ULTRA-LOW TEMPERATURE SHIPPING AND COLD CHAIN MANAGEMENT OF 'FUERTE' AVOCADOS (*Persea americana* Mill.) GROWN IN THE KWAZULU-NATAL MIDLANDS

by

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Abstract

'Fuerte' makes up 25% of the avocados exported from South Africa to European markets and requires shipping periods of up to 28 days and a correctly managed cold chain. A temperature of 5.5°C and expensive CA and 1-MCP treatments are currently used to delay ripening over this lengthy cold chain; however, fruit still appear on the European market showing signs of softening and physiological disorders. Increased competition on the global market and the disadvantage of a particularly long distance to the European market has challenged the South African export industry. These challenges have necessitated improved road and sea transport logistics, co-ordination with producing countries which supply fruit to European markets over similar periods as South Africa, and research into ultra-low temperature storage to possibly enable future access to new lucrative markets in the USA, China and Japan. It is also known that there are various 'weak links' in this cold chain and that cold chain breaks are detrimental to fruit quality, but further research into the negative effects of these cold chain breaks at ultra-low temperatures was needed. Thus, the objective of the study was to determine the potential for shipping 'Fuerte' avocados at temperatures of 2°C as well as determining the effects of cold chain breaks on fruit quality, throughout the growing season and possibly for an extended period of 56 days. 'Fuerte' avocados were harvested at three different maturity stages reflecting early-, mid- and late-season fruit, with moisture contents of 74%, 68% and 63%, respectively. Fruit were stored at 2°C or 5.5°C, treated with 1-MCP and waxed. Additionally cold chain breaks (24 hour delay and break at 14 days) were implemented. Fruit softening, mass loss, days-to-ripening, external and internal quality as well as antioxidant levels and total sugar levels were determined.

The first aim was to determine whether a lower than currently used storage temperature could be a successful alternative to 1-MCP use. A storage temperature of 2°C provided good internal quality as well as reduced mass loss and fruit softening, which is related to the slightly reduced use of C7 sugars at 2°C compared with 5.5°C. Although the overall occurrence of external chilling injury was relatively low, 2°C storage caused a notably higher occurrence of external chilling injury than 5.5°C storage, particularly early in the season, but extended the days-to-ripening. Unfortunately, no correlation between the anti-oxidants in the exocarp and external damage was found. Waxing significantly reduced the external damage on fruit stored at 2°C, so much so, that the treatment combinations of '2°C, no 1-MCP,

waxed' showed no external chilling injury throughout the season. Further, waxing fruit at 2°C could eliminate the need for 1-MCP, delivering a product of the required shelf-life and quality. Best results were achieved for mid-season fruit stored at 2°C. Late-season fruit would potentially be the most profitable to store at this low temperature, however, body rots (anthracnose and stem-end rot) were more common in the late-season. Storage at 2°C can therefore maintain the internal quality over a storage period of 28 days and be a potential alternative to 1-MCP use as the season progresses.

The effect of cold chain breaks on fruit quality was then investigated and showed that both a delay and a break in the cold chain increased mass loss and fruit softening, reduced days-to-ripening and increased external chilling injury, especially early in the season. Water loss was the main contributor to the decreased fruit quality which resulted from the delay in cooling, increasing external damage significantly, particularly early in the season. The break at 14 days had a marked effect on physiological activity of fruit during storage, seen mainly in the increased metabolic activity, resulting in increased fruit softening and water loss during storage and a decrease in C7 sugars and thus shelf-life, particularly for fruit stored at 5.5°C. Importantly, 1-MCP use and storage at 2°C reduced the effects of cold chain breaks with respect to fruit softening, however, lowering the storage temperature had a greater negating effect than 1-MCP and could be a successful alternative to the use of 1-MCP. The internal quality throughout the experiment was very good, with few internal disorders and no significant treatment effects on internal quality and C7 sugar concentrations. Overall, a break in the cold chain, before and during cold storage, resulted in a marked reduction in fruit quality.

The storage temperature of 5.5°C should not be used for a 56 day storage period as it resulted in significant fruit softening during storage, even when 1-MCP was used, and resulted in significantly more external chilling injury in the mid- and late-season than at 2°C. Storage of 1-MCP treated, waxed fruit at 2°C, resulted in the best shelf-life and fruit quality, particularly mid-season fruit which had negligible external chilling injury and 100% sound fruit. Early-season fruit suffered significant external chilling injury at 2°C and late-season fruit had the highest body-rots and internal disorders at this storage temperature. Although mid-season fruit could be successfully stored at 2°C for 56 days, the use of a 56 day storage period is not recommended as a practical storage period, due to the high risk of external damage,

particularly if maturity levels are not optimum and trees and fruit are not of the highest quality.

Overall this thesis has shown that 1-MCP treatment can play an important role early in the season when fruit are susceptible to external damage, however, storage at 2^{o}C results in good quality fruit and, when used in conjunction with waxing, appears to be a viable alternative to the use of 1-MCP, particularly later in the season. Further, the negative effects of cold chain breaks on fruit quality have been demonstrated and, importantly, the storage temperature of 2^{o}C negates the fruit softening effects of these breaks, even if 1-MCP is not used.

DECLARATION

I, Andre Lütge, declare that the research reported in this thesis is my own original work. Where the work of others has been used, this is appropriately and duly acknowledged in the text. This thesis has not been submitted for any degree or examination at any other university.

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Andre Lütge

I certify that the above statement is correct.

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Dr Isa Bertling
Supervisor

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This thesis has been compiled as separate chapters for publication in scientific journals however the references have been combined into one section placed at the back of the thesis.

Table of Contents

CHAPTER 1

LITERATURE REVIEW

TABLE OF CONTENTS

1. INTRODUCTION

The South African avocado industry has shown continued growth in recent years and, being largely export-orientated, depends heavily on the European markets. South African avocados are exported to France, the UK, Germany, Netherlands, Scandinavia and other parts of Eastern Europe, with France and the UK being the largest buyers. In the past five growing seasons, approximately 73% of South African export avocados were shipped to Central Europe and 24% to the UK (PPECB, 2011). Approximately 12 million 4 kg cartons (48 000 tons) were exported in the 2008 season (www.avocado.co.za), approximately half the total annual production. In 2009, the total exported volume decreased slightly to 10.5 million 4kg cartons (42 000 tons), and in 2010, approximately 11.25million 4kg cartons (45000 tons) were exported (www.avocado.co.za).

Production occurs mainly in the warm subtropical areas of the Limpopo and Mpumalanga provinces in the northeast of the country, and to a lesser degree in KwaZulu-Natal, where cooler conditions prevail (Vorster, 2001). In the cooler production areas in KwaZulu-Natal, avocados are harvested approximately two months later than those grown in the warmer production areas of the Northern Province (Donkin *et al*., 1994). 'Hass' and 'Fuerte' cultivars constitute approximately 50% and 25% of the total exported avocado crop, respectively, with the remaining 25% being greenskins other than 'Fuerte' (Pers. com. 1). Owing to the climatic variations in the different growing areas of South Africa, avocados are harvested over an extended period, from late February to early November. This aids in higher returns for the South African avocado industry in terms of exporting and marketing over this extended period, ensuring that there is not an over-supply of South African avocados on the European market at any given time during the season (Vorster, 2001). The bulk of production takes place from March to September but growers receive substantially higher prices for very early- and late-season fruit, owing largely to climatic variations of the different production areas as well as the selection and planting of 'early' and 'late' fruiting cultivars.

In order to delay the onset of ripening, while ensuring fruit quality is maintained, avocado fruit are currently shipped using low temperatures, lowered from 7.5^{o}C to 3.5^{o}C as the season progresses (Vorster *et al*., 1990), in conjunction with controlled atmosphere (CA) or 1-Methylcyclopropene (1-MCP) treatment. However, fruit still appear on the European market with signs of softening and physiological disorders, both internally and externally. Increased environmental awareness, coupled with the perception that agro-chemicals are harmful to the

environment and humans, has resulted in a trend towards organic and eco-friendly agricultural commodities and processes, and thus prompted research into techniques to preserve post-harvest produce quality without the use of agro-chemicals. Cold treatment as well as heat, irradiation and controlled atmosphere treatments have all been investigated (Wills *et al.*, 2007). As a perfect treatment method has not yet been found, the opportunity to improve currently used technologies exists.

Storage temperatures below the currently implemented average temperature of 5.5^{o}C could negate the need for chemicals such as 1-MCP, but there are fears that temperatures below 5.5^{o}C will cause severe chilling injury and poor fruit quality. However, previous investigations have indicated that shipping at lower than the presently used protocol temperatures, is not only possible but also relatively successful. These lower temperatures have delivered good internal quality in 'Pinkerton' when stored at 2^{o}C (Bower and Magwaza, 2004; Van Rooyen and Bower, 2006; Van Rooyen and Bower, 2002), and 'Hass' when stored at 1^{o}C (Zauberman and Jobin-Decor, 1995; Bower, 2005b; Van Rooyen, 2009). Internal fruit quality was not compromised by these lower temperatures, even without the use of CA and 1-MCP as post-harvest treatments. Cold chain breaks can contribute further to fruit softening during storage as well as a reduced shelf-life, and are detrimental to avocado fruit quality (Blakey and Bower, 2009; Lütge *et al.*, 2010), with particularly damaging effects on pathology (Lemmer and Kruger, 2010). Some of these effects can be negated by treatments such as 1-MCP or CA, as well as lower shipping temperatures. Recent focus on the negative effects of cold chain breaks (Blakey and Bower, 2009), the influence of 1-MCP and CA (Lemmer and Kruger, 2010) as well as the interaction of cold chain breaks with ultra-low temperatures (Blakey and Bower, 2009; Lütge *et al.*, 2010) has provided valuable and much needed information. Of great importance is the interaction between post-harvest water loss and skin damage, and further research was necessary to determine the effects of cold chain breaks on fruit quality at these ultra-low temperatures. This suggests that focus needs to be placed on the effects of cold chain management on fruit quality, with the objective of shipping at lower temperatures, possibly in conjunction with currently adopted post-harvest treatments, ultimately reducing shipping costs without negatively affecting fruit quality.

In the future, as the South African avocado industry expands into new markets which have phytosanitary certification requirements, storage temperatures close to freezing will be required and temperature fluctuations in the cold chain will not be tolerated in these protocols. A successful cold storage treatment of 2^{o}C for 'Fuerte' is a step closer to enabling

South African avocados to reach new markets and ultimately bring higher profits to the grower. The success of cold storage, and thus the avocado export industry, depends on the knowledge and understanding of fruit physiology, the principles and mechanisms of cold storage, careful management of the cold chain and effective post-harvest treatments and management (Bezuidenhout *et al*., 1992).

Therefore, by measuring physical quality parameters as well as physiological analysis of fruit tissues, this project aims to fulfil the following three objectives:

1) To determine whether storage at 2^{o}C (as opposed to 5.5^{o}C) is comparable to 1-MCP treatment during simulated shipping.
2) To determine the physiological effects of cold chain breaks on final fruit quality for 'Fuerte'
3) To investigate the possibility of storing 'Fuerte' avocados at 2^{o}C for 56 days, allowing exporters to fetch much higher prices later in the season.

2. THE AVOCADO

The avocado (*Persea americana* Mill.) is a subtropical fruit which has its centre of origin in the highland forest regions of central America and southern Mexico. The commercial avocado fruit consumed today has changed very little from its wild ancestors that were cultivated for centuries in this centre of origin (Menge and Ploetz, 2003). The avocado belongs to the family Lauraceae and has been classified into three botanical races, namely, *Persea americana* var. *americana*, a fruit of West Indian origin; *Persea americana* var. *guatemalensis*, a fruit of Guatemalan origin; and *Persea americana* var. *drymifolia*, a fruit of Mexican origin (Knight, 1980). Many of the commercially grown cultivars today are hybrids of these three races and show large variability in fruit traits, not only between races, but between cultivars within each race (Woolf *et al.*, 2002). 'Hass', 'Fuerte', 'Ryan' and 'Pinkerton' are the four main cultivars grown commercially in South Africa. 'Hass' is predominantly of Guatemalan origin with some Mexican germplasm, and 'Pinkerton' is of Guatemalan origin, while 'Fuerte' and 'Ryan' are natural hybrids of the Guatemalan and Mexican races (Bergh, 1975).

Origin is one of the most important factors influencing cold tolerance of a race, with the West Indian race being least tolerant to cold, the Mexican race being most tolerant and the Guatemalan race being of intermediate tolerance to cold (Bergh, 1975; Whitmore, 1986). The Mexican and Guatemalan races are therefore more tolerant to cold storage temperatures than the West Indian race (Bergh, 1992). Cold tolerance is important when cold storage for up to 30 days is required, in order for fruit to reach distant markets without suffering from chilling injury.

2.1 The 'Fuerte' cultivar

In 1911,the 'Fuerte' cultivar was introduced into California as budwood from southern Mexico by Carl B. Schmidt (Newett *et al.*, 2002). 'Fuerte' spread around the world and initiated a worldwide trade in avocados (Menge and Ploetz, 2003). The fruit is typically pear-shaped with a distinct neck but can vary in shape from elongated with a long thin neck, to dumpy with a short broad neck (Newett *et al.*, 2002). The fruit is medium to large in size (170-500 g) and the skin is dull green, thin in relation to other cultivars, slightly pebbled and leathery (Newett *et al.*, 2002; Bijzet, 2001). 'Fuerte' is easily marketable due to its excellent quality and flavorsome, nutty aftertaste (Newett *et al.*, 2002).

Figure 1: Typical shape of 'Fuerte' fruit

'Fuerte' dominated early production in South Africa, but due to the European preference for 'Hass' in recent times, 'Hass' has become the dominant cultivar, and thus old 'Fuerte' orchards are slowly being phased out. 'Fuerte' has lost favour due to its high tendency to alternate bearing and fruit rot problems, but has retained its importance in low humidity climates where insect pressure is low (Newett *et al.*, 2002). It is considered very susceptible to anthracnose and other physiological disorders during cold storage (Bijzet, 2001). 'Fuerte' constitutes approximately 25% of the total exported avocado crop in South Africa, but makes up a fairly low percentage of total production in other major avocado producing countries (Newett *et al.*, 2002), as a result of the increased popularity of black-skinned cultivars in these countries.

'Fuerte' still plays an important role in the South African avocado industry as it comes onto the market 2-4 weeks earlier than 'Hass' and thus helps exporters lengthen the export season and marketing time of South African avocados (Donkin, 2007). Some consumers may still be drawn to 'Fuerte' avocados as the shape and colour of this cultivar are what they recognise as a 'typical' avocado. 'Fuerte' remains green when ripe and thus does not have the advantage of undergoing a rind colour change during ripening (as is the case with cultivars which blacken when ripe, such as 'Hass') which masks the blackened lesions caused by chilling injury. External appearance is extremely important when marketing avocados, and when lengthy shipping times are required to reach these markets, preventing external cold damage (or chilling injury) is critical, especially for greenskins such as 'Fuerte'.

3. RIPENING PHYSIOLOGY

Biale (1975) described ripening as the processes which cause changes in the colour, taste and texture of fruit, which make the fruit acceptable for consumption. Ripening in avocados occurs between fruit maturity and senescence. Avocado fruit will only ripen, and acquire normal softening and acceptable taste, once a certain maturity level has been reached (Bower, 1985). At the correct maturity, the fruit is harvested and from this point on, the ripening process commences. Burg and Burg (1962) suggested that on-tree ripening is naturally inhibited by a ripening inhibitor, possibly an anion, which moves from the leaves to the fruit. Bower (1985) cites a further possible explanation by Tingwa and Young (1975) that some substance, also possibly an anion, acts to regulate ripening and moves either to or from the fruit pedicel after the fruit has been removed from the tree. Once the ripening process has begun it cannot be reversed, but only slowed by various methods.

An understanding of avocado ripening physiology is necessary in the search for causes of post-harvest disorders which occur as a result of cold storage. The strongly climacteric nature of avocado fruit means that there is a marked rise and fall in respiration rate (CO_2 production) and ethylene production during fruit ripening (Wills *et al.*, 2007, Figure 2).

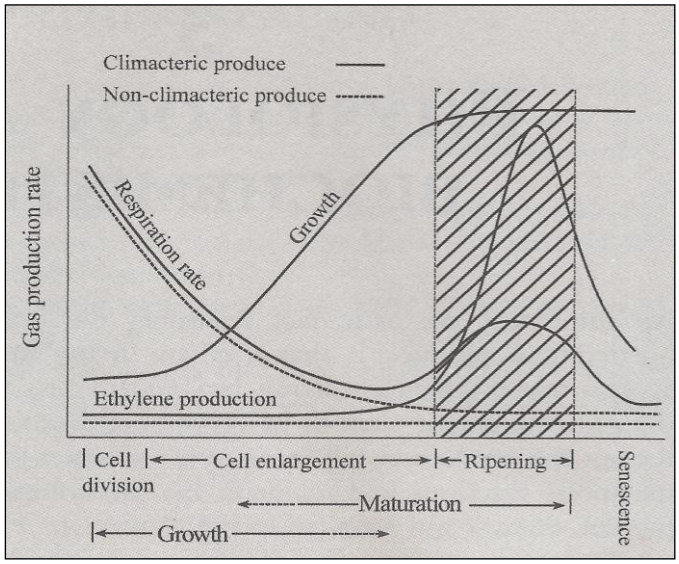

Figure 2: Growth, respiration and ethylene production of climacteric and non-climacteric plant organs (Wills *et al.*, 2007)

3.1 Structural Changes

Fruit ripening involves complex physical and physiological changes. This includes many catabolic and anabolic processes which require high levels of energy as well as prolonged membrane integrity (Bruinsma, 1981). The cell membrane as well as the plasma membrane, are important structural boundaries involved in fruit ripening (Bower and Cutting, 1988). The softening of the fruit, which occurs during ripening, is due to loosening and eventual degradation of cell walls (Platt-Aloia and Thomson, 1981). Awad and Young (1979) describe the most obvious sign of ripening after harvest as "the rapid transition of the mesocarp from a hard to a soft, butter-like consistency with an apparent total loss of structural integrity". Avocado ripening studies have shown that cellulose degradation was not evident, although some loss of fibrillar structure did occur (Pesis *et al.*, 1978; Platt-Aloia and Thomson, 1981). Thus the main structural changes which occur during softening are related to the loosening of cell walls and the loss of cell to cell cohesion, rather than cell wall degradation (Witney, 1985).

During ripening, generally, anabolic processes are responsible for the production of new pigments or flavor volatiles, while catabolic processes are linked to breakdown of chloroplast thylakoids or the breakdown of cell walls during softening. Platt-Aloia and Thomson (1981) found that chloroplasts, dictyosomes, microbodies, the nucleus, vacuoles, and ribosomes did not alter significantly in their structure and retained their structural integrity during avocado ripening. Platt-Aloia and Thomson (1981) concluded that although ripening may be the final anabolic process in the life of many fruit, ripening in avocados is not characterized by degradation of cytoplasmic organelles. Meir *et al*. (1991) suggested that lipid peroxidation is one of the earliest detectable processes in avocado fruit ripening, and found this occurrence measurable through the fractionation and analysis of fluorescent compounds (FCs) in the rind.

Platt-Aloia and Thomson (1981) also observed ultrastructural changes during ripening such as the breakdown of the cell wall and swelling and vesiculation of the rough endoplasmic reticulum, which Bower and Cutting (1988) suggested shows considerable enzyme synthesis, with the membrane systems remaining undisturbed. Structural studies using an electron microscope were able to correlate the breakdown of cell walls with this increased enzyme activity during softening (Platt-Aloia and Thomson, 1981).

3.1.1 Enzyme activity

The changes in cell wall structure during ripening are probably caused by increased synthesis and activity of certain enzymes. Several cell wall-degrading enzymes have been studied, of which the most important enzymes found to be correlated to the ripening process are cellulase, polygalacturonase (PG) and pectinmethylesterase (PME) (Awad and Young, 1979). A cytochrome P450 protein, which acts as a mono-oxygenase enzyme, has also been suggested to be part of the ripening process in avocados (Bozak *et al*., 1990). A definite correlation between the activity of cellulase, PG and PME and fruit softening in avocados has been demonstrated by Zauberman and Schiffmann-Nadel(1971).

Ripening of avocados is accompanied by softening of the fruit, which is believed to be a result of the loss of cell to cell cohesion with the cell walls (Platt-Aloia *et al.*, 1981). Scott *et al*. (1963) found that cellulose is the major constituent of avocado cell walls. Pesis *et al*. (1978) reported that cellulase increased during fruit softening, a process closely related to the respiratory climacteric and ethylene production peak. The same authors highlighted the impact of ethylene on cellulase activity, as cellulase activity increased when avocado fruit was placed in an ethylene-rich environment.

As ripening in avocados proceeds, cellulase and PG activities have been found to increase, while PME activity decreases (Awad and Young, 1979). Subsequently, Hatfield and Nevins (1986) were able to purify avocado cellulase and identified the enzyme as(1-4)-β-D-glucanase. These authors also found (1-4)-β-D-glucanase hydrolysed only (1-4)-β-glycosyl linkages and not the cellulose polymers found in mature avocados, which meant that cellulase activity could not be solely responsible for the breakdown of avocado cell walls. Hatfield and Nevins (1986) proposed that cellulose fibrils are hydrolysed, which would be consistent with observations of changes in cellulose fibres, as seen under a microscope. During ripening, hydrogen bonding to other polysaccharides in the cell wall may be altered, thus disturbing the cell wall matrix and allowing enzymes to break down the polygalacturans (Hatfield and Nevins, 1986). This might explain why PG activity increases after cellulase activity first increases (Awad and Young, 1979). The strong correlation between cellulase activity and softening seems to suggest that cellulase is responsible for the early stages of fruit softening (Hatfield and Nevins, 1986), which is controlled in part by ethylene, while PG seems to be responsible for final fruit softening (Bower and Cutting, 1988).

