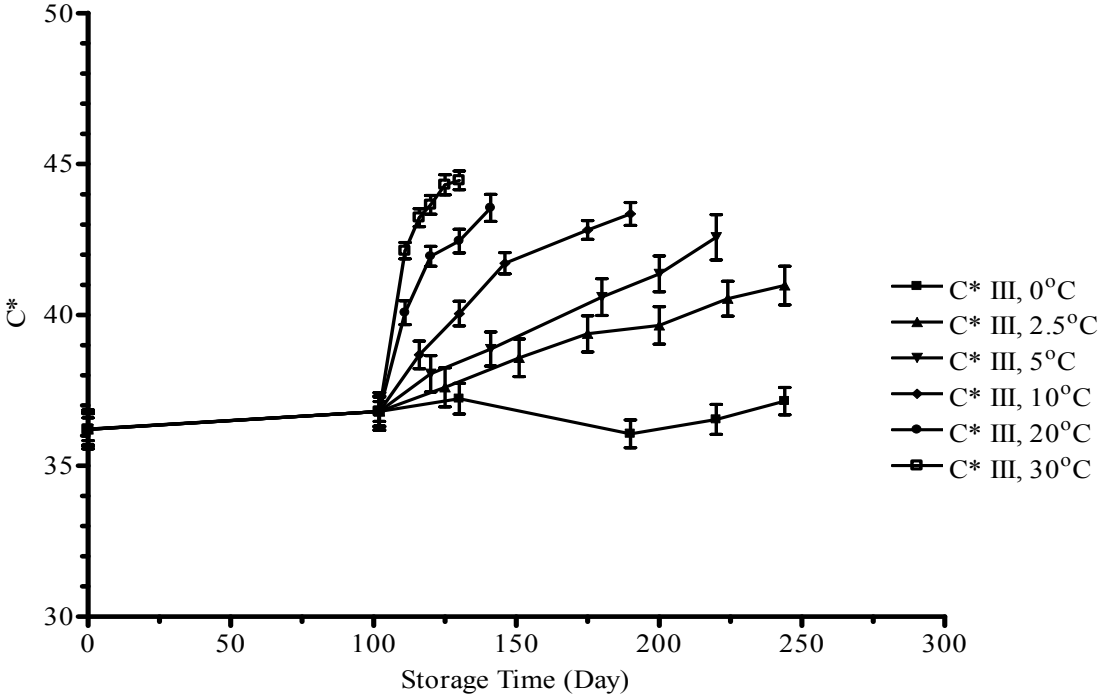

C*
Storage Time (Day)
C* II, 0°C
C* II, 2.5°C
C* II, 5°C
C* II, 10°C
C* II, 20°C
C* II, 30°C

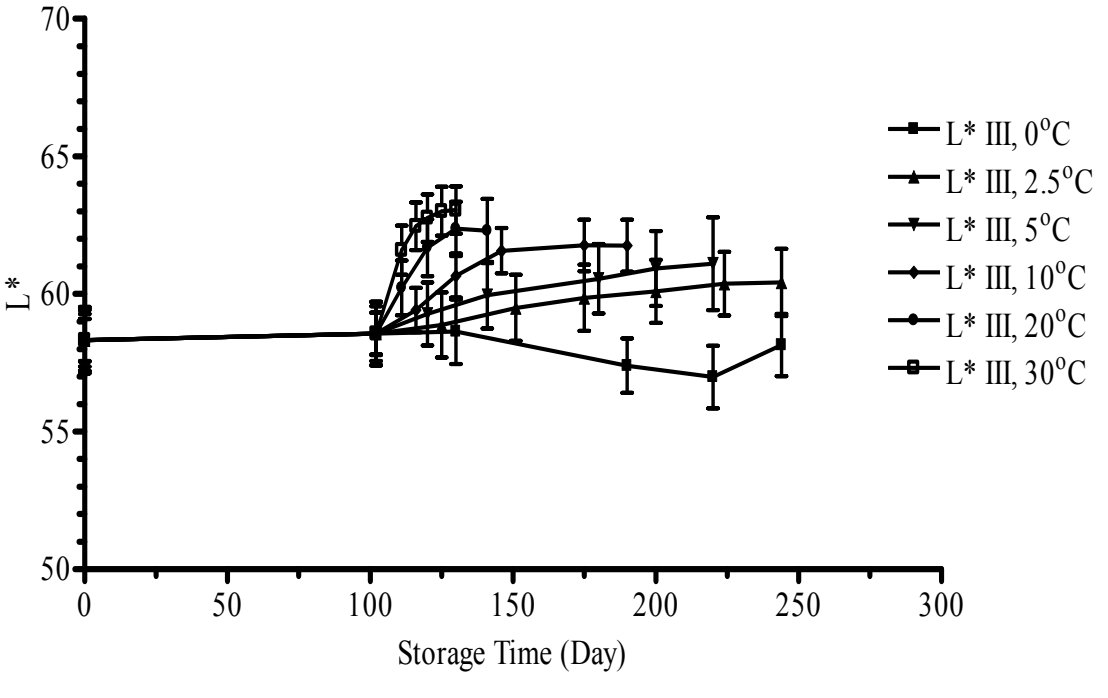
c


L*
Storage time (Day)
L* II, 0°C
L* II, 2.5°C
L* II, 5°C
L* II, 10°C
L* II, 20°C
L* II, 30°C

d


C*
Storage Time (Day)
C* III, 0°C
C* III, 2.5°C
C* III, 5°C
C* III, 10°C
C* III, 20°C
C* III, 30°C

e


L*
Storage Time (Day)
L* III, 0°C
L* III, 2.5°C
L* III, 5°C
L* III, 10°C
L* III, 20°C
L* III, 30°C

f