Enzyme activity has been shown to increase rapidly after cold storage (Blakey *et al*., 2010), with a high probability of some enzyme activity occurring during storage. Assuming that cellulase and PG are responsible for fruit softening through cell wall degradation, the activity of these enzymes needs to be minimised in order to reduce fruit softening during storage. Typically, the most logical method of reducing any enzyme activity is to reduce the temperature. As some fruit softening occurs at 5.5^{o}C, it can be assumed that the storage temperature is not low enough to shut down metabolic activity of these enzymes completely, thus additional treatments are currently used by the avocado industry to further minimise this activity during storage.

3.2 Role of plant growth regulators (PGRs) in ripening

PGRs play an important role in avocado fruit ripening (Bower and Cutting, 1988). The following information is presented to acknowledge that there are many components in the ripening process and to explain the importance of ethylene in avocado ripening.

3.2.1 Ethylene

As with many other fruit, ethylene is known to play an important role in the ripening process of avocados. Yang (1981) considered ethylene formation to be essential in the ripening of climacteric fruit, as the ethylene peak usually precedes the respiratory climacteric. Rhodes (1981) defined the climacteric as "a period in the ontogeny of certain fruits during which a series of biochemical changes is initiated by the autocatalytic production of ethylene, marking the change from growth to senescence and involving an increase in respiration and leading to ripening".

There is contrasting evidence on the exact role of ethylene in avocado ripening. Ethylene is widely thought of as one of the triggers which initiates the ripening process (Starrett and Laties, 1993). Zauberman *et al.* (1988), on the other hand, showed that successful ripening of avocados requires ethylene to be present continuously throughout the ripening process. Bower and Cutting (1988) provided a detailed account of the role of ethylene and concluded that ethylene is neither the initiator nor the cause of ripening, but does, however, play an important role in ripening. It is known that post-harvest applications of ethylene cause an earlier climacteric peak and, thus, earlier ripening (Eaks, 1966). This makes the manipulation of respiration and ethylene levels critical to the delay in ripening, as required during export,

and the subsequent application so that softening can occur after arrival overseas. Breaks in the cold chain may be significant enough to increase respiration and trigger ethylene production, which could result in 'soft arrivals'. Wounds on fruit stimulate ethylene production (Zauberman and Fuchs, 1981; Starrett and Laties, 1993), therefore only undamaged fruit should be stored.

One of the key enzymes of ethylene biosynthesis is 1-Aminocyclopropane-1-carboxylic acid (ACC) synthase, which converts S-adenosyl-methionine (SAM) to ACC, the precursor of ethylene (Figure 3). Blumenfield *et al*. (1986) postulated that on-tree ripening is prevented by the inhibition of ACC synthase and, thus, ethylene cannot be produced. Blumenfield *et al*. (1986) also found an increase in ACC synthase activity in avocados after harvest, which causes the accumulation of ACC. Once fruit is harvested, this ripening inhibitor is removed and, thus, ACC synthase activity increases, resulting in ACC production and, therefore, ethylene synthesis increases (Donkin, 1995). The biosynthesis of ethylene is autocatalytic, meaning that ethylene stimulates its own synthesis, which magnifies the effects even a small amount of ethylene in the storage atmosphere can have on the ripening of avocados (Mohr and Schopfer, 1995).

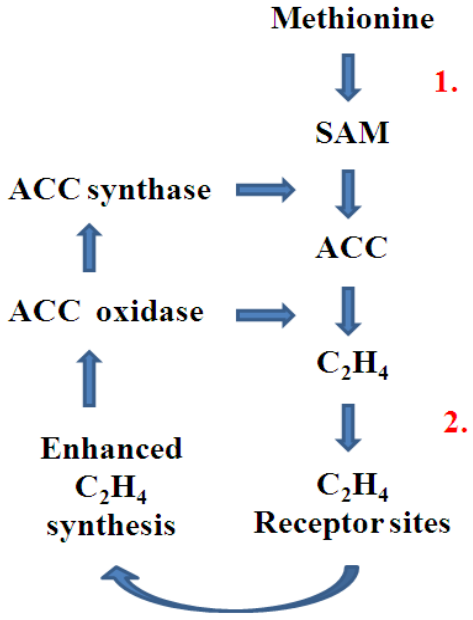

Figure 3: The biosynthesis of ethylene and possible areas of intervention (1 and 2) where steps can be taken to decrease the production of ethylene.

3.2.2 Other PGR's

In addition to ethylene, several other plant growth regulators play important roles in the avocado fruit ripening process including auxins, cytokinins, gibberellins and abscisic acid (ABA) (Bower and Cutting, 1988). Like ethylene, ABA promotes ripening, while auxins, cytokinins and gibberellins are ripening inhibitors in many fruit (Rhodes, 1981). In avocados, ABA appears to play a key role in ripening as well as fruit quality as the ABA content in avocados increases with maturity and ripening (Cutting *et al.*, 1988; Cutting *et al.*, 1990) and exogenous applications of ABA stimulate ethylene synthesis and fruit ripening (Vendrell and Palomer, 1977). Cytokinins appear to be involved in regulating calcium concentrations and movement (Ferguson, 1984), however, the importance of this in avocado ripening is debatable, as the involvement of cytokinins in avocado ripening seems to be limited (Wolstenholme *et al.,* 1985; Bower and Cutting, 1988). The role of gibberellins in avocados is similarly debatable, as Lieberman (1977) found that exogenous applications of gibberellins have a minimal effect on avocado fruit ripening.

3.3 Effect of ripening temperatures

Once fruit are removed from cold storage, the temperature at which avocado fruit are ripened is critical (Eaks, 1978; Hopkirk *et al*, 1994). Ripening temperatures affect the metabolic rate of the fruit, particularly the ripening enzymes, and is also linked to ethylene production which occurs only in a certain temperature range (Donkin, 1995). The uncontrolled presence of ethylene, in high concentrations, during cold storage is undesirable, and the reduction of ethylene in the commercial storage of many fruits is practiced (Hofman *et al*., 1995). However, once the fruit has been removed from cold storage, ethylene treatment enhances ripening without injury (Zauberman *et al*., 1988).

The temperature at which avocado fruit is ripened also affects the incidence of various disorders. Hopkirk *et al*. (1994) found that body rots, stem-end rot, vascular browning and uneven ripening increased as the ripening temperature increased from 20^{o}C to 30^{o}C, whilst these were at a minimum at 15^{o}C. Fitzell and Muirhead (1983) found that ripening temperatures above 24^{o}C increased the levels of anthracnose significantly in 'Fuerte' fruit, and recommended a ripening temperature of 17^{o}C for this cultivar. Thus, to balance optimal ethylene production and limit the incidence of these disorders, avocado fruit are typically

allowed to ripen at ambient temperatures (18-20^{o}C), after removal from cold storage (Hopkirk *et al*, 1994).

3.4 Role of fruit minerals in ripening

Van Rooyen (2005) provides a detailed report of the important fruit minerals in avocados and their effect on fruit quality. For the purpose of this study, the effects of the different fruit minerals on fruit quality will not be discussed, however, the role of calcium will be summarized as it plays a critical role in fruit ripening. Calcium is important in the development of the cell wall and influences the rate of fruit softening (Tingwa and Young, 1974; Cutting *et al.*, 1992). The role of calcium in avocado ripening is aptly described by Bangerth (1979) as being the effect that calcium has on enzymes, membranes, cell walls, and the interaction with PGR's. The review by Bower and Cutting (1988) provides a more in-depth discussion on the role of calcium in avocados as is currently understood, as very few new studies have been done on calcium since this review. Ferguson (1984) concluded that the important aspects of calcium in ripening and senescence are related to membrane and cell wall structure as well as function. Calcium is known to be important in the maintenance of membrane permeability control as well as membrane stability (Ferguson, 1984). It appears that not only the concentration of calcium, but also the position of calcium in the cell is important in the ripening process. Ferguson (1984) commented that it seems important that a high concentration of calcium is maintained outside the cytosol, and that the longer this condition is maintained, the slower the rate of ripening and senescence.

Once the calcium concentration in the cytosol reaches a certain level, the calcium combines with calmodulin to form a calcium-calmodulin complex (Poovaiah, 1985). This complex then combines with a receptor protein which creates an active enzyme complex, capable of controlling other enzymes (Cheung, 1982; Poovaiah, 1985). Calmodulin may also be involved in the active removal of excess calcium from cells (Cheung, 1982). Poovaiah (1985) found that the calcium-calmodulin complex plays a key role in plant growth including the onset and rate of senescence development. Ultimately, calcium concentration in the fruit is influenced by many pre-harvest factors, primarily calcium uptake from the soil and the effect that photosynthesis, vegetative growth and water flow through the tree have on calcium transport to the fruit.

In terms of the mineral levels at harvest, although dependant on the production area, the current guidelines for South African growers, set up by Sjnider *et al*. (2003), are that fruit calcium levels should be above 0.150 % (1500 ppm) in November, 0.070 % (700 ppm) in January/February, with a gradual decline until harvest. From a post-harvest perspective, the infiltration of calcium into mature fruit has been shown to significantly reduce the respiration rate and depress the peak of ethylene production, ultimately reducing overall ethylene evolution and, thus, extending the shelf-life of avocados (Wills and Tirmazi, 1982; Tingwa and Young, 1974; Eaks, 1985). Post-harvest applications of calcium, via orchard sprays and post-harvest dips, do not provide adequate calcium uptake, but vacuum infiltration is a more effective technique. Davenport (1984) showed that vacuum infiltration of calcium into avocado fruit increased the time taken to reach 'eating soft' stage after removal from cold storage by 50%, however, it is not useful commercially (Wills and Tirmazi, 1982; Eaks, 1985).

3.5 Sugars involved in the ripening process

The avocado contains relatively high concentrations of two naturally rare sugars, namely mannoheptulose (D-manno-2-ketoheptose) and its sugar-alcohol perseitol (D-glycero-D-galcto-heptitol) (Liu *et al*., 1999b). The concentrations of these seven-carbon (C7) sugars in avocados are higher than the common six-carbon (C6) sugars (sucrose, fructose and glucose) found in most other fruits (Bertling and Bower, 2005). The exact functions of these C7 sugars has yet to be clearly defined, however, several suggestions regarding their importance in avocado ripening have been put forward (Liu *et al*., 1999a; Liu *et al*., 1999b).

Liu *et al*. (2002) suggested that C7 sugars could control the ripening process, acting as ripening inhibitors when still attached to the tree, only allowing for the commencement of ripening when sugar levels decreased after harvest. These C7 sugars may also be sources of carbon and energy, used by the fruit during ripening (Liu *et al*., 1999a; Meyer and Terry, 2010; Tesfay, 2009; Tesfay *et al*., 2010). Blakey *et al.* (2009) stated that the only sugars found in adequate amounts, to supply the required postharvest energy needed for ripening, were mannoheptulose and perseitol, while common sugars, such as sucrose, fructose and glucose, were present in negligible quantities and showed no clear postharvest trends. Similarly, Liu *et al*. (1999) reported that the common sugars and mannoheptulose in the exo- and mesocarp significantly decreased during postharvest cold storage (1 or 5^{o}C), as these sugars are probably used as an energy source for respiration. Bertling *et al.* (2007) proposed

that the reduction in these sugars, as the fruit nears harvest maturity, could be related to deterioration in post-harvest quality, ultimately affecting the ripening process. Ogata *et al*. (1972) studied the mannoheptulose content of four avocado cultivars and found that unripe mesocarp tissue contained relatively high levels (0.64-2.5%), while ripe mesocarp tissue contained lower levels (0.03-0.5%). Blakey *et al.* (2009) found that mannoheptulose and perseitol decreased rapidly in the first week after harvest simultaneous to a rapid increase in proteins, presumably to synthesize ripening enzymes. If these energy reserves are depleted before complete ripening has occurred, proteins may get broken down as an alternative energy source, which could result in decreased fruit quality (Blakey *et al.*, 2009).

Bertling *et al.* (2007) and Tesfay *et al*. (2010) highlighted the important antioxidant abilities of these sugars and suggested their importance during the post-harvest cold storage of avocados. This is discussed further in section 1.5.4.1. If the concentrations of C7 sugars could be increased or maintained in the mesocarp of avocados, final fruit quality may be improved.

4. POST-HARVEST AND EXPORT LOGISTICS

Picking and handling of the fruit is extremely important as disregard for the importance of these tasks can be detrimental to final fruit quality. 'Fuerte' fruit should be clipped from the pedicel, whereas 'Hass' fruit can be snap-picked without negatively affecting fruit quality (Köhne and Kremer-Köhne, 1995). After picking, the fruit should be kept in the shade and transported to the packhouse within two hours with minimum vibrations (Kremer-Köhne, 1998). Zauberman *et al*. (1969) found that damage *en route* to the packhouse could be reduced significantly by using bulk bins instead of lug boxes because of the lower number of fruit in contact with the sides and bottom of the containers (where the most physical damage occurs). The same authors also found that the number of injured fruit is reduced by 50% when the bins are lined with canvas.

At the packhouse, the fruit is sorted, pre-cooled to a recommended temperature of between 16^{o}C and 18^{o}C (Eksteen, 2001), packed, and then immediately cooled to a fruit pulp temperature of 5-8^{o}C, depending on fruit maturity (Dodd *et al*., 2007; Kremer-Köhne, 1998). Once the set temperature is reached, the fruit is stored in the cold store whilst awaiting transport. Avocados are climacteric fruit, and when inadequate cooling occurs, ethylene is produced by the fruit, ripening is triggered and the ripening process occurs rapidly. It was shown that 'Fuerte' avocados which have started to ripen during storage have a greater chance of developing quality defects when ripening continues under cold storage conditions (Bezuidenhout, 1983). The cold storage facilities at the packhouse are the only link in the entire cold chain where the fruit can be effectively cooled to delay the onset of ripening (Eksteen, 2001).

Avocados are primarily exported by sea in refrigerated containers under controlled atmosphere (CA). Treatment with 1-MCP (SmartFresh®) is used for fruit that are exported to markets where avocados are not ripened before being sold to the consumer. Fruit are either packed into refrigerated trucks at the packhouse or into refrigerated containers for transport via road or rail to the port. Fruit transported in refrigerated trucks are packed into containers at the port and loaded onto the ship, whereas fruit transported in containers from the pack-house avoid the packing at the port and the containers are loaded directly onto the ship. The majority of export avocados are shipped from Cape Town, which is approximately 2000km from the larger production areas in the Northern Province (Figure 4) and takes approximately 40 hours to the port in Cape Town via road transport (Dodd *et al*., 2007). The sea voyage to

Europe takes 12-14 days (Donkin, 2007) and an average of 25-28 days in total to reach the overseas destination from the time of picking (Bezuidenhout *et al.*, 1992). Once arriving at the European markets, it is the importing agent's responsibility to collect the consignment as soon as possible, but within a maximum of 2 hours after discharge from the vessel (Eksteen, 2001). Should the importer be dissatisfied with the condition of the fruit, a quality surveyor must be immediately contacted to provide a detailed technical report on fruit quality (Eksteen, 2001). Containers need to be unpacked, re-sorted and packed based on ripeness and quality, and finally distributed to the supermarket. This entire process may take up to30 days (from the packhouse to the European retailer) and thus fast cooling, thorough management of the cold chain as well as optimal temperature regimes are crucial in maintaining high quality fruit (Eksteen, 2001).

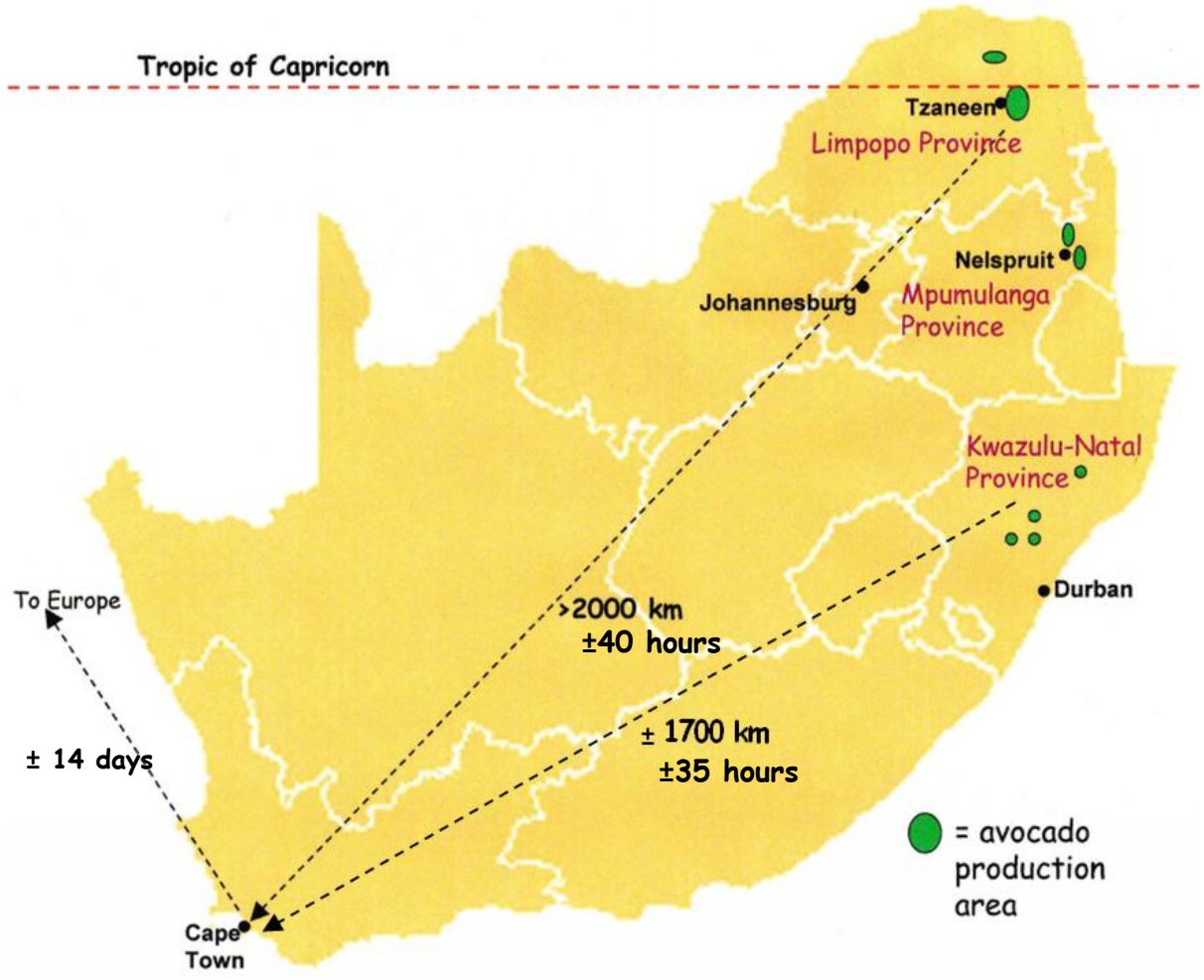

Figure 4: Production areas and approximate transport distance to the port for export to Europe (After SAAGA, 2007)

5. QUALITY CONTROL AND POST-HARVEST STORAGE

Once a fruit has been harvested, it continues respiration and associated metabolic activity and thus the aim of post-harvest storage is to reduce the metabolic rate of the fruit. Once harvested, avocado fruit are cut off from their supply of carbohydrates and water and, thus, need to be harvested when both are at an optimum level for long-term storage. The fruit continues to be metabolically active and continues to utilize stored sugars as an energy source, resulting in reduced fruit quality and storage time. Low temperatures, CA and 1-MCP are currently used in an attempt to slow down the fruit's metabolic activity and consequently delay ripening. Reducing water loss in fruit, once it has been harvested, is also vital for successful storage at low temperatures. Water lost from the fruit cannot be replaced and thus post-harvest conditions should attempt to minimize water loss.

5.1 Harvest maturity

Harvesting time for avocados is determined by the minimum maturity standard for the cultivar, the required storage and transport time, and market prices (Hofman *et al.*, 2002). Kaiser *et al*. (1995) define physiological maturity in avocados as "that stage of development at which the fruit, once detached from the tree, will ripen and result in a product desirable for eating". Harvesting avocados prior to their mature physiological state may result in irregular ripening, off-flavours and physiological disorders (Swarts, 1979; Zauberman *et al.*, 1977). One of the major determinants of post-harvest fruit quality is whether fruit was harvested at the correct maturity, which is reflected by the water or oil content of the fruit at harvest (Milne, 1998).

The best measure of maturity is oil concentration, which is directly related to the percentage dry matter (Swarts, 1978); however, this method is time consuming and detailed (Hofman *et al*., 2002). In South Africa, fruit water content at harvest is usually used to measure maturity, as water content and total oil content are reciprocal and generally add up to a constant value in each cultivar (Swarts, 1978). The mesocarp moisture content is known to decrease from early to late harvested fruit, while the oil content increases as the fruit increases in maturity (Pearson, 1975; Kruger *et al*., 1995). This maturity index in avocados is simpler and more practical to measure than oil content readings (Kruger and Claassens, 2001). Fruit maturity will vary greatly within a tree, due to different fruit set periods, thus selective picking is

recommended (Milne, 1998). The time-to-ripening is also influenced by the moisture content of the fruit, with fewer days-to-ripening with increasing maturity (Adato and Gazit, 1974).

The Perishable Products Export Control Board (PPECB) along with the South African Avocado Growers' Association (SAAGA) have clearly defined the optimal fruit moisture content to assist growers and exporters in the correct application and interpretation of this maturity index (Eksteen, 2001). The maximum stipulated moisture content for 'Fuerte' is reported to be 80% (Eksteen, 1999). However, Mans *et al*. (1995) found that 'Fuerte' fruit from the cooler production areas of KwaZulu-Natal were able to ripen to a desirable quality at a moisture content of 75%, 5% lower than the suggested moisture content in the warmer production areas of the Northern Province. One concern with measuring fruit maturity with moisture content, is that this method is open to manipulation. Early-season sample fruit can be harvested from water stressed trees and fruit which reduces the mean moisture content of an orchard, implying that the orchard is suitably mature for harvest, allowing growers higher prices for immature fruit.

5.2 Post-harvest water loss

Water loss is one of the main factors influencing deterioration of export avocado fruit (Milne, 1998). Maintaining a high relative humidity (RH), via humidification, prevents water loss from avocado fruit and significantly reduces the incidence of pathological disorders as well as internal physiological disorders (Bower *et al.*, 1989). Vapour pressure deficit (VPD), the differential in water content between the fruit and the surrounding air, can be used to explain why a high RH (85-95%) is desirable when storing avocados (Woolf *et al*., 2002). When the RH is low (high VPD), the moisture in the fruit will tend to move from the fruit into the surrounding air, resulting in water loss from the fruit. A high VPD between the fruit and the storage atmosphere results in an increased rate of water loss from the fruit while a low VPD results in minimal water loss to the surrounding atmosphere. Cold air can hold substantially less water than warm air and thus cold air has a low VPD, which illustrates the importance of rapid cooling in minimizing fruit water loss. The findings by Arpaia *et al*. (1992) that mesocarp discolouration increased in severity and incidence with increased time before cold storage, is probably a result of fruit water loss soon after harvest.

Post-harvest water loss must be minimized throughout the export procedure, and thus RH recommendations and protocol have been set between 90 and 95%. However, it is extremely difficult to maintain 95% RH and is rarely achieved in the South African export market. Shipping containers are not airtight and therefore humid air moves out of the containers, resulting in water loss from the container atmosphere, and thus water loss from the fruit. This situation can be modified with the use of polyethylene bags or other forms of packaging, as the surrounding air in the bag reaches almost 100% RH due to the fruit transpiring. This results in a low VPD and less water is lost from the fruit (Bower and Magwaza, 2004).

5.3 Controlled atmosphere (CA) and 1-MCP

The production of ethylene ultimately leads to the onset of ripening, which is undesirable when fruit is in cold storage. In general, there are two reactions in the biosynthetic pathway of ethylene where one can limit the effect of ethylene during storage (Figure 3). The primary area of intervention (1.) includes possible treatments of lowering temperatures or the use of CA, as low storage temperatures decrease the overall enzyme activity and, thus, prevent or slow the formation of various compounds in the biosynthetic pathway of ethylene. Similarly, CA acts to reduce the respiratory activity of fruit by manipulating the storage atmosphere in order to limit the reaction involved in respiration. By reducing fruit respiration, the subsequent production of various compounds, required for the production of ethylene, is also slowed. The second area of intervention (2.) is where 'patch-up' methods are applied, which hide or counteract the effects of ethylene production. These 'patch-up' methods include the use of ethylene scrubbers which remove ethylene from the storage atmosphere, as well as the use of 1-MCP which binds irreversibly to ethylene receptor sites to reduce the effect of any ethylene present in the storage atmosphere.

At the currently adopted storage temperatures, refrigeration is not the only treatment available for adequate quality control. Post-harvest treatments such as CA and 1-MCP are also currently used to slow avocado metabolism and delay the onset of ripening. The use of CA and 1-MCP in avocado export has been deemed to be highly successful, but also have negative impacts on fruit ripening and quality (Burdon *et al*., 2008). One of the main disadvantages of using these post-harvest treatments is the high cost involved. These treatments cost US$ 800 - 1500 per container to implement (Pers. comm. 1), which needs to be justified by the increase in fruit quality provided by these treatments. Further, CA and 1-MCP can be, and are, used to mask or 'cover up' problems which occur in the cold chain.

Perhaps the application of 1-MCP may not be required if cold chain breaks are avoided or lower shipping temperatures can be applied successfully. Dodd *et al*. (2007) suggest that, for certain cultivars and at certain physiological maturities, improved management of the cold chain may negate the need for these costly technologies. Economically, an ideal situation would involve successfully exporting avocados of a high external and internal quality, using only cold storage.

5.3.1 CA

The use of CA storage is both common and effective (Spalding and Reeder, 1975; Eksteen and Truter, 1985). This form of cold storage involves manipulating the concentrations of CO_2 and O_2, and the ratio of these gases, either by setting them at a particular level at the start of storage (static CA) or controlling these gases during storage (dynamic CA) (Burdon *et al*., 2008). Use of CA has been found to reduce post-harvest disorders and chilling injury during storage by reducing respiration and ethylene production and thus extending the time to ripening. Decreased O_2 and elevated CO_2 has been shown to decrease respiration and, if implemented correctly, extend the time to reach the climacteric peak (Faubion *et al.*, 1992). The broad, recommended composition of the storage atmosphere, for avocados in general, is 2-5% O_2 and 3-10% CO_2 (Eksteen and Truter, 1985; Thompson *et al*., 1998; Burdon *et al*., 2008). The optimum levels for CA-shipped avocados are 2% O_2 and 10% CO_2 when stored at 5.5^{o}C (Hatton and Reeder, 1972; Truter and Eksteen, 1987). The main advantages of low O_2 levels in storage are delayed softening, and reduction in respiration and ethylene production at standard storage temperatures (Woolf *et al.*, 2002). Elevated CO_2 levels may result in delayed softening and reduced sensitivity to external chilling injury, and thus allow for lower storage temperatures (Faubion *et al.*, 1992).

However, disadvantages are that CA is expensive and can increase the avocado's susceptibility to decay (Eksteen and Truter,1985). Further, incorrect gas levels can result in internal and external fruit damage. If O_2 levels are too low, external injury occurs, visible as irregular brown to dark brown patches on the rind, while internal browning of the flesh below these affected areas can develop (Woolf *et al.*, 2002). When CO_2 levels exceed 10% skin discolouration and off-flavours can occur, especially when O_2 levels are less than 1% (Woolf *et al.*, 2002). Arpaia *et al*. (1990) found that even 1ppm ethylene in the CA may cancel out any positive effects of CA. Faubion *et al.* (1992) confirmed these findings.

It seems that although CA is useful in long distance shipping, the high expense of CA has prompted investigations into various alternative methods of delaying the onset of ripening. One such alternative is the use of 1-MCP, presently used in certain avocado consignments.

5.3.2 1-MCP

The chemical compound 1-MCP (SmartFresh®) binds irreversibly to ethylene receptor sites, and acts by blocking these receptor sites and thus slows down the ripening process (Lemmer and Kruger, 2003). The use of 1-MCP to extend the post-harvest life of ethylene-sensitive produce has been widely documented; however, the efficacy varies with crop. The primary reasons for 1-MCP use in avocado exports are to reduce the amount of soft fruit arriving at overseas markets and to reduce the incidence of physiological disorders, such as grey pulp (mesocarp discolouration) and pulp spot (Kruger and Lemmer, 2007). Lemmer *et al.* (2002) found that 'Hass' and 'Fuerte' fruit treated with 1-MCP, took twice as long to ripen once removed from cold storage than fruit which were not treated with 1-MCP. The use of 1-MCP has become an effective alternative to CA, following the first commercial implementation of this treatment in South African avocados in 2003 (Kruger and Lemmer, 2007). Nelson (2005) reported that in 2004, 1-MCP treatment was effective but only if applied to the fruit shortly after harvest.

Application of 1-MCP is usually conducted at room temperature and involves the gassing of fruit after harvest (Mare *et al.*, 2002). The ability of the fruit to ripen following application of 1-MCP is due to the production of new ethylene receptor sites (Adkins *et al*., 2005), as the fruit is only sensitive to ethylene when new receptors have been synthesised (Jeong and Huber, 2004). The transport time from South Africa to European markets is sufficient to allow for this production of new ethylene receptors. Sisler *et al*. (1996) stated that 1-MCP treatment at low temperature requires higher concentrations of 1-MCP to achieve the same results, than if applied at room temperature. If applications are not correct, and higher concentrations than recommended are applied, the treated fruit may exhibit excessive delays in ripening, likely resulting in increased disease probability. Woolf *et al*. (2005) found that 1-MCP concentrations of 50-100 nL L^{-1} (ppb) for 12-24 hours gave the best balance between reducing internal disorders and reducing the risk of excessive ripening times.

It appears that 1-MCP treatment affects different aspects of fruit quality, having both positive and negative effects. Roets *et al*. (2009) evaluated different atmospheric conditioning

treatments using 'Hass' fruit from three production areas of South Africa, including fruit from the same production area as this study. Treatment of these fruit (Wartburg area) with 1-MCP resulted in a significantly higher occurrence of anthracnose and a slightly (but significantly) higher occurrence of stem-end rot and vascular browning than when fruit were stored under regular atmosphere (RA). The authors also found that the occurrence of grey pulp was significantly reduced by 1-MCP treatment, as was found in a recent study by Lemmer and Kruger (2010), particularly in late-season fruit (65% moisture content (MC)) and at higher temperatures (6-8^{o}C). Lemmer and Kruger (2010) also found that 1-MCP significantly reduced the incidence of anthracnose and stem-end rot in a batch of 'early' season (75% MC) fruit stored at all temperatures between 4 and 8^{o}C, although this was not the case in the 'mid' (70% MC) and 'late' (65% MC) season fruit due to overall low disease incidences. Maré *et al*. (2002) showed that 'Hass' avocado fruit had a higher percentage of 'sound fruit' (without physiological or pathological damage)when stored using normal air (91.3% sound fruit) than when stored using 1-MCP (85% sound fruit), although this difference was not statistically significant.

Results are not consistent as the effect of 1-MCP is highly dependent on the source of the fruit and can result in mixed ripening (Adkins *et al*., 2005). Although results are variable, South African avocados have been commercially treated with 1-MCP since 2003 at a recommended dose rate of 300-600 nL.L^{-1} (Lemmer and Kruger, 2007), as it is believed that the reduced risk of internal browning disorders, exceeds the possible problem of delayed ripening. If applied at the recommended rates, 1-MCP is a valuable tool in the long distance export of avocados, but control of exact effects is difficult.

5.4 Cooling methods

Room cooling, forced air cooling and hydrocooling are different methods to cool commodities, and while most commodities respond best to a particular method, each method has its advantages and disadvantages. As discussed earlier, water loss has a marked influence on chilling injury susceptibility and thus the correct cooling process is very important in maintaining high fruit quality. An important aspect is the time taken for effective cooling, as longer cooling times result in a greater loss of fruit moisture (Van Rooyen, 2005).

5.4.1 Room cooling / static cooling

Room cooling involves cold air being blown over the top of the produce and then drawn back past the fruit at low velocity, to a fan which removes the air. This is a common method used in the South African avocado industry. The advantage of room cooling is that the fruit can be cooled and stored in the same room, which reduces storage facility requirements. The disadvantage is that this method cools the fruit too slowly and therefore the fruit lose large amounts of water. Another disadvantage is that the fruit within the stack are cooled at different rates, depending on the position in the stack, leading to softening during shipping and uneven ripening after arrival (Ginsberg, 1985). Storage in these cooling rooms requires more air flow and a greater air velocity than an ideal storage environment, and thus the fruit loses more water than if stored in storage rooms separate from cooling rooms (Mitchell, 1992).

5.4.2 Forced air cooling

In this method, developed by Guillo, Mitchel and Parsons (1972), air is forced through the containers which allows for faster removal of heat and, therefore, quicker cooling. An exhaust fan is used to create a pressure gradient between the stack faces, which draws air down the gradient and past the fruit at a high velocity (Van Rooyen, 2005). Hofman *et al*. (2002) cite Watkins and Ledger (1990) who report that forced air cooling of avocados can be achieved in 8 or 12 hours with air flow rates of 1.0 and 0.5 Ls^{-1} $kg^{-1}$of fruit, respectively. The disadvantage of this quicker cooling process is that the fruit is subjected to a larger velocity of air and thus suffers greater water loss. Slabbert and Toerien (1984) found that this method of cooling improved the shelf-life of 'Fuerte' avocados compared with room cooling, but also found that it caused an increase in severity of external chilling injury. Forced air coolers are the least energy efficient coolers, but are commonly used because they are adaptable to many commodities and packaging types as well as providing more rapid and uniform cooling than room-cooling.

5.4.3 Hydrocooling

Hydrocooling involves the bulk cooling of avocados using cold water as the cooling medium. This method results in rapid cooling and decreased water loss. Bower and Magwaza (2004) agreed that hydrocooling would result in less water loss than either forced air cooling or static

cooling, and may thus reduce the incidence and severity of chilling injury. In room cooling and forced air cooling there is a high atmospheric demand for water and therefore water loss occurs from the fruit, whereas hydrocooling limits water loss and, thus, may be an important factor in reducing chilling injury at low temperatures (Donkin and Cutting, 1994).

A disadvantage of hydrocooling is that once the fruit is cooled, to a temperature close to the final storage temperature, the fruit may experience a rise in temperature during the packing process (Mitchell, 1992). Another logistical disadvantage of hydrocooling is that avocado fruit need to be cooled before packaging as the standard cartons are not water-proof, whereas other cooling techniques are able to cool the packed product. This poses various logistical problems with respect to general, current packhouse operations as the fruit will need to be pre-cooled and subsequently dried before being packed. However, Thompson (2002) recognised that hydrocooling may be feasibly integrated into the pack-line. Some packhouses may already be using water to lift avocados out of the picking bins by submerging the bins in water so as to limit the possibility of lenticel damage and other external blemishes which might result from tipping fruit onto the pack-line (Pers. Comm. 2). This system may allow for simultaneous hydrocooling and entry onto the packline with minimal delays, if fruit can be cooled rapidly enough.

Hydrocooling may, in part, provide a valuable and fairly inexpensive answer to minimizing fruit water loss and maintaining fruit quality. In the future, investigations into the required rate of cooling and feasibility of hydrocooling in South Africa should be carried out.

6. COLD STORAGE AND CHILLING INJURY

Cold storage is the main approach to post-harvest storage of avocados and is used to delay ripening, increase shelf-life, and ensure a high quality product upon arrival in overseas markets. Traditionally, the standard temperature for the export of avocados has been 5.5^{o}C (Donkin, 1995). Unfortunately, these low temperatures during storage may result in physiological disorders (Eaks, 1976; Wills *et al*. 2007, Sevillano *et al*., 2009). In practice, exporters in South Africa adjust the shipping temperature throughout the season, based on fruit maturity, experience and various other factors (Eksteen, 2001).

There is a fine line between the successful shipping of firm fruit and fruit which will suffer chilling injury (Bezuidenhout *et al*., 1992), and thus temperature regimes, treatments and shipping conditions need to be precise if low temperature shipping to distant markets is to be successful. Lyons and Breidenbach (1987) define chilling injury as "the permanent or irreversible physiological damage to plant tissues, cells or organs, which results from the exposure of plants to temperatures below some critical threshold for that species or tissue", and a chilling temperature as "any temperature below the critical threshold temperature (but above freezing) that causes injury". The critical/threshold temperature for avocado fruit is 8^{o}C (Lyons, 1973); however, the optimum temperature and storage period for avocados depends on a number of factors.

6.1 Types of chilling injury and visible symptoms

Chilling injury can be externally or internally present, and usually occurs when fruit is stored below a critical temperature for extended periods of time. All forms of chilling injury are a result of the disruption of the fluidity and structure of the membrane lipids, affecting the function and interaction with associated enzymes (Lyons and Raison, 1970). Internal chilling injury symptoms may appear as mesocarp discolouration, vascular browning, pulp spot or grey spot (Bower, 2005a). Fruit which show signs of more than 1% chilling injury can lead to fruit being rejected at the final destination (Dodd *et al*., 2007). Chilling injury may also cause a failure of the fruit to ripen normally (Couey, 1982).

6.1.1 Internal chilling injury

Mesocarp discolouration severity ranges from a light grey discolouration in parts of the fruit (particularly at the base of the fruit around the seed) to a blackening of the entire fruit (Van Rooyen, 2005; Woolf *et al.*, 2002). Swarts (1984) classified pulp spot as localised spherical grey spots in the mesocarp and grey spot (grey pulp) as a browning or general grey discolouration of the mesocarp. Nelson (2010) reported that internal greying of the fruit flesh (grey pulp or mesocarp discolouration) has long been, and is currently, a problem for late season South African avocados sold in Europe, particularly 'Fuerte'. Internal chilling injury is often associated with vascular browning which, as the name suggests, involves browning of the vascular bundles. Vascular browning starts at the base of the fruit and should not be confused with the browning of the vascular system as a result of stem-end rot, which occurs from the stem end (Woolf *et al.*, 2002).

6.1.2 External chilling injury

External chilling injury can be seen in avocados as black sunken lesions (which is known as 'pitting'), scalding and blackening, and is similar to apple scald (Kader and Arpaia, 2000; Van Rooyen, 2005; Woolf *et al.*, 2002). The damage first occurs in the inner cell layers of the rind and then the outer layers (Woolf, 1997). External chilling injury can be observed during storage but usually increases in severity after removal from cold storage (Woolf *et al.*, 2002).

The South African avocado industry has adopted certain terms to describe the symptoms of external damage resulting from cold temperatures, particularly in the case of Chilling Injury which is known in South Africa as Cold Injury and can include Black Cold Injury, Brown Cold Injury and Dusky Cold Injury. The South African avocado industry uses these terms so that all role-players will be able to identify which physiological disorders are being referred to, and thus are briefly described below.

6.1.2.1 Black cold injury

Black Cold Injury (Figure 5a) is probably the most common external damage caused by low temperatures in avocados exported from South Africa. The term is used to describe the shiny black lesions on the exocarp, and is most commonly found near the distal end of the fruit. The lesions are slightly sunken and have clear edges, but in the majority of cases these lesions do not affect the internal quality of the avocado. This type of cold damage is normally visible immediately upon arrival in Europe. The primary cause of Black Cold Injury is inappropriately low storage temperatures. Physiological maturity is one of the main factors which can increase the susceptibility of the fruit to Black Cold Injury, as less mature fruit (early-season fruit) are more susceptible than more mature fruit (SAAGA, 2007).

6.1.2.2 Brown cold injury

Brown Cold Injury (Figure 5b) can be described as brown discolourations on the exocarp, where the blemishes are well defined but are not sunken as in Black Cold Injury. These brown blemishes do not affect the lenticels which remain green and healthy within the brown area (Figure 5a).This symptom is found primarily in 'Fuerte' and Edranol' and is rarely seen in other greenskin cultivars. The symptoms can be visible hours after removal from cold storage, but can also develop when placed in cold storage after arrival in Europe, especially when ripening occurs in refrigeration (SAAGA, 2007).

6.1.2.3 Dusky cold injury

As the name suggests, this type of cold-related damage is visible upon ripening as dusky brown discolourations found at the distal end of the fruit (Figure 5c). These symptoms usually develop after excessive periods of cold storage and when fruit have ripened under refrigeration. This disorder does affect the internal quality of the fruit, resulting in discolouration of the mesocarp (SAAGA, 2007).

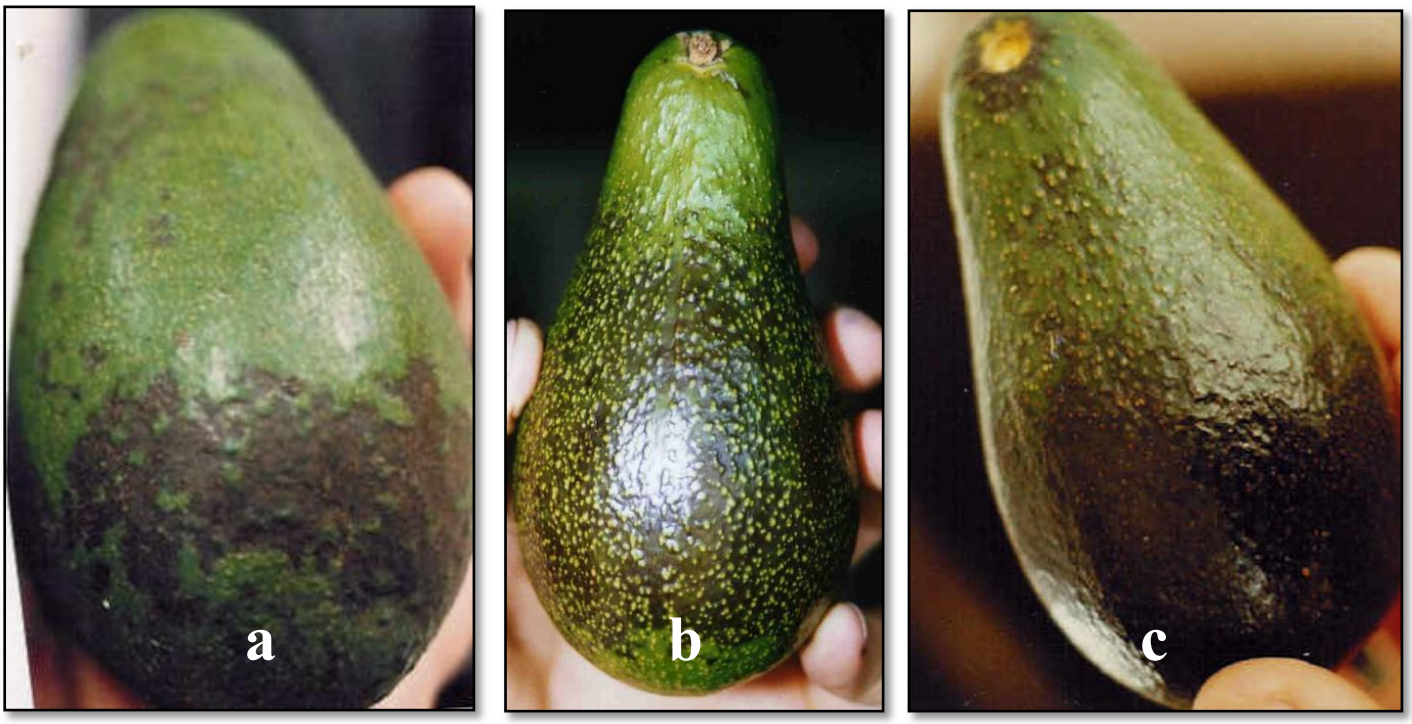

Figure 5: Visual symptoms of (a) Black Cold Injury, (b) Brown Cold Injury and (c) Dusky Cold Injury on greenskin avocados (SAAGA, 2007)

6.2 Factors affecting chilling injury

Susceptibility to chilling injury, as well as other physiological disorders, is influenced by numerous pre-harvest cultural factors such as cultivar, rootstock, production area, fruit maturity, tree vigour, crop load and mineral nutrition, as well as numerous post-harvest factors (Ferguson *et al*., 1999). Some of these factors will be dealt with briefly to grasp the complexity involved in trying to avoid chilling injury, with special attention placed on water loss and length of cold storage due to the relevance to this study.

6.2.1. Fruit origin

The nature and extent of chilling injury varies between different avocado cultivars as well as with fruit origin. 'Fuerte' is slightly less cold tolerant than Hass' (Eaks, 1976), and should thus be stored at slightly higher temperatures than 1^{o}C, a temperature that has recently been found to be relatively successful for stage of 'Hass' fruit (Van Rooyen, 2009). Vorster *et al*. (1987) suggested that a temperature of 3.5 ^{o}C, late in storage, may depress the activity of enzymes involved in the browning of avocados. Van Rooyen and Bower (2007) found that 'Pinkerton' could be shipped at 2^{o}C with minimal mesocarp discolouration but with some external injury occurring.

6.2.2. Orchard temperature

Ginsberg (1985) cite Swarts (1979) and Smith and Lunt (1984) who found that the temperature sensitivity of avocados, especially 'Fuerte', was greatly influenced by pre-harvest orchard temperatures. They found that susceptibility to chilling injury decreased as the number of hours that the orchard temperatures fell below 17^{o}C increased.

6.2.3. Storage temperature

Although many factors affect chilling injury it is assumed that fruit injury is directly related to the stress imposed during cold storage. The threshold storage temperature is reported to be 8^{o}C in avocados (Lyons, 1973). It is generally accepted that 'Hass' and 'Fuerte' can be stored at 5-6^{o}C for 28 days, without severe physiological disorders. It has been reported that fruit which are susceptible to chilling injury, in this case peaches and nectarines, are prone to more severe and faster development and expression of chilling injury symptoms when exposed to temperatures in a temperature range of 2.2^{o}C-7.6^{o}C (coined the 'killing temperature zone') than when stored below these temperatures but above freezing point (Harding and Haller, 1934; Crisosto *et al*, 1999). Lowering the storage temperature, to below these temperatures, has the potential to reduce the incidence of internal disorders (Dixon *et al.*, 2004) but may increase external chilling injury. Low storage temperatures do not suppress all cellular activity to the same extent (Wills *et al*., 2007). Kosiyachinda and Young (1976) proposed that low temperature storage may alter the activity of regulating enzymes, causing an accumulation of intermediates to concentrations that are toxic to cells. It is proposed that these regulating enzymes have different critical temperature thresholds and that a 'killing zone' (Crisosto *et al*, 1999; Blakey, 2011), perhaps of a similar temperature range, may exist in avocados where storage temperatures de-activate certain enzymes while others remain active, resulting in an imbalance in cell metabolism and eventual cell death (Wills *et al*., 2007). Ultra-low temperature storage (2^{o}C for greenskins and 1^{o}C for 'Hass') has repeatedly resulted in better maintenance of internal quality than currently implemented protocol temperatures (average of 5.5^{o}C), reducing the incidence of browning disorders (Bower and Magwaza, 2004; Lütge *et al.*, 2010; Van Rooyen, 2009; Van Rooyen and Bower, 2003; Van Rooyen and Bower, 2006; Van Rooyen and Bezuidenhout, 2010). This suggests that ultra-low storage temperatures (1 and 2^{o}C) fall outside of this 'killing zone', effectively de-activating the majority of the enzymes associated with ripening.

6.2.4. Fruit maturity

The water and oil content, which are correlated to the maturity of the avocado, have also been found to have an effect of chilling injury. The initial water that the fruit contains is converted into oils as the fruit matures (Pearson, 1975). Early-season fruit have been found to be more sensitive to low temperatures than late-season fruit (Toerien, 1986), probably related tooil content (fruit maturity) or orchard temperatures (Smith and Lunt, 1984). Early-season fruit also tend to have a higher occurrence of pulp spot than late-season fruit, while late-season fruit have a higher potential for grey spot infection (Swarts, 1980; Kremer-Köhne, 1998). "Step down" temperature regimes were first proposed by Toerien (1986), but an integrated approach in which fruit maturity determines the degree of temperature decline was proposed by Vorster *et al*. (1990) and is currently used for the export of South African avocados by sea (Donkin *et al*., 1994). Temperatures are typically reduced by 1-2^{o}C every week, with the final temperature not lower than 3.5^{o}C (Woolf *et al.*, 2003). However, Donkin (1995) found that step down temperature regimes produced fruit of quality no better than storage at 5.5^{o}C for 4 weeks. Early-season fruit will thus be expected to suffer more damage than later season fruit at low temperatures, and therefore, early-season fruit may need to be stored at slightly higher temperatures than those found successful for late-season fruit.

6.2.5. Calcium

Many physiological disorders in fruit and vegetables are related to a lack of calcium in the tissues of the plant, mainly due to inefficient distribution within the plants and not due to poor uptake (Wills *et al*., 2007). As calcium is transported in the xylem, uptake and transport into the fruit is influenced by transpiration rates, thus conditions which cause reduced transpiration (low atmospheric demand for water) and water stress will cause reduced calcium accumulation in the fruit. Avocado fruit with low levels of calcium have been found to be more susceptible to chilling injury (Chaplin and Scott, 1980). Chaplin and Scott (1980) found that the distal end of the avocado fruit, which is generally the area with the highest mesocarp discolouration, had a lower concentration of calcium than the proximal end. Eaks (1985) found that dipping 'Fuerte' avocados into calcium solutions ($CaCl_2$) of varying concentrations (0.05-0.5M Ca), did not have any significant effect on chilling injury. Eaks (1985) was able to successfully reduce mesocarp discolouration in 'Fuerte' avocados by vacuum infiltration of calcium, but this did result in exocarp browning. It has also been found that there is a coinciding demand for calcium by the fruit and leaves, and thus competition

between the two. Bower and Cutting (1988) showed that the terminal bud of the spring flush has a larger sink-strength than the fruit, which results in less calcium being transported into the fruit. It would thus be advisable to restrict excessive spring flush which ultimately reduces the quality of the fruit. It appears that in years where vegetative growth is vigorous, less of the available calcium is transported to the fruit (Donkin *et al.*, 1994), which poses a particular problem in orchards characterised by alternate bearing, as the 'off year' will produce fruit which are more susceptible to cold storage damage than the 'on year'. It is clear that the physiology of plants is influenced strongly by calcium levels during growth and development (Jones and Lunt, 1970).

6.2.6. The climacteric

The sensitivity and timing of chilling injury with respect to the climacteric is also important to note. Kosiyachinda and Young (1976) showed that the chilling sensitivity of 'Fuerte' avocados is closely linked to the occurrence of the climacteric. They found that 'Fuerte' avocados which were placed into storage 36 to 48 hours after reaching the climacteric peak, were able to be stored at 2^{o}C for 6 to 7 weeks. Mature pre-climacteric fruit could only be stored at 8^{o}C for 3 to 4 weeks without damage (Kosiyachinda and Young, 1976). The fruit's sensitivity to cold was found to increase from relatively high sensitivity before the climacteric, to a maximum at the climacteric peak, followed by reduced sensitivity after the climacteric peak (Kosiyachinda and Young, 1976). Similarly, Bezuidenhout (1983) found that when 'Fuerte' fruit were subjected to excessive cold just prior to the climacteric, chilling injury and pulp spot development was favoured. Lee and Young (1984) found that the sensitivity of avocado fruit to chilling injury increased when the fruit were stored in an atmosphere containing 100µl.l^{-1} of ethylene. This suggests that the increased sensitivity to chilling temperatures at the climacteric peak may be partly due to ethylene in the storage atmosphere. It is thus essential to prevent ethylene production and ripening during storage, which can be commercially achieved by the use of ethylene scrubbers.

6.2.7 Water loss

Pre-harvest as well as post-harvest water loss has been found to affect fruit ripening physiology significantly. Bower and Cutting (1987) showed that ripening rate and fruit quality were both affected by the rate of water loss during storage. Donkin and Cutting (1994) found that after 14 days of cold storage at 5.5^{o}C the severity of external chilling injury

increased with an increase in moisture loss before storage in 'Fuerte' avocados (Figure 6). Cutting and Wolstenholme (1992) found that passive infusion of water through the pedicel of avocado fruit, during cold storage at 5.5°C for 28 days, totally inhibited mesocarp discolouration. Bower (2005b) found that 2°C storage for 30 days resulted in internally sound fruit and also noted that by minimizing fruit mass loss during storage, the chance of chilling injury could potentially be reduced. Adato and Gazit (1974) showed a reduction in chilling injury occurrence when relative humidity levels were maintained above 95%. Bower and Cutting (1988) suggested that water loss may initiate softening, and result in an increase in respiration and thus a reduction in carbohydrate reserves. Bower and Magwaza (2004) confirmed the important correlation between water content and chilling injury, and showed that early water loss increased the fruit's sensitivity to chilling injury, whilst also predicting that physiological damage probably occurs during the first 10 days of cold storage if fruit is subjected to water loss.

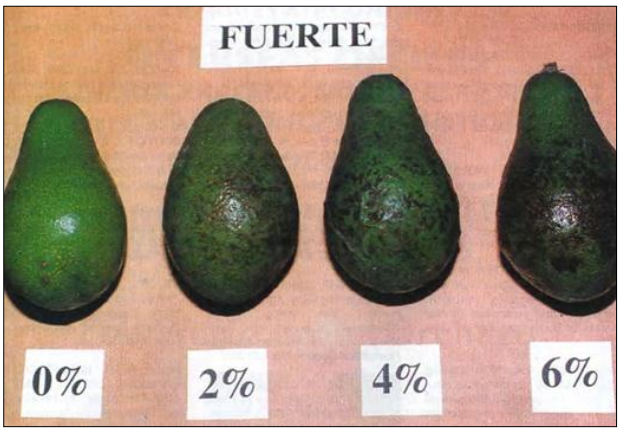

Figure 6: Chilling injury symptoms of 'Fuerte' fruit after 14 days at 5.5°C after different levels of moisture loss before storage (Donkin and Cutting, 1994)

Bower and Magwaza (2004) proved that a shipping temperature of 2°C for 'Hass' resulted in less mass loss (assumed to be mostly water loss) than at 5.5°C and 8°C. Bower and Magwaza (2004) also showed that external chilling injury occurred at a storage temperature of 2°C, but the use of polyethylene bags reduced this external injury. It seems that both, water loss reduction as well as storage temperatures, lower than presently implemented, result in better internal quality.

6.2.8 Time-temperature interaction

The time-temperature interaction is extremely important in maintaining high quality fruit during the lengthy shipping period to overseas markets. Threshold storage temperature and storage duration are the primary factors affecting chilling injury as the longer the duration of cold storage, the greater the potential for chilling injury (Van Rooyen, 2005). Saltveit and Morris (1990) indicated that the severity of chilling injury is a function of the period for which the fruit are stored at a certain temperature in the range from 3.3°C to 7.6°C. As the storage time is increased, at a certain storage temperature, the incidence of chilling injury increases (Table 1). Vorster *et al*. (1990) found a drastic increase in physiological disorders as the time of storage was increased, and suggested that the post-harvest handling period be kept to a minimum to avoid excessive storage periods. Lemmer *et al*. (2006) found that 'Fuerte' avocados could be stored for 2 months when combining 1-MCP treatment and CA, using a step down temperature regime with fruit stored at 7°C for the first month and 6°C for the second month. This indicates the potential for extended cold storage and requires further investigation into the possibility of success at temperatures lower than 6°C under RA.

Table 1: Occurrence of chilling injury following certain storage periods at different temperatures (Saltveit and Morris, 1990)

Storage Temperature (°C)	Storage Period (days)	Chilling Injury (%)
3.3	15	12.5
	28	66.0
	34	82.0
4.4	15	3.6
	28	26.0
	34	43.3
5.6	15	0.0
	28	10.2
	34	20.2
6.7	15	0.0
	28	1.9
	34	1.9
7.6	15	0.0
	28	0.0
	34	0.0

6.3 Physiological and biochemical effects of chilling stress

Chilling stress can cause various physiological and biochemical alterations as well as membrane dysfunctions (Wang, 1982; Sevillano *et al*., 2009). It was initially believed that chilling stress brings about a phase change of the lipid portions of the membranes in plants. Membrane lipids undergo a phase transition (solid-liquid) at a certain temperature (Mohr and Schopfer, 1995), and plants have the ability to alter membrane fluidity (to continue their metabolic functioning) by changing the saturation and unsaturation of fatty acyl groups and glycerolipids. As storage temperatures decrease, unsaturation of membrane lipids increases, the primary event in chilling injury (Lyons and Raison, 1970; Wang, 1982). Further research showed that less than 10% of the membrane lipids underwent this phase transition (Wills *et al*., 2007), thus a revised theoryonlipid-phase transition was developed. This revised theory is based on the presence of heterogenous domains in membranes undergoing liquid-crystalline to gel-phase transitions (Stanley, 1991). This temperature-induced phase change is completely reversible, but the effect on the whole organism is only reversible up to the point where injury occurs to the membrane (Platt-Aloia, 1980). Once the integrity of the membrane system is compromised, the damage is often reflected in ion leakage from the cell (Van Rooyen, 2005). In avocados, an example of such physiological damage is the leakage of phenols from the cytoplasm and subsequent oxidization by PPO, resulting in blackening of the mesocarp (Van Rooyen, 2005).

6.3.1 Chilling injury model

Raison and Orr (1990) devised a 'chilling injury model' and proposed that the process of chilling injury, and related membrane alterations, be divided into two stages (Figure 7). The primary event is likely to be the temperature-induced membrane phase change, and is initiated when storage temperatures are reduced below a certain threshold temperature (a), 8^{o}C in avocados (Lyons, 1973). The primary phase initiates metabolic dysfunctions that lead to tissue damage in the secondary phase (b). If the chilling stress is removed and the tissue is returned to warmer temperatures before the loss of cellular integrity, the process is reversible (c). If the tissue is returned to warmer temperatures in the reversible phase, there is a sudden increase in metabolic activity, related to the catabolism of intermediates accumulated due to reduced metabolic activity during chilling. This reduced metabolic activity during chilling restores the metabolic balance (d). However, if the stress is prolonged and cellular integrity is lost in the irreversible phase, and membrane dysfunctions occur, the higher temperature accelerates the development of visible chilling injury symptoms(e) because the anabolic repair processes do not outweigh the catabolic processes.

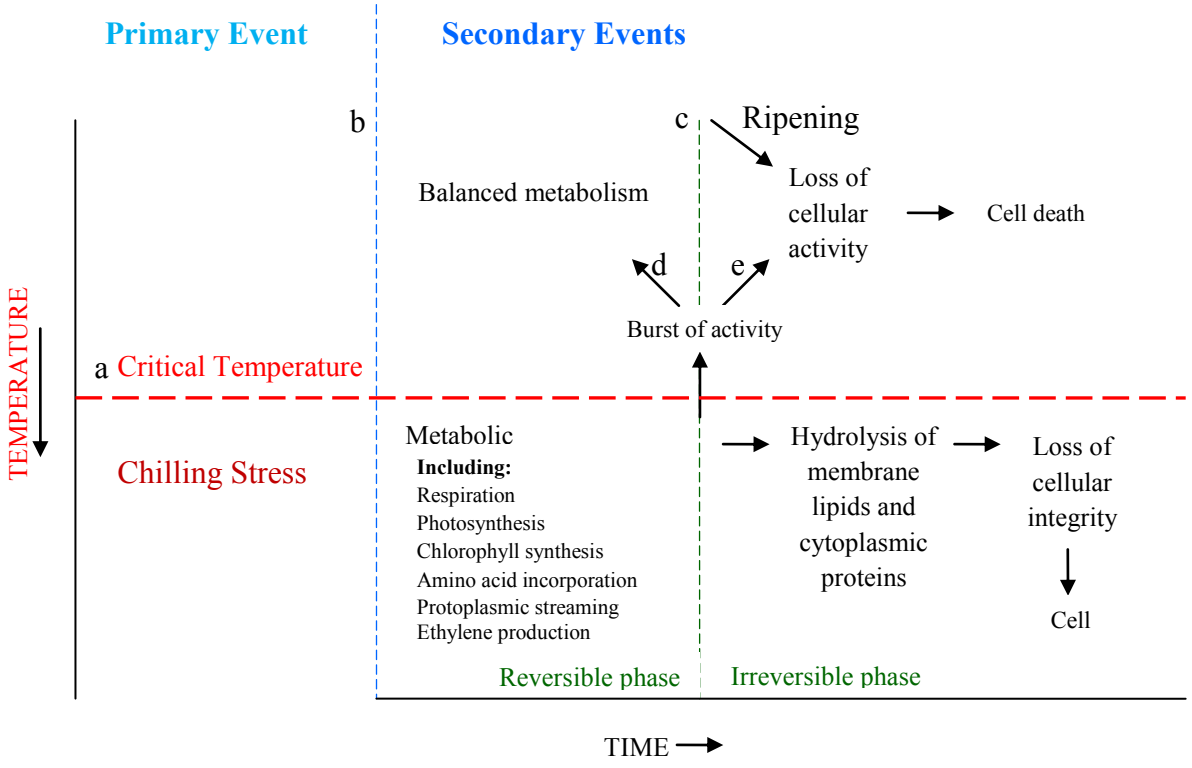

Figure 7: Schematic representation of primary and secondary chilling injury events (After Raison and Orr, 1990)

6.3.2 Internal damage

Browning of fruit tissues occurs when phenols leak from the vacuole into the cytoplasm, as a result of increased membrane permeability, where the phenols are oxidised by polyphenoloxidases (PPOs). The browning reactions in the fruit are correlated to the phenol levels, the activity of the oxidative enzymes (PPOs), or a combination of these factors (Kahn, 1977). The role of phenols and PPO in mesocarp discolouration is complex as these compounds often interact as a result of damage to cell membranes during cold storage.

Kahn (1975) found that in three avocado cultivars, the rate of browning was directly related to the PPO activity in mature fruit. Avocado cultivars which were more susceptible to mesocarp discolouration had higher PPO activities than less susceptible cultivars. PPO oxidises o-diphenols to o-quinones which are then oxidised to brown melanin pigments (Bower and Cutting, 1988). In undamaged tissue, PPO is present in highest concentrations in the thylakoid membranes of chloroplasts and is also present in leucoplasts, proplastids and amyloplasts, where it is latent (Vaughn and Duke, 1984). Chilling injury damages these cells which release PPO into the cytoplasm, and allows the browning reactions to proceed. Golan and Sadovski (1977) reported that under low temperature storage, avocados showed an increase in PPO activity as the time of storage was increased. Cutting *et al.* (1992) found that the total phenols in 'Fuerte' avocados increased with increasing fruit maturity, as did mesocarp discolouration in that particular year.

6.3.3 External damage

External chilling injury is highly correlated with increased electrolyte leakage of skin tissue (Woolf, 1997). This leakage is a criterion often used to indicate the degree of damage which may occur as a result of various stresses (Levitt, 1980). This leakage or loss of control of permeability indicates that damage has occurred to the plasmalemma, and thus its function is compromised (Platt-Aloia and Thomson, 1992). Although there is no indication of damage to the plasma lemma during avocado ripening, there is, however, a definite change in the organisation of this membrane in fruit that show low temperature injury (Platt-Aloia and Thomson, 1981; 1992). Platt-Aloia and Thomson (1992) proposed that the loss of selective permeability, which occurs during the lipid phase change during chilling stress (Moeller *et al.,* 1981), results in membrane dysfunction and cell death, causing visible chilling symptoms.

6.4 Important anti-oxidants in avocados and their function

Anti-oxidants are produced in cells to protect cellular structures against extremely reactive and damaging compounds, particularly reactive oxygen species (ROS) such as the superoxide radical, the hydroxyl radical, other free radicals and hydrogen peroxide (Fang *et al.*, 2002; Vranová *et al*., 2002). Anti-oxidants function to scavenge ROS which are formed during oxidative stress. The balance that exists between anti-oxidants and ROS needs to be maintained for normal cell metabolism within the plant. Under stress conditions, this balance can be easily disturbed and result in an accumulation of ROS (Foyer and Noctor, 2005). An accumulation of ROS, to damaging concentrations, results in oxidative stress which can cause membrane rigidification, peroxidation of membrane lipids, protein denaturation and DNA mutation (Borg and Schaich, 1988). To avoid permanent tissue damage, the accumulated ROS need to be removed from the cells by anti-oxidant systems within the fruit. These anti-oxidant systems occurring in various fruit include low molecular mass non-enzymatic anti-oxidants (e.g. ascorbic acid, vitamin E, β-carotene and phenols) and enzymatic anti-oxidants, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases (POX) (Fang *et al*., 2002; Blokhina *et al*., 2003).

The antioxidant activity in fruits can be influenced by factors such as maturity at harvest, genetic differences, pre-harvest environmental conditions, postharvest storage conditions and processing (Connor *et al.*, 2002). For 'Hass', Tesfay (2009) reported higher levels of total anti-oxidants in the exocarp and seed than in the mesocarp. Bertling and Bower (2005) found higher concentrations of C7 sugars in the exocarp of 'Fuerte' and 'Pinkerton' than in 'Hass', which may be due to the differences in the exocarp characteristics of these cultivars or due to genetic differences. Bertling and Bower (2005) suggested that the thinner, less protective exocarp of 'Fuerte' and 'Pinkerton', in comparison to 'Hass', may require higher levels of these C7 sugars to be transported to the exocarp in order to possibly aid in stress resistance. Plants respond to changes in environmental conditions by altering their antioxidant metabolism (Litchtentaler, 1996). However, very little is known about the changes in anti-oxidant systems in avocados which occur during low temperature storage.

Total anti-oxidant capacity (TAOC), according to Benzie and Strain (1996), and total anti-oxidant activity (TAOA),according to Re *et al*. (1999),are used to determine hydrophilic and lipophilic anti-oxidants, respectively. Tesfay *et al*. (2009a) reported that lipophilic anti-oxidants did not make up a large portion of the total anti-oxidant pool in the mesocarp of pre-

harvest ‘Hass’ avocados, as TAOC and TAOA results were very similar, thus TAOC will be used in this study. The concentration of C7 sugars will be of interest in the edible portion of the fruit and how these anti-oxidants affect the internal quality, while TAOC and ascorbic acid (AsA) will be of interest in terms of identifying their effects on external damage of the exocarp at ultra-low storage temperatures.

6.4.1 C7 Sugars

It was postulated that early fruit growth in avocados involves the accumulation of fructose, glucose, mannoheptulose and perseitol reserves (Liu *et al*., 1999). These sugars have not only been proposed as an energy source required by avocados after harvest (Liu *et al*., 1999; Meyer and Terry, 2010; Tesfay, 2009; Tesfay *et al*., 2010), but are also proposed as important anti-oxidants which reduce the effects of unfavourable environmental conditions such as cold storage temperatures and water stress conditions (Popp and Smirnoff, 1995).

Liu *et al*. (1999) reported that mannoheptulose and perseitol are the main non-structural carbohydrates present in avocado fruit. Cowan (2004) proposed that the C7 sugars have various important functions, including the protection of key enzymes from damage by ROS. Tesfay *et al*. (2009a) studied various sugars and anti-oxidants in ‘Hass’ avocado fruit and showed that the main anti-oxidant found in the edible portion of the avocado fruit (mesocarp) was mannoheptulose. Perseitol is the reduced polyol form of mannoheptulose, and has been suggested to act as a fairly efficient anti-oxidant(Jennings *et al*., 1998), however, not as effective as mannoheptulose (Tesfay *et al.*, 2010a). Polyols are osmotically active solutes, which increase in concentration in response to stress so as to compensate for reduced water potential inside the cell (Popp and Smirnoff, 1995), helping to protect enzyme activities and membranes under water-deficit conditions (Tesfay *et al*., 2010a).

Shaw *et al.* (1980) quantified various sugars in the mesocarp of 21 avocado cultivars by high-performance liquid chromatography (HPLC) analysis, and found that sugar content varied widely among the cultivars studied. The same authors detected only trace amounts of fructose and did not detect any glucose, mannoheptulose or perseitol in the mesocarp of ‘Fuerte’ avocados. Tesfay *et al*. (2010) found that mannoheptulose in ‘Hass’ avocados declined in concentration as the fruit approached harvest maturity, strengthening previous assumptions made by Bertling and Bower (2006) that this C7 sugar plays an important role in fruit quality. Further, Tesfay *et al*. (2010b) suggested that if mannoheptulose acts as the major respiratory

substrate, and declines during the ripening period, this sugar is likely to play a vital role in the ability to withstand stress and prevent browning in the mesocarp after harvest.

6.4.2 Ascorbic acid

Ascorbic acid (AsA), also known as Vitamin C, has an important role to play in environmental stress resistance in fruit. It not only acts as a broad spectrum anti-oxidant, providing photo-oxidation of free radicals thus reducing oxidative damage (Foyer, 1993), but also helps regenerate α-tocopherol, another important anti-oxidant compound that limits membrane damage (Tesfay *et al*., 2010; Thomas *et al*., 1992). Burton and Ingold (1981) reported the α-tocopherol is one of the most potent *in vitro* anti-oxidants. AsA has the ability to donate electrons in many enzymatic and non-enzymatic reactions, making it the main ROS-detoxifying compound in the aqueous phase (Blokhina *et al*., 2003).

Sun *et al.* (2002) investigated the total anti-oxidant activities of common fruits and found that ascorbic acid made up a relatively small portions of total anti-oxidants, the highest being 8.6% of total anti-oxidant activity in grapefruit, however this was in the edible portion of these fruit. Bertling *et al.* (2007) showed that AsA was the main anti-oxidant found in the exocarp and seed of 'Hass' avocado fruit. Tesfay *et al*. (2010a) investigated the AsA concentration in the exocarp, mesocarp and seed of 'Hass' avocados and found that, as was the case for TAOC, the highest concentrations were found in the exocarp and seed whilst the lowest concentrations were found in the mesocarp. In general, it is accepted that ascorbic acid concentrations vary greatly between fruit species (Davie *et al*., 2000). Larrigaudière *et al.* (2004) investigated the effect of 1-MCP and cold storage on the ascorbic acid content of pears, reporting that ascorbic acid concentrations in both treated and untreated fruit decreased during the first two weeks of cold storage at 0.5^{o}C and then increased to a level similar to the level at harvest, indicating the fruit's ability to increase levels of antioxidants in response to stress. However, knowledge on the effect of storage temperature on ascorbic acid concentrations in avocados is limited.

6.5 Prevention or reduction of chilling injury

It is very important to maintain an optimal cold chain as well as ensuring that pre-harvest conditions and tree health are managed efficiently, in an attempt to reduce chilling injury. Numerous experimental treatments such as low temperature conditioning, step down temperature regimes, vapour heat treatment, waxes, packaging as well as treatments with calcium and other chemicals, such as ethylene, abscisic acid, and other natural compounds (Van Rooyen, 2005) have been tested with varying success. CO_2 shock treatment has been found to be successful in delaying softening and chilling injury development in 'Fuerte' avocados (Truter and Eksteen, 1987). Fuchs *et al*. (1995) remark that post-harvest treatments can be applied in an attempt to reduce chilling injury, but these treatments cannot 'cure' poor quality fruit.

The effect of cold storage on fruit quality may vary from season to season, depending on prevailing climatic condition and pre-harvest management of avocado trees. For this reason, feedback from overseas markets is crucial and should include the information on the fruits' firmness upon arrival, occurrence of external chilling injury and the temperature of the consignment upon inspection (Bezuidenhout *et al.*, 1992; Kremer-Köhne, 1998). This will allow exporters to trace the cause of the injury or disorder and correct the mistake made.

6.5.1 Low temperature pre-conditioning

Low temperature pre-conditioning is used to make cold-sensitive tissues more tolerant to damaging low temperatures. This involves holding the fruit at temperatures just above the threshold at which injury to tissues occurs (Woolf *et al*., 2003). This technique has been successful in reducing chilling injury in many fruit and vegetables (Wang, 1993). Pre-conditioning of avocados at low temperatures prior to cold storage has achieved success in alleviating chilling injury (Hofman *et al.*, 2003; Van Rooyen, 2009; Van Rooyen and Bezuidenhout, 2010; Van Rooyen and Bower, 2007; Woolf *et al.*, 2003). The key factors appear to be the difference in temperature between the pre-conditioning temperature and storage temperature, as well as the duration of the pre-conditioning treatment (Woolf *et al.*, 2003). Low temperature conditioning, at 6^{o}C for 3 days, before cold disinfestation, was found to eliminate external chilling injury in 'Hass' avocados (Hofman *et al.*, 2003). Van Rooyen and Bower (2007) studied the effect of various temperature pre-conditioning treatments on 'Pinkerton' avocados and found the most successful treatment, in terms of alleviating chilling

injury during long term storage at 2°C, was when fruit were held at 10°C for 2 days. Van Rooyen and Bower (2007) postulated that temperature pre-conditioning may increase the degree of unsaturation of fatty acids in membranes, and in turn, affect membrane fluidity and permeability. The authors do, however, acknowledge that further studies are needed to elucidate the effect that temperature pre-conditioning has on the physiology and biochemistry of avocados.

6.5.2 Waxing and packaging

A high level of fruit water loss causes stress during storage, resulting in an increase in physiological and pathological disorders (Bower and Cutting, 1987). Waxing as well as packaging, of individual fruit or bags of fruit, has been found to be effective in reducing chilling injury in avocados (Lunt *et al*., 1981; Eksteen and Truter, 1985; Wang, 1993; Bower and Jackson, 2003; Bower and Magwaza, 2004; Bower and Blakey, 2008). Polyethylene wax emulsions are important as they reduce water loss, delay softening, and improve the appearance of 'Fuerte' avocados (Lunt *et al.*, 1981; Durand *et al.*, 1984). Joyce *et al*. (1995) reported that waxing caused a reduction in water loss and modification of the internal atmosphere, which resulted in an increase in shelf-life.

Bower (2005a) found polyethylene bags to be very effective in reducing water loss in 'Hass' and found no rind damage, consistent with previous results by Eksteen and Truter (1985). Bower and Magwaza (2004) found that packaging 'Fuerte' avocados in micro-perforated polypropylene bags was more effective in reducing chilling injury than waxing. These authors found that an effective and inexpensive method of reducing external chilling injury was to use micro-perforated film-packaging either for individual fruit or bags of fruit while avoiding condensation. These semi-perforated film packaging options subject the fruit to a modified atmosphere which increases CO_2 levels and decrease O_2 levels as well as maintaining a high relative humidity around the fruit. These conditions are thought to prevent or minimize the risk of cell collapse in the epidermal and underlying cells (Van Rooyen and Bower, 2007). Scott and Chaplin (1978) found a decrease in mesocarp discolouration in 'Hass' and 'Fuerte' avocados stored in sealed polyethylene bags. Bower and Blakey (2008) reported that individually wrapped fruit can be cooled acceptably; however, these authors showed that wrapped fruit took significantly longer to reach the desired temperature in cold storage, and suggested that fruit may need to be cooled before packaging.

Although waxing and packaging does result in a reduction of chilling injury, problems such as increased incidences of pathological disorders have been reported (Eksteen and Truter, 1985; Van Rooyen and Bower, 2007), and may negate any increase of external fruit quality. Bower and Papli (2006) noted the use of waxes to be questionable as this may create other stresses, such as limited gas exchange, resulting in external damage. Waxing has also been shown to alter the gas exchange characteristics of fruit (Amerante and Banks, 2001; Joyce *et al*., 1995), which Bower and Blakey (2008) suggested may result in slower and less uniform ripening than non-waxed fruit. Durand *et al.* (1984) found that the internal CO_2, O_2 and C_2H_4 concentrations after two weeks of cold storage (5^{o}C) were not significantly influenced by waxing, however, waxing did reduce mass loss significantly. Bower and Magwaza (2004) report that avocado importers found that even ripening in waxed fruit is more difficult to achieve than in non-waxed fruit for fruit sold on the 'ripe-and-ready' market.

7. COLD CHAIN BREAKS

The South African export cold chain for avocados is the longest in the world (Pers. com. 1), comprising many role players as the fruit moves from the orchard to the retailer. Shipping is the main form of transport to the European markets and thus the fruit requires storage for up to 30 days (Bower and Cutting, 1988). Airfreight is seldom used, due to the high costs, and only viable when export prices are abnormally high (Donkin, 2007). The South African regulations for the export of avocados are strictly enforced and stipulate that the avocado pulp temperature cannot differ more than 2^{o}C from the specified export temperature (Dodd *et al*., 2007). If the temperature of the consignment is outside this 2^{o}C tolerance, the exporter must rectify the pulp temperature which, if temperatures are too high, requires an expensive and time-consuming re-cooling process. Proper management of the cold chain can help avoid these additional costs and delays, and result in fruit with a better post-storage quality and shelf-life.

7.1 Problem areas

Dodd *et al*. (2007) analysed the avocado cold chain in South Africa, starting at the packhouse and included pre-cooling, consolidation, road transport loading and unloading, and finally loading into the shipping containers; however, the authors did not include the sea voyage links in the cold chain. The diagram below (Figure 9) illustrates the different role players involved in the transport of export avocados from the orchard to the port, with the scope areas analysed by Dodd *et al*. (2007) indicated by the dark block. This analysis identified various 'weak links' in the cold chain, where fruit may be subjected to temperature fluctuations. The process of loading the fruit from the packhouse into the road transport vehicles is the main weak link in the cold chain. Dodd *et al*. (2007) found that in the 2006 season, 33% of packhouses had off-loading temperatures 2^{o}C warmer than the set point and that 60% of refrigerated road transport loads had loading temperatures 2^{o}C or higher than the set point. It is clear that packhouses are often loading fruit which is much warmer than the recommended set point. This could be as a result of old and inadequate cooling equipment in the packhouses, or perhaps this equipment is not set to the correct temperature in an attempt to save cooling costs – either way, this is a poor start to the cold chain.

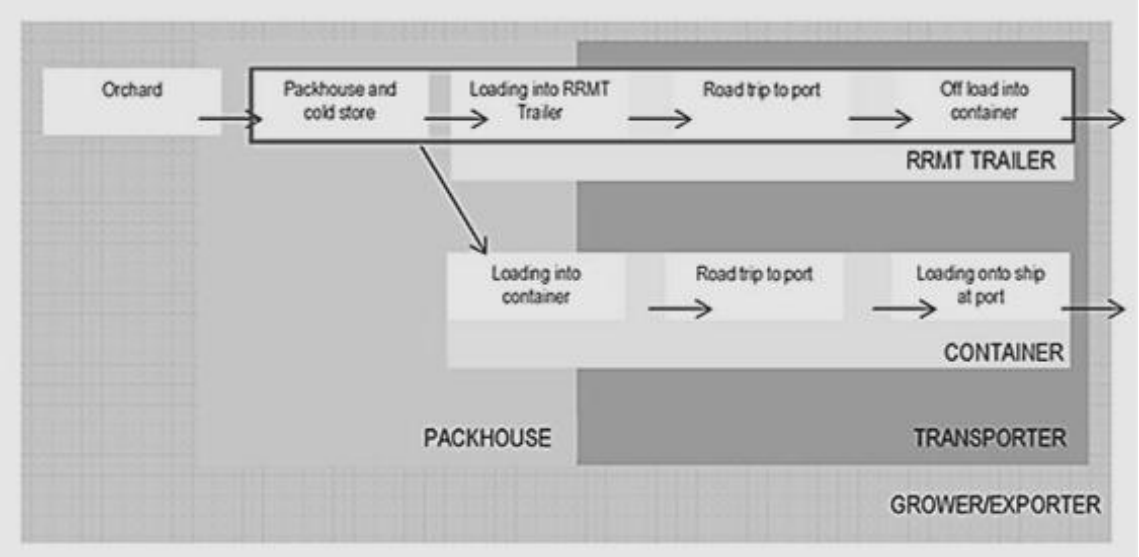

Figure 9: Role players involved in transporting avocados from the orchard to the port (Dodd *et al*., 2007). RRMT = refrigerated road motor trailers

Recent work by Lütge (2009), on cold chain breaks at various stages in the cold chain, identified the cooling phase as a major area of concern. 'Fuerte' fruit which underwent a 24 hour delay in cooling suffered a marked decline in fruit quality and an increased susceptibility to external chilling injury, presumably as a consequence of increased water loss from the fruit (Lütge, 2009).

7.2 Effects of cold chain breaks

Breaks in the cold chain have been shown to reduce avocado fruit quality and compromise shelf-life, and thus should be avoided (Dodd *et al*., 2007; Eksteen, 1995; Eksteen, 1999; Lemmer and Kruger, 2010; Lütge *et al*., 2010; Vorster *et al*., 1991). De Castro *et al*. (2005) showed that tomato quality is substantially reduced as a result of cold chain breaks, adding that cold chain breaks had the greatest influence on 'time to maturation' and 'disease susceptibility'. Cold chain breaks are, therefore, likely to compromise fruit shelf-life and increase fruit disease incidence in avocados.

Hofman *et al*. (2002) list the possible problems associated with cold chain breaks as being: re-cooling costs, condensation on the fruit (resulting in higher microorganism growth, an increase in chilling injury due to evaporative cooling, and weakening of cartons) and reduction in storage life. Nelson (2005), an overseas technical officer, noted that breaks in the cold chain caused a break in the inhibitory effect of 1-MCP on ripening, resulting in shorter shelf-life, especially during unloading in Europe. In 'Hass', Undurraga *et al*. (2007)

concluded that cold chain breaks for 2 or 3 days of 25^{o}C, during cold storage, caused mass loss, early softening and physiological disorders, all of which compromise the quality and shelf-life of exported avocados. Lemmer and Kruger (2010) studied the effect of cold chain breaks on 'Hass' fruit quality and ripening, and found that in general, the effect of cold chain breaks increased as fruit maturity increased, suggesting that cold chain breaks later in the season are more detrimental to fruit quality than early in the season. Swarts (1981) demonstrated that a break late in the cold chain had a greater softening effect than early in the cold chain. Similarly, Lemmer and Kruger (2010) found that the mean days-to-ripening (shelf-life) was shorter when cold chain breaks were introduced after 20 days of cold storage than when a similar break was introduced after 5 days of cold storage, indicating that a break early in the cold chain is less detrimental than later in the cold chain as was found by Lütge *et al.* (2010). Further, as the length of the break increased, the increase in respiration and reduction in firmness became more pronounced, and the mean days-to-ripening value was decreased (Lemmer and Kruger, 2010).

Lemmer and Kruger (2010) found that cold chain breaks resulted in a significant increase in the incidence of stem-end rots as well as anthracnose, though the latter increase in pathology was slightly less severe than the former. However, cold chain breaks did not influence the occurrence of grey pulp which was found to be more prevalent under higher storage temperatures (5-8^{o}C) and in the mature fruit (65% moisture content). This suggests that the main effects of cold chain breaks are possibly due to condensation which occurs as a result of the temperature break, and thus increases the likelihood of pathological disorders.

Until recently, investigations into the physiological effects of breaks at various points in the cold chain were lacking, particularly information on the interaction of cold chain breaks and ultra-low shipping temperatures. Blakey and Bower (2009) investigated this interaction on 'Hass' avocado fruit and showed that storing fruit at 1^{o}C provided a buffer against cold chain breaks, possibly because the lower pulp temperature would cause fruit to take longer to warm up and exceed critical levels than fruit stored at the current industry standard of 5.5^{o}C. Lütge *et al.* (2010) showed, on 'Fuerte' avocados, that cold chain breaks caused increased fruit softening, water loss and susceptibility to external cold damage, especially when a delay in cooling occurred.

7.3 Solutions

Eksteen and Bester (1987) provide some practical suggestions for improved cold chain management, including harvesting, pre-cooling, storage temperature recommendations, air circulation, carton ventilation and container loading. The primary solution to cold chain breaks is improved management. Planning and organisation needs to be precise and execution of individual tasks needs to be strictly monitored.

The leading exporters of avocados in South Africa use temperature monitors which record temperature and relative humidity in a consignment. One such system is the 'Xsense® Perishables Quality Monitoring System' used by 'Westfalia' for 95% of their export avocado consignments (Pers. Comm., 2). These 'Xsense®' monitors (Manufactured by 'StePac') use radio frequency to upload the stored data automatically onto an internet website, whenever the sensors reach a facility which has a 'Communication Unit', such as at the Cape Town port. Alert emails are also sent whenever a container arrives at its destination with the temperature or relative humidity outside the set thresholds. This allows for compensative action to be taken by the exporters before it is too late. This system allows exporters to track individual refrigerated road motor trailers (RRMT) and containers, and be aware of the exact conditions present in each consignment. Packhouse cooling equipment needs to be able to cool fruit to the required storage temperatures, and should be inspected periodically. Dodd *et al*. (2007) suggest that the air flow dynamics need to be examined, and that packaging design and palletisation play an important role in fruit cooling. Cool air must move freely around the fruit and thus packaging design may need to be modified so that maximum air contact with the fruit is achieved. Dodd *et al*. (2007) explain how critical horizontal and vertical airflow is, and how all four sides as well as the base of the carton need to allow for proper airflow. Improved packaging may make cooling and maintaining fruit pulp temperature somewhat easier in the future. The main goal in road transport to the port should be to avoid the expensive re-cooling process at the port due to higher than stipulated pulp temperatures.

The main benefit of ensuring that no cold chain breaks occur *en route* to distant markets is that ripening is not initiated during storage. The negative effects of cold chain breaks reported in various recent studies (Blakey and Bower,2009; Kok *et al*., 2010; Lütge *et al.*, 2010; Lemmer and Kruger, 2010) clearly reduce the quality of export avocados. One of the main effects of cold chain breaks, which is often overlooked, is condensation which results in increased pathological disorders in these export consignments.

8. PATHOLOGICAL DISORDERS

All the pathological disorders which occur in South African export avocados have been reported in detail by various authors, and thus the purpose of this section is not to describe each disorder, but rather to identify the most common disorders and then discuss the effect of storage temperature and certain treatments on these disorders.

8.1 Fruit rots

Fruit rots are very seldom visible at harvest or during storage but can increase rapidly during ripening and fruit softening (Woolf *et al*., 2002). Fruit rots can be categorized into two types based on the location on the fruit, namely stem end and body rots (Snowdon, 1990).

8.1.1 Stem-end rots

As the name suggests, stem-end rots enter at the stem end or peduncle of the fruit (Figure 10). Most infections of the stem-end are dormant at harvest and only manifest themselves when fruit ripening begins (Pegg *et al.*, 2002). Stem-end rots are a particular problem when the cold chain is sub-optimally maintained (Manicom, 2001). Symptoms are first visible as slightly shrivelled areas around the stem button of the fruit (Menge and Ploetz, 2003). The infection moves down the fruit causing discolouration of the mesocarp, often with associated browning of the vascular system (Johnson and Kotze, 1994). The infected areas are dark-brown to black in colour and have clearly defined margins (Menge and Ploetz, 2003). Infected fruit eventually become shrivelled, watery and are often covered with fungal mycelium (Johnson and Kotze, 1994).

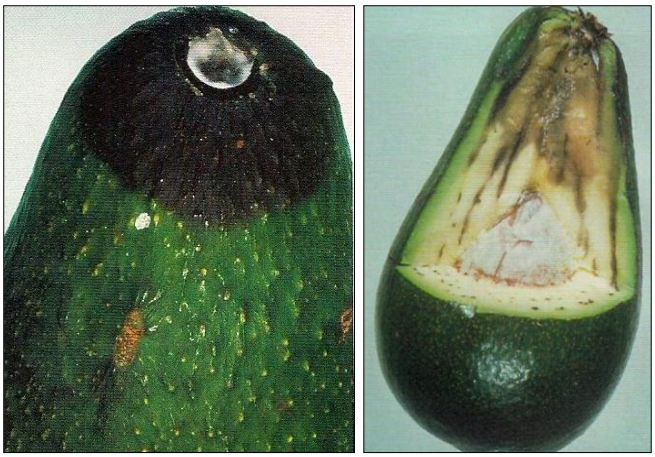

Figure 10: Early (left) and late (right) symptoms of stem-end rot of 'Fuerte' avocados. Note the darkening of vascular strands which begins from the stem-end. (Whiley *et al.*, 2002)

8.1.2 Body rots

Body rots infect the fruit through the skin and cause circular brown spots that may become covered with pink spores in the later stages of infection (Figure 11, Woolf *et al*., 2002). The decay enters the mesocarp through the skin and causes areas of discoloured flesh. Anthracnose is the most common fruit rot in mature avocados, and is a large problem in most avocado producing areas of the world (Menge and Ploetz, 2003). Losses of up to 37% of harvested fruit have been reported by Fitzell (1987), and 'Fuerte' has been found to be more susceptible to anthracnose than 'Hass' (Menge and Ploetz, 2003).

Figure 11: External (left)and internal (right) symptoms of anthracnose in 'Fuerte' avocados. Note the pinkish spores on the surface and the hemispherical lesion extending through the flesh (Whiley *et al.*, 2002).

8.2 Effects of post-harvest treatments on pathological disorders

Post-harvest temperature management and controlled ripening, using ethylene applications, can greatly influence anthracnose development. A strong correlation between ripening time and anthracnose development has been shown in avocados (Darvis, 1985; Hopkirk *et al*., 1994). Thus cold storage and other treatments which lengthen the time to ripening will increase the risk of anthracnose infection, while treatments such as ethylene application, after removal from cold storage, will reduce this risk. The temperature during ripening has been found to influence the levels of anthracnose in 'Fuerte' avocados (Fitzell and Muirhead, 1983, section 1.3.5). Lemmer and Kruger (2003) found that 'Fuerte' avocados treated with 1-MCP had significantly higher incidences of anthracnose and stem-end rots than untreated fruit, primarily because of the lengthening of the storage period as is the case with cold storage, CA and MA. Mare *et al*. (2002) found that the combination of CA and 1-MCP resulted insignificantly higher levels of anthracnose than fruit stored under RA, illustrating the above correlation between ripening time and anthracnose development. Van Rooyen and Bower (2007) found that 'Pinkerton' fruit stored at 2^{o}C had a significantly higher incidence of anthracnose than fruit stored at 5.5^{o}C, and also found that waxed fruit and those packaged in polypropylene bags had a significantly higher incidence of anthracnose than non-waxed fruit, which agrees with the findings of Eksteen and Truter (1985) for 'Fuerte' avocados. Cold chain breaks also have the potential to increase the occurrence of numerous pathological disorders as a result of condensation on the fruit surface, which promotes fungal development as was found with cold chain breaks on tomatoes (de Castro *et al*., 2005).

Ultimately, any treatment which extends the shelf-life of avocados could result in a higher incidence of pathological disorders, and thus a balance is needed between providing sufficient time to market the fruit and the risk of high infection rates due to an excessive ripening period.

9. PHYTOSANITARY ISSUES

Disinfestation treatments are required for fruit entry into certain world markets such as the USA. These treatments have been largely centred on chemical fumigation and dips, but are no longer accepted by many importing countries. Safer alternative methods are currently being developed and involve the use of heat, cold, irradiation and controlled atmospheres, either individually or in combination (Hofman *et al*., 2003). Many avocado producing areas require a disinfestation treatment against fruit flies, if these areas intend to export these fruit to certain markets. Because avocados do not tolerate high temperature disinfestation treatments, and irradiation has been found to be unsuitable (Du Rand *et al*., 2010), the most viable approach is low temperature disinfestation.

Cold disinfestation of 1^{o}C for 16 days is the approved quarantine treatment for 'Hass' avocados exported to New Zealand, as it is an accepted treatment for many fruit fly species (Hofman *et al*., 2003; Jessup, 1994). At present, the USDA-APHIS requirements state that a storage temperature of 1^{o}C, for a continuous period of 21 days, needs to be met for 'Hass' avocados, as discussed by Van Rooyen (2009). Van Rooyen (2009) found that cold sterilization (at 1^{o}C for a minimum of 21 days) of pre-conditioned 'Hass' fruit did not render an unacceptable quality, and if this treatment is found to achieve the right degree of insect mortality, this treatment may provide access to new markets for South African avocado producers. Ware *et al*. (2011) proved that a cold disinfestation period of 22 days at 2^{o}C (±0.5^{o}C) could be used successfully as a phytosanitary treatment against 'False Codling Moth' for 'Hass' avocados. These authors illustrated that a total 101135 individual larvae were cold-treated and no live larvae were detected, potentially offering access to new markets in the future.

Although this study is focussed on 'Fuerte' avocados, and there is a real possibility that these low temperatures may result in highly visible external damage in greenskins, the progress in this aspect of cold storage is important to follow. The good internal quality reported in greenskins such as 'Fuerte' (Lütge, 2009) and 'Pinkerton' (Van Rooyen and Bower, 2006) stored at 2^{o}C, suggests that the possibility of meeting such phytosanitary requirements does exist if mitigating treatments for the external chilling injury are economically viable. Further research as well as governmental co-operation is required before any shipments to these potential markets can materialise.

10. DISCUSSION AND CONCLUSION

Currently, the South African avocado industry is reliant on CA and 1-MCP to ensure that fruit arrive at their final destination without significant quality deterioration. The costs of CA and 1-MCP treatments are extremely high. Low temperatures during post-harvest storage, slows the metabolic activity of the fruit and thus delays the onset of ripening (Wills *et al*., 2007). The ideal low temperature for such storage appears to be that temperature below which chilling injury occurs (Van Rooyen, 2005), and thus the line between chilling injury and successful cold storage needs to be identified.

It would appear that the current protocol shipping temperature, which averages 5.5^{o}C through the season, is not low enough to prevent fruit softening during shipping. The proposed temperature of 2^{o}C for 'Pinkerton' (Van Rooyen and Bower, 2002) may be a more suitable temperature for 'Fuerte', if chilling injury can be prevented. If this storage temperature is successful, the industry could realise large financial savings, as this method is significantly cheaper than using 'patch-up' methods, such as 1-MCP, to prevent softening during storage. If the use of 1-MCP could be avoided, there may also be reduced incidences of uneven ripening, enabling importers of avocados to sort and distribute quality avocados more efficiently.

Thus, successful shipping of 'Fuerte' avocados at 2^{o}C seems possible, based on preliminary experiments (Lütge *et al*., 2010) as well as on research conducted on greenskins and 'Hass' at ultra-low temperatures (Bower and Magwaza, 2004; Lütge *et al.*, 2010; Van Rooyen, 2009; Van Rooyen and Bower, 2003; Van Rooyen and Bower, 2006; Van Rooyen and Bezuidenhout, 2010). This temperature could easily be achieved in the packhouse, and the capital expenses of the additional cooling required would be significantly lower than the current costs of CA and 1-MCP. Research is needed on whether different situations, particularly differences in fruit maturity through the avocado season, will require low temperature to be used in conjunction with other current methods of extending the shelf-life of avocados. Relatively successful semi-commercial trials on the risks of shipping 'Hass' avocados at 1^{o}C for 28 days (Van Rooyen, 2009) seem to indicate that temperature precondition of these fruit is necessary to reduce the external damage which can occur at this low temperature.

Post-harvest moisture loss has been implicated as a factor affecting chilling injury in avocados (Bower and Cutting, 1987). Fruit moisture loss, both before and during cold

storage, needs to be considered, when attempting to identify the optimum cold storage temperature. One needs to consider the cooling method used to cool the fruit, as this can have an effect on moisture loss and chilling injury occurrence. Waxes are also currently used to reduce water loss and to increase the aesthetic appeal of the fruit; however, there may be complications with the use of waxes, such as limited gas exchange in the fruit, resulting in external injury (Bower and Papli, 2006). There have also been reports of uneven ripening (Bower and Magwaza, 2004). Previous results indicated that waxing reduces the occurrence and severity of external chilling injury of 'Fuerte' at 2^{o}C (Lütge, 2009).

There is scarce literature available on the effects of breaks in the cold chain of 'Fuerte' avocados, particularly when fruit are stored at ultra-low temperatures. The effect of cold chain breaks when using 2^{o}C compared with 5.5^{o}C needs to be investigated for 'Fuerte' avocados. Breaks in the cold chain are undesirable and methods of reducing these breaks need to be explored.

Overall, the avocado industry needs to continually improve to ensure that quality fruit arrive at their export destination. To remain competitive and expand as an industry, shipping and storage technologies need to constantly improve or evolve as further information becomes available. It will be highly beneficial for the industry to expand into new markets, such as the USA and Japan, but to do this, methods will have to conform to current import regulations and current protocols need to be adapted. A shipping temperature of 2^{o}C may help the industry to realise the goal of accessing these markets with phytosanitary regulations, in the future, if chilling injury can be minimised.

CHAPTER 2

Fruit quality of 'Fuerte' avocados following ultra-low temperature storage for 28 days and its potential as an alternative to 1-MCP use

Andre Lütge, Isa Bertling and John Bower

An average storage temperature of 5.5°C throughout the export season and expensive CA and MA treatments are currently used to delay ripening of 'Fuerte' avocados; however, fruit still appear on the European market showing signs of softening and physiological disorders. Recently Lütge *et al*., (2010), showed that 2°C storage of 'Fuerte' avocados could be a potentially successful alternative shipping method and a potential cold sterilization treatment required to enter new markets with phytosanitary requirements. The objective of this study was to determine the potential for storing 'Fuerte' avocados at temperatures of 2°C for 28 days, throughout the growing season. 'Fuerte' avocados were harvested at three different maturity stages reflecting early-, mid- and late-season fruit, with moisture contents of 74%, 68% and 63%, respectively. Fruit were subjected to treatments of 1-MCP and waxing and stored at 2°C and 5.5°C for 28 days. Fruit softening, mass loss, days-to-ripening, external quality and internal quality were recorded. A storage temperature of 2°C provided good internal quality as well as reduced mass loss and reduced fruit softening. The 2°C storage caused a notably higher occurrence of external chilling injury than the 5.5°C storage but extended days-to-ripening. Waxing significantly reduced the external damage on fruit stored at 2°C, so much so, that the treatment combinations of '2°C, no 1-MCP, waxed' showed no external chilling injury throughout the season. Best results were achieved for mid-season fruit stored at 2°C. Late-season fruit would potentially be the most profitable to store at this low temperature, however, body rots (anthracnose and stem-end rot) were more common in the late-season fruit. Storage at 2°C can therefore maintain the internal quality over a storage period of 28 days. Further, waxing fruit and subsequent storage at 2°C could eliminate the need for 1-MCP, delivering a product of the required shelf-life and quality. However, external damage at a storage temperature of 2°C remains a concern, suggesting that at present 1-MCP and 5.5°C storage are required for greenskins, particularly early in the season, with an opportunity to lower temperatures as fruit maturity increases later in the season, possibly in conjunction with pre-conditioning treatments.

1. INTRODUCTION

Exporting South African avocados to European markets requires that firm, high quality fruit reach the overseas ports after a sea voyage of 12-14 days (Donkin, 2007) and an average of 25-28 days in total to reach the overseas destination from the time of picking (Bezuidenhout *et al.*, 1992). Temperature regimes revolving around a storage temperature of 5.5°C as well as expensive post-harvest treatments of 1-Methylcyclopropene (1-MCP) and controlled atmosphere (CA) are currently being used to delay ripening and achieve a desirable days-to-ripening (DTR). However, under the current commercial export protocols, at an average conventional storage temperature of 5.5°C, fruit may occasionally appear on the European market showing signs of softening, uneven ripening and physiological disorders (Nelson, 2010). Although these cases are fairly infrequent, these occurrences can lead to reduced European consumer confidence in South African avocados, and thus export shipping conditions and treatments need to be constantly monitored and improved.

Shipping of greenskin avocados at lower than the standard temperature of 5.5°C is not only possible but also relatively successful when fruit are stored under regular atmosphere (RA) at 2°C (Bower and Magwaza, 2004; Van Rooyen and Bower, 2007; Lütge *et al.*, 2010). Such low temperature storage can be used as a measure to reduce premature softening and moisture loss. Lower temperatures have also been repeatedly shown to result in better fruit quality for various commercial cultivars when stored for 28 days (Bower and Jackson, 2003; Van Rooyen, 2009; Van Rooyen and Bower, 2006). Justifiably, fears of extensive external chilling injury (ECI) have prevented the use of these ultra-low temperatures to date. The use of 1-MCP has become an effective alternative to CA, following the first commercial implementation of this treatment in South African avocados in 2003 (Kruger and Lemmer, 2007). Waxing of fruit is a common practice in the avocado industry as a treatment to reduce water loss, delay softening, and improve the appearance of 'Fuerte' avocados (Lunt *et al.*, 1981; Durand *et al.*, 1984). If successful, lowering the shipping temperature could negate the need for expensive 'patch-up' treatments, such as 1-MCP, and may help gain access to new markets with phytosanitary disinfestation requirements (Bower, 2005b).

The objectives of this research were, therefore, to determine the potential for storing 'Fuerte' avocados at 2°C and to determine whether 2°C could become a viable alternative to the use of 1-MCP, by investigating the effect of temperature, 1-MCP and waxing on fruit quality.

2. MATERIALS AND METHODS

2.1 Fruit and Treatments

Export grade 'Fuerte' avocado fruit were obtained from Cooling Estate, located in Wartburg (29°27'S, 30°40' E, KwaZulu-Natal, South Africa). The fruit were collected on 25/06/2010 (early-season), 12/08/2010 (mid-season) and 16/09/2010 (late-season) in order to obtain differing maturity levels, namely moisture contents of 74%, 68% and 63%, respectively. The fruit were harvested from the same block. All waxed fruit were of uniform 'count 16' size (204.4g-365.8g), while the non-waxed fruit were packed before passing through the packline and were, in general, slightly smaller in size (192.3g-351.4g). Post-harvest operations such as grading and sizing, 1-MCP treatment, waxing and forced air cooling were carried out at the packhouse as well as the weighing, firmness measurement and labelling of individual fruit before being placed into storage or treated with 1-MCP so as to minimise any breaks later in the cold chain. The 1-MCP treatment was applied at the registered rate (500 ppb) for 16 hours in cold storage at 5.5°C, whilst the untreated fruit were stored under regular atmosphere at the same temperature for the same period. All fruit were transported in a non-refrigerated vehicle but were enclosed in a canopy and transported within 40 minutes to the laboratories of Horticultural Science at the University of KwaZulu-Natal (UKZN) and immediately prepared for simulated shipping. This did constitute a short cold chain break for all fruit, however, this was completed in a very short period of time and was not deemed to be a significant cold chain break. For each harvest date, ten addition fruit were collected from the same orchard to estimate the moisture content. Moisture content was measured by slicing a longitudinal strip of the entire avocado, of approximately 3mm in width, and weighing this sample before and after freeze drying the material. Fruit were randomly placed into export shipping cartons, each containing 10 fruit. Each carton was randomly assigned to one of the 24 treatment combinations, including treatments of temperature (2°C and 5.5°C), 1-MCP (treated and untreated) and waxing (waxed with Avoshine® and non-waxed) over three harvest dates. Fruit were placed into cold storage containers in regular atmosphere (RA) for 28 days, at air delivery temperatures of 2°C (±1°C) and 5.5°C (±1°C). To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used.

2.2 Data collection

Storage Data

Visual, external observations were made before entering cold storage, during break periods, when removed from cold storage and during ripening. Before entering cold storage, fruit mass, fruit softness, CO_2 evolution, ethylene evolution and overall fruit condition were measured. Fruit were visually rated for shrivel, sunburn, netting, carapace skin, and external damage before storage to be able to accurately distinguish between ECI and pre-storage damages. After 28 days, fruit were removed from cold storage and fruit softening, mass loss, CO_2 evolution and ethylene evolution were calculated. For the ripening period, the fruit were allowed to ripen in a laboratory at room temperature (18-22^{o}C).

2.3 Fruit softness

A hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip was used to measure fruit firmness (ripeness) on a scale of 85-90 (hard, unripe; ≡ 8.06 N) to <60 (soft, ready to eat; ≡ 5.05 N). Four equally spaced readings were taken around the circumference of each fruit and the average reading recorded. Ripening time was calculated as the number of days from harvest until 'eating soft' stage, which corresponded to an average densimeter reading of less than 60 (5.05 N). Fruit softening during storage was calculated as the difference in fruit firmness before and after cold storage, and expressed as a percentage of the initial firmness of each fruit [% fruit softening = ((before – after) / before) x 100].

2.4 Mass loss

During cold storage, the main contributor to mass loss is most likely water loss, because of the reduced respiration rate and metabolic activity (thus the low usage of dry matter energy reserves) at low temperatures (Bower and Jackson, 2003), therefore, mass loss will be assumed to be primarily water loss for the purpose of this study. Fruit mass loss was calculated as the difference in fruit mass before and after cold storage, and expressed as a percentage of the initial mass of each fruit. Individual fruit were weighed at the packhouse prior to any cold chain break treatment and again weighed after removal from cold storage, allowing sufficient time for condensation to evaporate so as not to influence measurements.

2.5 Internal Quality

Upon ripening, all 10 replications in each treatment fruit were cut longitudinally and assessed for anthracnose, stem end rot, vascular browning and mesocarp discolouration. Internal assessments were made on a scale of 0 (no visible symptoms) to 5 (extremely severe, area completely infected or discoloured). Sound fruit were classified as fruit which ripened to the eating ripe stage with a rating of 0 for all internal disorders and body rots, (i.e. free from any disorders and diseases)

2.6 External Quality

The severity of ECI was rated on a scale of 0 (no blemishes) to 10 (fruit surface area entirely blemished). The fruit were visually assessed for ECI once the fruit had reached room temperature, allowing for sufficient time for symptoms of chilling injury to be expressed.

2.7 Statistical Analysis

Statistical analyses were conducted using GenStat® version 12.1 (VSN International, Hemel Hempstead, UK). The data collected was statistically analysed in the form of a factorial design, where each treatment combination consisted of 10 fruit, each constituting a single replication. Analysis of variance and Least Significant Difference (LSD) values were computed to identify significantly different treatment combinations at a confidence level of 95%.

3. RESULTS

3.1 Fruit Softening

Fruit softening was significantly reduced by storing fruit at 2°C (P<0.001) and by the use of 1-MCP (P<0.001 (Table 2 and 3). When stored at 5.5°C, fruit treated with 1-MCPsoftened significantly less than untreated fruit (P=0.003). A storage temperature of 2°C caused the least fruit softening, irrespective of whether 1-MCP was used or not, but this was not significantly different from fruit stored at 5.5°C with 1-MCP (Figure 12). Waxing had the tendency to reduce fruit softening during storage. Fruit softening was significantly affected by season (P<0.001), with both early- and late-season fruit, softening significantly more than mid-season fruit (Table 4).

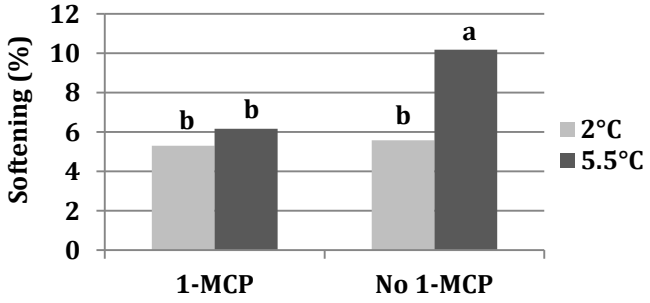

Figure 12: Mean percentage fruit softening of 1-MCP treated and untreated 'Fuerte' avocados following 28 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05).LSD = 1.205

3.2 Mass Loss

Early-season fruit suffered significantly higher mass loss (P<0.001) than mid- and late-season fruit. Fruit stored at 5.5°C showed significantly higher mass loss than those stored at 2°C (P<0.001) (Table 3). In all three harvest dates, fruit stored at 2°C had significantly less mass loss than those at 5.5°C (Figure 13). The highest mass loss occurred in early-season fruit stored at 5.5°C, and the lowest in mid- and late-season fruit stored at 2°C. Waxing significantly reduced mass loss when compared with non-waxed fruit (P<0.001; Table 3), which was particularly evident in the mid- and late-season fruit but there was no significant difference in the early-season fruit (Table 4). The least mass loss occurred from mid-season waxed fruit (Table 4).

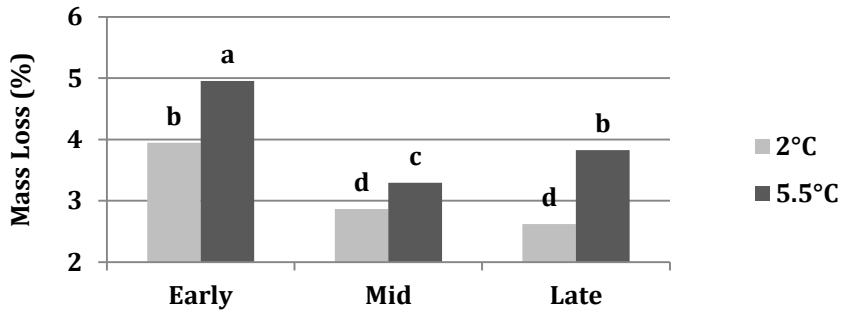

Figure 13: Mean percentage mass loss of 'Fuerte' avocados of different seasons following 28 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.321

3.3 Days-to-ripening (DTR)

A storage temperature of 2°C resulted in significantly more DTR than storage at 5.5°C (P<0.001; Table 3), because of the reduced fruit softening during storage. Further, treatment of fruit with 1-MCP resulted in significantly more DTR throughout the season (P<0.001; Table 4). The combination of lower temperature and 1-MCP resulted in significantly more DTR (P=0.003) than treatment with 1-MCP in fruit stored at 5.5°C (Figure 14). Untreated fruit stored at 5.5°C ripened faster, however, treated fruit stored at 5.5°C and untreated fruit stored at 2°C had a ripening time of approximately seven days, a desirable number of DTR. Waxing of fruit significantly increased the DTR (P=0.002; Table 3), extending DTR by 1 day. The effect of water loss in non-waxed fruit was lower when stored at 2°C, resulting in more DTR, while non-waxed fruit stored at 5.5°C ripened fastest (Table 3). Mid-season fruit took significantly longer to ripen than early- and late-season fruit (P<0.001).

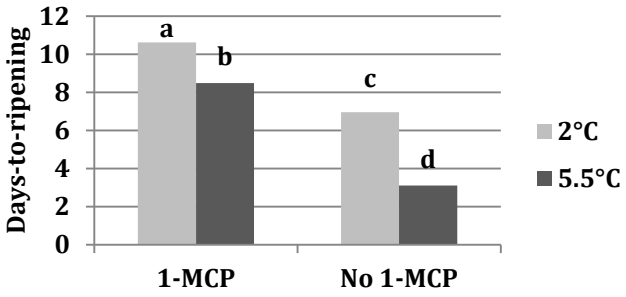

Figure 14: Mean days-to-ripening for 1-MCP treated and untreated 'Fuerte' avocados after 28 days of cold storage at 2°C or 5.5°C.Different letters indicate significant differences between treatments (P≤0.05).LSD = 0.802

3.4 External Quality

Overall, the external damage measured in this experiment was fairly low, as the grand mean rating of ECI in the control fruit was only 0.188 on a rating scale from no blemishes (0) to entirely blemished (10). Early-season fruit suffered significantly more external damage than mid- and late-season fruit (P= 0.018). Early-season, non-waxed fruit stored at 2°C had a significantly higher occurrence of ECI than those stored at 5.5°C (Figure 15), and although the mean severity would not constitute rejection upon arrival, individual fruit did suffer damage which would be of commercial significance and result in a reduced price and increased re-sorting costs for the exporter. Mid-season fruit had the least ECI, but not significantly less than late-season fruit. Waxing and 1-MCP had a tendency to reduce the amount of ECI (Table 3).The best treatment combination, in terms of external appearance, was the combination of '2°C, no 1-MCP, waxed fruit which, throughout the season, showed no ECI (Figure 16) and is extremely promising.

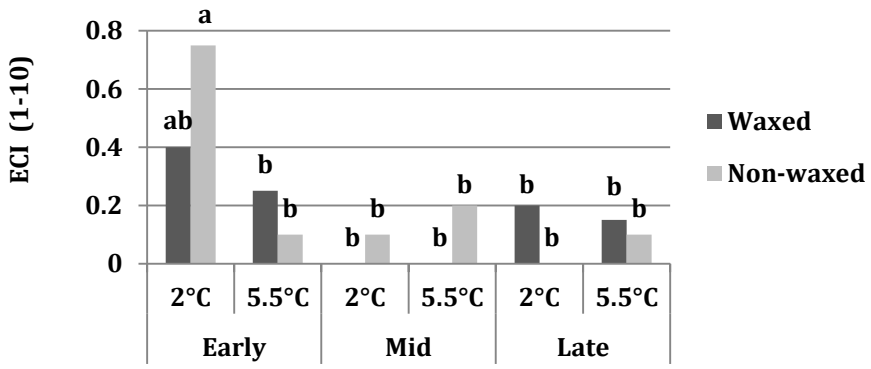

Figure 15: Mean external chilling injury (0-10) throughout the season for waxed and non-waxed 'Fuerte' avocado fruit after 28 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.449

Figure 16: External appearance of the best treatment combination for early-, mid- and late-season, waxed fruit stored at 2°C for 28 days, without the use of 1-MCP.

3.5 Internal Quality

Internal quality parameters were not significantly affected by treatment; this might have been due to the low occurrence of internal disorders throughout the experiment (Table 5). Of the 240 control fruit (no breaks), only 15 fruit showed any signs of internal quality disorders. There were three incidences of mesocarp discolouration, four incidences of vascular browning, four incidences of anthracnose and seven incidences of stem-end rot, with two fruit having more than one disorder. Although not significant, there was a notable trend towards higher incidences of anthracnose and stem-end rot in treatments that increased DTR, such as 1-MCP treated fruit stored at 2^{o}C. There were no significant differences in the percentage of sound fruit between the treatments (Table 5).

4. DISCUSSION

4.1 Fruit Quality

'Fuerte' avocados can be successfully stored at 2°C for 28 days without negatively impacting on the internal and external quality of the fruit, particularly when water loss during storage is reduced. Lowering the storage temperature to 2°C effectively minimized physiological changes during storage. Fruit stored at 2°C had a reduced rate of softening and mass loss compared with 5.5°C (Table 3), which is to be expected because of the reduced metabolic activity at this lower temperature. Blakey *et al*. (2010) reported reduced respiration and ethylene production under ultra-low temperature storage of 'Hass' avocados. Enzymes such as cellulase and polygalacturonase have been found to be responsible for cell wall degradation during the ripening process, which causes fruit softening (Bower and Cutting, 1988). The Q_{10} principle states that for a 10°C rise in temperature, the enzyme activity approximately doubles (Wills *et al.*, 2007). This is the basic principle behind the reduced softening as a result of lower temperatures, and the subsequent reduced activity of the enzymes which cause softening. This illustrates that storage temperatures can be reduced, particularly later in the season, to reduce the number of soft fruit following 28 days of cold storage. Lowering the storage temperature also becomes a useful tool for exporters to be able to adjust shipping temperatures in accordance with feedback from previous shipments, if, for example, fruit are arriving in a soft condition or a longer shelf-life is required.

Treatment of fruit with 1-MCP reduces the rate of softening during storage, particularly at the current average shipping temperature of 5.5°C under RA (Figure 12). This raises the question of whether the shipping temperature should not be lowered, as to reduce the metabolic activity of the fruit, thus less carbohydrate reserve usage and a longer shelf-life. Treatment of fruit with 1-MCP at 5.5°C can be viewed as a 'patch-up' method or 'insurance' against softening during shipping, as currently, this treatment allows higher shipping temperatures to be used, in an attempt to negate the risks of external damage. Results demonstrate that 1-MCP treatment is a valuable tool, particularly for early-season fruit which are more susceptible to external cold damage (Kosiyachinda and Young, 1976). However, in terms of fruit softening and water loss, a storage temperature of 2°C appears to be a potential alternative to this chemical treatment, especially where cold sterilization of fruit is a required treatment for access to new markets. At 2°C, 1-MCP treatment does not result in a reduction

in fruit softening (Figure 12) or water loss (Table 3) during storage, thus it would not yield any additional advantage to RA storage at 2°C. The reduction in metabolic activity probably suppresses production of ethylene and, subsequently, the activity of ripening enzymes during storage (Blakey, 2011).

It is important that mitigating treatments be implemented where possible to minimise the chance of external damage in consignments. 'Fuerte' avocados have a relatively thin skin, in comparison with 'Hass', which makes water loss an important issue during post-harvest storage of greenskins. Waxing plays an important role in reducing water loss, particular early in the season, subsequently reducing the external damage (Figure 15) which can occur as a result of such stress on the fruit (Donkin and Cutting, 1994; King and O'Donoghue, 1995). It should be noted that waxing formulations do vary considerably, and may affect fruit quality differently to the wax (Avoshine® 2010) used in this experiment. New waxes are currently being tested and used by the industry (Van Rooyen, 2011), as the EU 'food additive' law resulted in the banning of morpholine in November 2010, a component of some currently used waxes. The most promising mitigating treatments, shown to successfully reduce the risk of external damage at these ultra-low temperatures, include modified atmosphere packaging (Bower and Jackson, 2003; Bower and Magwaza, 2004; Bower and Blakey, 2008) as well as low temperature pre-conditioning (Hofman et al., 2003; Van Rooyen, 2009; Van Rooyen and Bezuidenhout, 2010; Woolf et al., 2003).

It has been found that pre-conditioning of 'Hass' is required to avoid occurrence of ECI when storing fruit at 0-1°C (Hofman et al., 2003; Van Rooyen, 2009; Van Rooyen and Bezuidenhout, 2010; Woolf et al., 2003). However, Blakey *et al*. (2010) reported that 'Hass' can be stored at 1°C without pre-conditioning. Further, results from this study show that waxed 'Fuerte' can be stored at 2°C without a pre-conditioning treatment, possibly because fruit grown in a cooler climate may be acclimated to the lower storage temperature, as was reported by Blakey *et al.* (2010). It should be noted that fruit in this experiment were pre-conditioned to some degree as fruit were stored at 5.5°C for 16 hours while the 1-MCP treatment was applied. Thus, commercially, pre-conditioning of fruit is suggested to minimise the risk of ECI in 'Fuerte', particularly early in the season.

4.2 Ripening Quality

The time taken to ripen (DTR) as well as the internal quality is linked to the physiological changes of the fruit during storage and thus it is important that the treatments which provide good storage quality, also achieve good ripening quality. There is a clear link between the effects of storage treatments on fruit softening and water loss, and the eventual ripening of the fruit. Higher fruit softening and water loss, results in a higher respiration rate and increased breakdown of carbohydrate reserves for energy during the ripening process, thus negatively affecting shelf-life (Bower and Cutting, 1988). In this experiment, storage of fruit at 5.5^{o}C, without 1-MCP, did not provide an acceptable DTR with some fruit recorded as soft upon removal from cold storage (Table 4). The 2^{o}C storage temperature showed a significant reduction in softening and water loss, and ultimately more DTR in comparison with the 5.5^{o}C storage temperature (Table 3). Thus, if fruit are destined to be sold in a firm state, storage temperatures need to be lowered, or at 5.5^{o}C, 1-MCP needs to be used in order in extend the DTR.

Further, a suitable DTR, of approximately seven to ten days, will be required for fruit undergoing cold sterilisation. Currently, fruit are required to remain in a firm state for up to 28 days (e.g. 14-18 days sea voyage and up to 10 days for distribution and sale), and thus fruit softening soon after removal from storage may still provide a suitable DTR, as distribution time is included in the cold chain. However, for cold sterilisation, no breaks in the cold chain are permitted and thus additional time is required for distribution and sale upon arrival at the overseas market, depending on the duration necessary for cold sterilization.

Lowering the storage temperature, treatment of 1-MCP and waxing increases the DTR (Figure 14; Table 3). There is a notable, additive effect of an increase in DTR when combining treatments. However, such an extension in DTR is not desirable as it increases the chance of fungal body rots (Hopkirk *et al.*, 1994), explained by the strong correlation found between ripening time and anthracnose development (Darvis, 1985; Eksteen and Truter, 1985). Thus, a suitable number of DTR can be achieved by identifying the optimum treatment combination. Waxing of 'Fuerte' avocados allows for approximately one day longer DTR, a result in agreement with Durand *et al*. (1984). Therefore, such treatment is highly recommended as it also maintains external quality more effectively (Figure 15). Ultimately, fruit stored at 5.5^{o}C require 1-MCP treatment, while fruit stored at 2^{o}C can achieve similar days to ripening without the use of 1-MCP, if the fruit are waxed. Thus, the

lower storage temperature is not only a suitable alternative to 1-MCP with regard to fruit quality, but also ripening quality.

The internal quality of 'Fuerte' avocados using 2^{o}C storage is particularly good, which is in agreement with the numerous ultra-low temperature storage studies in recent times (Bower and Magwaza, 2004; Van Rooyen, 2009; Blakey *et al*., 2010). The reduction in cellular metabolism at ultra-low temperatures reduces the incidence of internal disorders, such as mesocarp discolouration and vascular browning (Dixon *et al.*, 2004; Van Rooyen and Bower, 2006). The lowering of storage temperature, to below the 'killing zone' (Crisosto *et al*, 1999; Blakey, 2011), appears to minimise imbalances in potentially toxic levels of metabolic intermediates during cold storage, which can occur as a result of enzymatic activity imbalances (Kosiyachinda and Young, 1976). With low temperature storage (2^{o}C) the metabolic activity of fruit is reduced compared with 5.5^{o}C storage, and, ensuring that the majority of enzymatic activities are minimised, the internal quality of the fruit can be maintained over a longer period.

5. CONCLUSION

It can be concluded that waxed South African 'Fuerte' avocados can be stored at 2^{o}C under RA for 28 days without the use of 1-MCP. This treatment appears to be the best treatment combination as it reduces fruit softening and mass loss during storage significantly compared with fruit stored at 5.5^{o}C in RA. This treatment combination also provides a similar DTR to 1-MCP treated fruit stored at 5.5^{o}C, and provides good internal quality and acceptable external quality when fruit were waxed, particularly mid-season fruit.

Only few internal quality defects were recorded in this experiment; it is, therefore, suggested that fruit quality, for example, as a result of good pre-harvest orchard practices and nutrient management, is critical to the success of ultra-low temperature storage. Although good quality fruit can still suffer post-harvest damage, it is clear that post-harvest stress, particularly water loss stress, needs to be reduced where possible. If fruit is to be shipped to new markets with cold sterilisation as a phytosanitary requirement, mid-season fruit should be sent as these fruit have the least risk of external damage. Cold storage pre-conditioning of fruit may further reduce the risk of ECI, particularly early in the season, and is suggested for future research.

It seems likely that reducing the storage temperature is cheaper than the considerable costs involved in 1-MCP treatment, and since storage at 2^{o}C results in good quality fruit, this seems to be a viable alternative to the use of 1-MCP. Further research, with an economic focus, is recommended, including cost comparisons between 1-MCP treatment and the increased costs of maintaining lower temperatures during storage.

Table 2: Statistically significant interactions in the ANOVA for the various quality parameters measured after 28 days of cold storage. Percentage fruit softening, percentage mass loss, days-to-ripening (DTR) and external chilling injury (ECI) were significantly affected by treatments or interactions, while no significant treatment effects were found for the internal quality parameters after 28 days of cold storage.

Treatment/Interaction	% Fruit Softening	% Mass Loss	DTR	ECI
Harvest date	**	**	**	*
Temp	**	**	**	
1-MCP	**		**	
Wax		**	**	
Harvest date.Temp		**		
Harvest date.1-MCP			**	
Temp.1-MCP	**		**	
Harvest date.Wax		**		
Temp.Wax				
1-MCP.Wax				
Harvest date.Temp.1-MCP		*		
Harvest date.Temp.Wax			**	
Harvest date.1-MCP.Wax				
Temp.1-MCP.Wax				
Harvest date.Temp.1-MCP.Wax				
* = Significant (confidence level of 95%)				
** = Highly Significant (confidence level of 99%)				

Table 3: Percentage fruit softening, percentage fresh mass loss and external chilling injury (ECI) of 'Fuerte' avocado fruit at the removal from cold storage as well as days-to-ripening (DTR) at 20±2°C after removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates combined. *

Treatments			Fruit Softening (%)	Mass Loss (%)	ECI (0-10)	DTR (days)
2°C	1-MCP	waxed	4.84 c	2.94 de	0.17 a	11.57 a
		Non-waxed	5.77 bc	3.52 bc	0.23 a	9.67 b
	No 1-MCP	waxed	5.50 bc	2.85 e	0.20 a	7.03 d
		Non-waxed	5.63 bc	3.26 cd	0.37 a	6.87 d
5.5°C	1-MCP	waxed	5.47 bc	3.70 b	0.27 a	8.77 bc
		Non-waxed	6.87 b	4.36 a	0.00 a	8.20 c
	No 1-MCP	waxed	10.36 a	3.63 bc	0.03 a	3.60 e
		Non-waxed	9.97 a	4.41 a	0.23 a	2.60 e
LSD			1.71	0.37	0.37	1.14
* Each point represents the mean of 30 fruit.						
Different letters indicate significant differences within each column.						

Table 4: Seasonal changes in percentage fruit softening, percentage fresh mass loss and external chilling injury (ECI) of 'Fuerte' avocado fruit upon removal from cold storage, as well as days-to-ripening (DTR) at 20±2°C after removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

Treatment				Fruit Softening (%)	Mass Loss (%)	ECI (0-10)	DTR (days)
Early	2°C	1-MCP	waxed	7.13 cdefg	4.47 bcd	0.3 ab	11.4 b
			Non-waxed	7.05 cdefg	3.94 def	0.9 a	9.0 def
		No 1-MCP	waxed	6.70 cdefgh	3.80 ef	0.1 b	6.6 ghi
			Non-waxed	6.41 cdefgh	3.57 efg	0.0 b	4.7 jk
	5.5°C	1-MCP	waxed	7.14 cdefg	4.84 ab	0.0 b	7.9 defg
			Non-waxed	8.78 abcd	4.80 ab	0.4 ab	8.9 def
		No 1-MCP	waxed	11.12 a	4.96 ab	0.0 b	3.1 kl
			Non-waxed	11.30 a	5.20 a	0.0 b	2.7 l
Mid	2°C	1-MCP	waxed	2.54 i	2.07 m	0.5 ab	14.0 a
			Non-waxed	4.89 fghi	3.61 efg	0.5 ab	11.6 b
		No 1-MCP	waxed	5.26 efghi	2.33 lm	0.1 b	7.4 efgh
			Non-waxed	2.65 i	3.45 fgh	0.0 b	9.4 cd
	5.5°C	1-MCP	waxed	3.07 i	2.83 hijkl	0.0 b	11.3 bc
			Non-waxed	3.99 hi	3.63 efg	0.2 b	9.8 bcd
		No 1-MCP	waxed	9.36 abc	2.80 ijkl	0.0 b	5.1 ij
			Non-waxed	7.94 bcde	3.92 def	0.0 b	2.5 l
Late	2°C	1-MCP	waxed	4.85 ghi	2.29 lm	0.6 ab	9.3 de
			Non-waxed	5.37 efghi	3.01 ghijk	0.0 b	8.4 defg
		No 1-MCP	waxed	4.54 ghi	2.41 klm	0.0 b	7.1 fgh
			Non-waxed	7.84 bcdef	2.76 jkl	0.2 b	6.5 ghi
	5.5°C	1-MCP	waxed	6.21 defgh	3.43 fghi	0.0 b	7.1 fgh
			Non-waxed	7.82 bcdef	4.65 abc	0.2 b	5.9 hij
		No 1-MCP	waxed	10.61 ab	3.12 ghij	0.3 ab	2.6 l
			Non-waxed	10.68 ab	4.10 cde	0.2 b	2.6 l
LSD				2.953	0.6419	0.6349	1.965

* Each point represents the mean of 10 fruit.

Different letters indicate significant differences within each column.

Table 5: Internal quality of 'Fuerte' avocado fruit upon reaching eating ripeness at 20±2°C. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates are combined. *

Treatments			Anthracnose (0-5)	Stem-end rot (0-5)	Vascular browning (0-5)	Mesocarp discolouration (0-5)	Sound fruit (%)
2°C	1-MCP	waxed	0.03 a	0.00 a	0.00 a	0.00 a	96.7 a
		Non-waxed	0.03 a	0.07 a	0.13 a	0.07 a	86.7 a
	No 1-MCP	waxed	0.00 a	0.03 a	0.03 a	0.07 a	90.0 a
		Non-waxed	0.00 a	0.07 a	0.00 a	0.00 a	93.3 a
5.5°C	1-MCP	waxed	0.03 a	0.00 a	0.00 a	0.00 a	96.7 a
		Non-waxed	0.13 a	0.10 a	0.03 a	0.10 a	90.0 a
	No 1-MCP	waxed	0.00 a	0.00 a	0.03 a	0.00 a	96.7 a
		Non-waxed	0.00 a	0.00 a	0.00 a	0.00 a	100 a
LSD			0.1438	0.1018	0.1434	0.1346	13.52

* Each point represents the mean of 30 fruit.

Sound fruit are ripe and free from disorders and diseases.

Different letters indicate significant differences within each column.

CHAPTER 3

The effect of cold chain breaks on 'Fuerte' avocado fruit quality and the influence of 1-MCP and ultra-low temperature storage for 28 days

Andre Lütge, Isa Bertling and John Bower

The South African avocado industry is export-driven and the cold chain that needs to be maintained by this industry is one of the longest in the world, due to the multitude of role-players and handling points. It is known that there are various 'weak links' in this cold chain and that cold chain breaks are detrimental to fruit quality, but little research on the effects of these cold chain breaks exists, with no study on a break during ultra-low temperature storage. Thus the objectives of this study were to determine the effects of cold chain breaks on fruit quality after storage for 28 days and to determine how ultra-low storage temperatures and treatment with 1-MCP influence fruit quality following breaks in the cold chain. 'Fuerte' avocados were harvested from Wartburg during the early-, mid- and late-season and stored at 2°C or 5.5°C. Fruit were waxed, treated with 1-MCP and subjected to a 24 hour delay in cooling and a break in the cold chain after 14 days. Fruit softening, mass loss, days-to-ripening, and external and internal quality were determined. The delay in cooling and the cold chain break were found to increase mass loss and fruit softening, reduce days to ripening and increase external chilling injury, especially early in the season. Water loss seemed to be the main contributor to the decreased fruit quality which resulted from the delay in cooling, increasing the external damage significantly, particularly early in the season. The break at 14 days had a marked effect on the physiological activity of the fruit during storage, detectable as an increase in fruit softening and water loss during storage and a decrease in shelf-life, particularly for fruit stored at 5.5°C. Use of 1-MCP and storage at 2°C reduced the effects of cold chain breaks with respect to fruit softening; however, lowering the storage temperature had a greater effect on minimising fruit softening than 1-MCP and could be a successful alternative to 1-MCP. The internal quality throughout the experiment was very good, with few internal disorders and, hence, no significant treatment effects, although a trend towards increased body rots with extended ripening times was noted. Overall, a break in the cold chain, before and during cold storage, resulted in a marked reduction in fruit quality.

1. INTRODUCTION

The South African export cold chain for avocados comprises many role players as the fruit moves from the orchard to the overseas retailer, requiring up to 28 days cold storage. To maintain fruit of high quality over this lengthy period requires that the cold chain be maintained. Successful cold storage of avocados requires efficient management, field heat removal from the fruit as early as possible after harvest and the maintenance of optimal storage temperature throughout the cold chain (Ginsberg, 1985). It has been shown that cold chain breaks have numerous negative effects on both distribution costs and avocado fruit quality (Dodd *et al*., 2007; Eksteen, 1995; Eksteen, 1999). The process of loading the fruit from the packhouse into road transport vehicles was found to be the main weak link in the cold chain. Recent focus on the negative effects of cold chain breaks, including the influence of 1-MCP and CA (Lemmer and Kruger, 2010) as well as the interaction of cold chain breaks with ultra-low temperatures (Blakey and Bower, 2009; Lütge *et al.*, 2010) has provided valuable and much needed information, mainly for 'Hass' avocados. Cold chain breaks have been shown to cause fruit softening and mass loss during storage as well as a reduced shelf-life (Blakey and Bower, 2009; Kok, *et al.*, 2010; Lütge *et al.*, 2010; Undurraga *et al*., 2007), with particularly damaging effects on pathology (Lemmer and Kruger, 2010). Some of these effects can be mitigated by treatments such as 1-MCP or CA, however, these treatments are expensive and sometimes inefficient (Roets *et al*., 2009); therefore possible alternatives, such as ultra-low temperature storage need to be investigated.

Cold storage of 'Fuerte' avocados at 2^{o}C has resulted in better internal quality (Bower and Magwaza, 2004; Lütge *et al*., 2010) than that of fruit stored at the conventional 5.5^{o}C, as reported for other commercial cultivars (Van Rooyen and Bower, 2006; Van Rooyen, 2009). Reducing the storage temperature may also act as a buffer, and mitigate some of the negative effects of cold chain breaks on fruit quality, by maintaining the pulp temperature of the fruit below critical levels for longer periods during these breaks. Of importance, particularly for 'Fuerte avocados, is the interaction between post-harvest water loss and external damage (Donkin and Cutting, 1994), and further investigations were necessary to determine the effects of cold chain breaks on fruit quality at a storage temperature of 2^{o}C. The objectives of this study were to determine the effects of a cold chain break during storage and a delay in fruit cooling, on fruit quality, and to determine how ultra-low storage temperatures, and treatment with 1-MCP, influence fruit quality following a break and delay in the cold chain.

2. MATERIALS AND METHODS

2.1 Fruit material

Export grade 'Fuerte' avocado fruit were obtained from Cooling Estate, located in Wartburg (29°27'S, 30°40' E, KwaZulu-Natal, South Africa). The fruit were collected on 25/06/2010 (early-season), 12/08/2010 (mid-season) and 16/09/2010 (late-season) in order to obtain differing maturity levels, namely moisture contents of 74%, 68% and 63%, respectively. The fruit were harvested from the same block. All waxed fruit were of uniform 'count 16' size (204.4g-365.8g), while the non-waxed fruit were packed before passing through the packline and were, in general, slightly smaller in size (192.3g-351.4g). Post-harvest operations such as grading and sizing, 1-MCP treatment, waxing and forced-air cooling were carried out at the packhouse as well as the weighing, firmness measurement and labelling of individual fruit before being placed into storage or treated with 1-MCP so as to minimise any breaks later in the cold chain. The 1-MCP treatment was applied at the registered rate (500 ppb) for 16 hours in cold storage at 5.5°C, whilst the untreated fruit were stored under regular atmosphere at the same temperature for the same period. All fruit were transported in a non-refrigerated vehicle but were enclosed in a canopy and transported within 40 minutes to the laboratories of Horticultural Science at the University of KwaZulu-Natal (UKZN) and immediately prepared for simulated shipping. This did constitute a short cold chain break for all fruit, however, this was completed in a very short period of time and was not deemed to be a significant cold chain break. For each harvest date, ten addition fruit were collected from the same orchard to estimate the moisture content. Moisture content was measured by slicing a longitudinal strip of the entire avocado, of approximately 3mm in width, and weighing this sample before and after freeze drying the material. Fruit were placed into cold storage containers in regular atmosphere (RA) for 28 days, at air delivery temperatures of 2°C (±1°C) or 5.5°C (±1°C). To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used.

2.2 Treatments

Fruit were placed into export shipping cartons, each containing ten fruit. Each carton was randomly assigned to one of 24 treatment combinations, including treatments of temperature (2°C or 5.5°C), 1-MCP (treated or untreated) and waxing (waxed with Avoshine® or non-waxed) over three harvest dates. Cold chain breaks included a 24 hour delay in cooling and a

break after 14 days of cold storage, where fruit were placed in the laboratory for eight hours at 20±2^{o}C and then returned to cold storage. Temperatures during the delay in cooling were not controlled, however these fruit were stored in the packhouse overnight and packhouse temperatures during these delays were not notably different on the three harvest dates.

2.3 Data collection

Storage Data

Visual, external observations were made before entering cold storage, during break periods, when removed from cold storage and during ripening. Before entering cold storage, fruit mass, fruit softness, CO_2 evolution, ethylene evolution and overall fruit condition were measured. Fruit were visually rated for shrivel, sunburn and external damage before storage to be able to accurately distinguish between external chilling injury (ECI)and pre-storage damages. After 28 days, fruit were removed from cold storage and fruit softening, mass loss, CO_2 evolution and ethylene evolution were calculated. For the ripening period, the fruit were allowed to ripen in a laboratory at room temperature (18-22^{o}C).

2.4 Fruit softness

A hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip was used to measure fruit firmness (ripeness) on a scale of 85-90 (hard, unripe; ≡ 8.06 N) to <60 (soft, ready to eat; ≡ 5.05 N). Four equally spaced readings were taken around the circumference of each fruit and the average reading recorded. Ripening time was calculated as the number of days from harvest to the 'eating soft' stage, which corresponded to an average densimeter reading of less than 60 (5.05 N). Fruit softening during storage was calculated as the difference in fruit firmness before and after cold storage, and expressed as a percentage of the initial firmness of each fruit (% fruit softening = {[softness before storage – softness after storage] / softness before storage} x 100).

2.5 Mass loss

During cold storage, water loss is the main contributor to mass loss, because of the reduced respiration rate and metabolic activity (thus the low usage of dry matter energy reserves) at low temperatures (Bower and Jackson, 2003). Therefore, it was assumed that mass loss is equivalent to water loss for the purpose of this study. Fruit mass loss was calculated as the

difference in fruit mass before and after cold storage, and expressed as a percentage of the initial mass of each fruit. Individual fruit were initially weighed at the packhouse prior to any cold chain break treatment and again after removal from cold storage, allowing sufficient time for condensation to evaporate so as not to influence measurements.

2.6 Internal Quality

Upon ripening, all ten replications of each treatment were cut longitudinally and assessed for anthracnose, stem end rot, vascular browning and mesocarp discolouration according to Lutge *et al*., 2010. Internal assessments were made on a scale of 0 (no visible symptoms) to 5 (extremely severe, area completely infected or discoloured). Sound fruit were classified as fruit which ripened to the eating ripe stage with a rating of 0 for all internal disorders and body rots (i.e. free from any disorders and diseases).

2.7 External Quality

The severity of ECI was rated on a scale of 0 (no blemishes) to 10 (fruit surface area entirely blemished). The fruit were visually assessed for ECI once the fruit had reached room temperature, allowing for sufficient time for symptoms of chilling injury to be expressed.

2.10 Statistical Analysis

Statistical analyses were conducted using GenStat® version 12.1 (VSN International, Hemel Hempstead, UK). The data collected was statistically analysed in the form of a factorial design, where each treatment combination consisted of ten fruit, each constituting a single replication. Analysis of variance and Least Significant Difference (LSD) values were computed to identify significantly different treatment combinations at a confidence level of 95%.

3. RESULTS

3.1 Fruit Softening

Overall, cold chain breaks had a statistically significant effect on fruit softening($P<0.001$), as both breaks resulted in significantly more softening than the control. Harvest date significantly altered the effect of cold chain breaks on fruit softening ($P<0.001$). The 24 hour delay softened significantly more than the control in the mid- and late-season fruit, but not significantly more in the early-season. The break during storage resulted in significantly more softening than the control in the early- and late-season fruit, but not significantly more in the mid-season fruit (Figure 17).

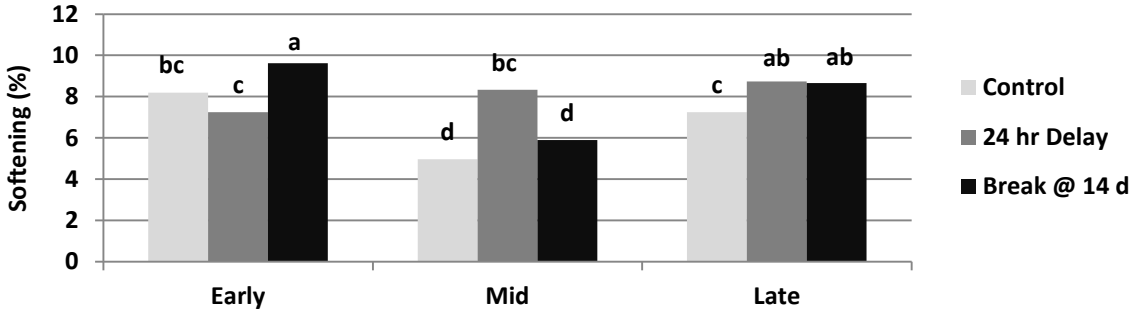

Figure 17: Effect of a delay in cooling and a cold chain break on percentage fruit softening of 'Fuerte' avocados of different harvest dates following 28 days of cold storage at both 2°C and 5.5°C.Different letters indicate significant differences between treatments ($P\leq0.05$). LSD = 1.183

At 5.5°C, both the cold chain break and the delay in cooling resulted in significantly more softening than the control; however, at 2°C, the cold chain breaks had no significant increase in softening, irrespective of 1-MCP treatment (Figure 18). Late-season fruit, stored at the higher storage temperature, tended to softened more as a result of cold chain breaks than early- and mid-season fruit (Table 7). Therefore, storage temperature significantly affected the response to cold chain breaks ($P=0.004$).

While at the warmer 5.5°C storage temperature 1-MCP significantly reduced the degree of softening, at 2°C no decrease in softening was recorded following 1-MCP treatment. When fruit were not treated with 1-MCP, both the cold chain break and the delay in cooling resulted in increased softening (Figure 18), particularly non-waxed fruit which softened significantly more than waxed fruit (Table 7). The use of 1-MCP at 5.5°C had a less negating effect on the

delay in cooling than the break in the cold chain (Figure 18). It should be noted that delay in cooling occurred before the treatment of 1-MCP and lower storage temperatures and thus any negative effects of the delay would not be countered by these treatments during the period of the delay (24 hours), only during storage. Fruit which softened most as a result of cold chain breaks were late-season, non-waxed fruit, stored at 5.5°C without 1-MCP treatment (Table 7).

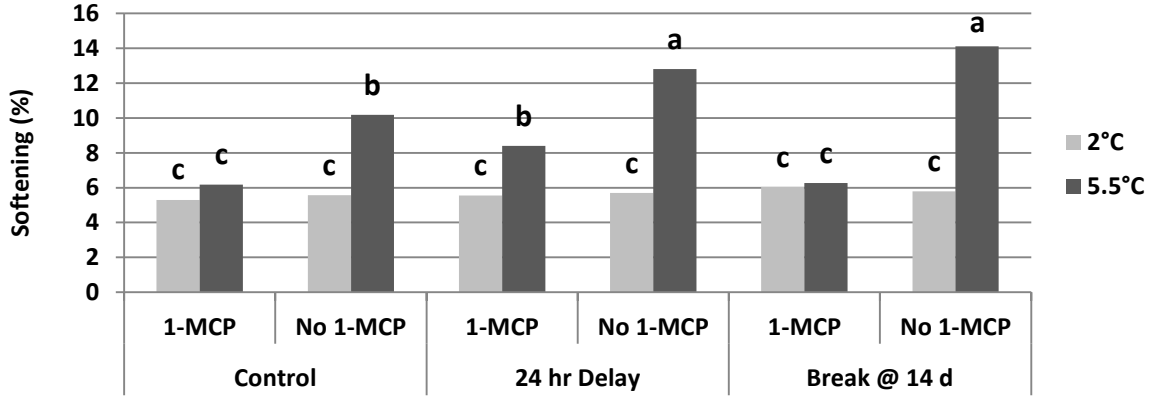

Figure 18: Effect of a delay in cooling and a cold chain break on the percentage fruit softening of 1-MCP treated and untreated 'Fuerte' avocados following 28 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments ($P \leq 0.05$). LSD = 1.366

3.2 Mass Loss

The 24 hour delay resulted in higher water loss than the break at 14 days, particularly late in the season where this difference was significant (Figure 19). Waxing resulted in a reduction in mass loss as a result of cold chain breaks, with this reduction being significant for the delay in early- and mid-season fruit as well as the break during storage in late-season fruit (Table 7). Cold chain breaks had a statistically significant effect on mass loss ($P<0.001$). Both the break in the cold chain and the delay in cooling resulted in a significant increase in water loss. Cold chain breaks resulted in significantly more water loss than the control in all three seasons, except the break in the late-season fruit where the mass loss was not significantly higher than the control (Figure 19).

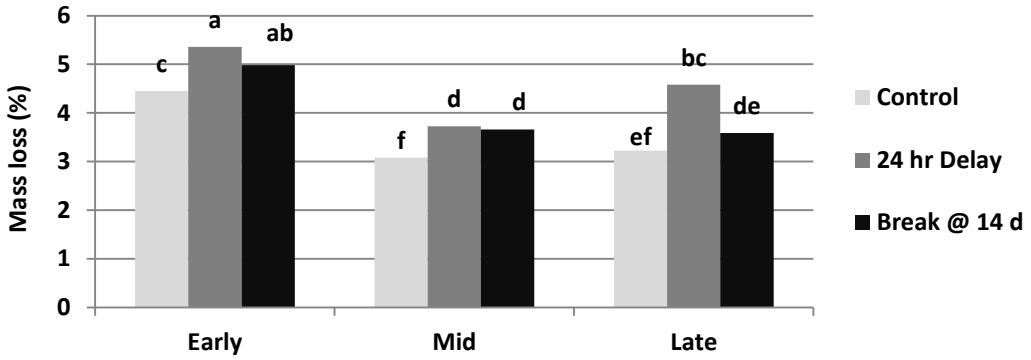

Figure 19: Effect of a delay in cooling and a cold chain break on percentage mass loss of 'Fuerte' avocados of different harvest dates following 28 days of cold storage at both 2°C and 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.400

3.3 Days-to-ripening

Cold chain breaks significantly affected DTR (P<0.001), with a significant effect of harvest date (P<0.001). The 24 hour delay resulted in a reduction in DTR, an average reduction of approximately 6 days averaged over the three harvest dates. Mid- and late-season fruit had the shortest DTR (approximately 5 days) (Table 8). The break during storage also resulted in a significant reduction in DTR, although not as much as the 24 hour delay in cooling. The mid-season DTR results for the control, as well as the break during storage were higher than expected, primarily because a number of these fruit did not ripen at all, which increased the mean DTR. These fruit were mainly 1-MCP treated fruit stored at 5.5°C, underlining concerns with this treatment. While the lower storage temperature and 1-MCP reduced the negative effects of cold chain breaks on fruit softening, the effects of temperature and 1-MCP on DTR were erratic and no substantial trends were noted (Table 8).

3.4 Internal Quality

Neither the cold chain break nor the delay in cooling resulted in a significant increase in any of the internal disorders analysed and no treatments had a significant effect on internal fruit quality, due to the low occurrences of internal disorders through the experiment (Tables 9 and 10). Surprisingly, the control had a significantly lower percentage of sound fruit than the break and the delay in cooling (P=0.038; Table 10).

3.5 External Quality

As the overall level of ECI in this experiment was fairly low, few statistically significant differences with respect to cold chain breaks were found; however, cold chain breaks had a significant effect on ECI (P=0.041). Both the delay in cooling and the break during storage resulted in a higher ECI level than the control, with the break after 14 days causing significantly more ECI than the control (Figure 20).

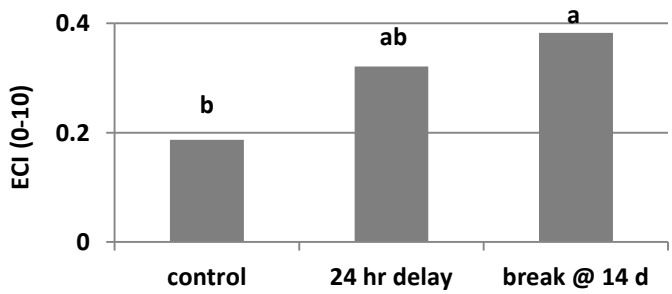

Figure 20: Effect of a delay in cooling and a cold chain break on external chilling injury (ECI) of 'Fuerte' avocados following 28 days of cold storage at both 2^{o}C and 5.5^{o}C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.155

The highest ECI was recorded for early-season, non-waxed fruit stored at 2^{o}C and treated with 1-MCP, which scored a mean rating of 2.3 out of 10 for the box of 10 fruit (Table 8). This treatment combination also resulted in the highest rating for fruit subjected to the 24 hour delay in cooling (Table 8). Although the average ECI for the cold chain breaks (Figure 20) would be likely not to result in rejection of fruit upon arrival, the ECI present in the more severely affected treatment combinations, such as the combination mentioned above, would require resorting and result in a lower price receive.

Although the interaction between harvest date, break and waxing (Figure 21) is not statistically significant (P=0.074), the interaction identified that the break during storage (break at 14 days) caused early-season, non-waxed fruit to suffer significantly higher levels of ECI than the control. The 24 hour delay in cooling tended to cause early-season, non-waxed fruit to experience twice as much ECI than the control, although not significantly more.

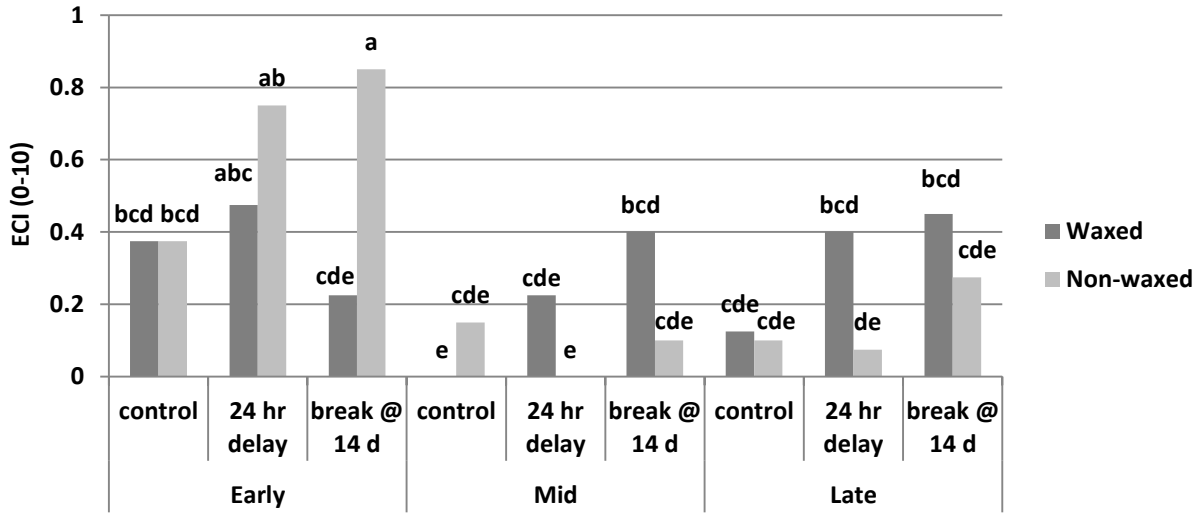

Figure 21: Effect of cold chain breaks and waxing on external chilling injury (ECI) of 'Fuerte' avocados of different harvest dates following 28 days of cold storage at both 2^{o}C and 5.5^{o}C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.379

4. DISCUSSION

Results showed that cold chain breaks are detrimental to avocado fruit quality. Overall, cold chain breaks caused significantly increased fruit softening, mass loss and ECI during storage and decreased DTR after removal from storage, when compared to uninterrupted cold storage (Table 7 and 8). These results agree with previous findings on 'Hass' (Blakey and Bower, 2009) and 'Fuerte' (Lütge *et al*., 2010). The high percentage of fruit softening for the early-season control (Figure 17) was not expected, as these fruit were expected to soften the least during storage and soften more as fruit maturity increased through the season (Wills *et al*., 2007). The negative effects of cold chain breaks on fruit physiology were more severe in more mature fruit, as reported by Lemmer and Kruger (2010), with the exception of ECI, where early-season fruit were more susceptible than fruit harvested later in the season. For both cold chain breaks, waxing reduced the amount of water lost by the fruit and subsequently aided in reducing the amount of ECI, as was found by Donkin and Cutting (1994) and shown in chapter 2. The DTR for waxed fruit, stored at 2^{o}C and not treated with 1-MCP (proposed best combination in chapter 2) resulted in an average DTR through the season of approximately 5.8 days and 6.5 days for the 24 hour delay and break during storage, respectively. This is significantly lower than the control (7 days), however, still acceptable.

4.1. Fruit quality following a break in the cold during storage

The cold chain break during storage (break at 14 days) resulted in quicker fruit softening, higher mass loss during storage and less DTR than the control (Table 7 and 8), clearly indicating that the break affected fruit physiology. Bower and Cutting (1988) suggested that water loss may initiate softening, and result in a spike in respiration, thus a reduction in carbohydrate reserves, which in turn will reduce the shelf-life of the fruit. This spike in respiration, owing to the increased temperature during the break, was illustrated by Blakey and Bower (2009) as well as Undurraga *et al*. (2007) for 'Hass' avocados, and may trigger premature initiation of ripening by the synthesis of ripening enzymes responsible for softening in avocados (Awad and Young, 1979). Thus, when compared with uninterrupted cold storage, the higher ECI resulting from a break in the cold chain may be ascribed not only to the increased water loss (Donkin and Cutting, 1994) but also to fruit softening during storage.

The use of 1-MCP at 5.5°C reduces the effects of cold chain breaks on fruit softening during storage, and results are in agreement with Lemmer and Kruger (2010). However, by lowering the storage temperature, softening due to a break in the cold chain is completely negated without using 1-MCP. This confirms that the lower temperatures act as a buffer against the negative effect of cold chain breaks on fruit softening during storage. When stored at lower temperatures, fruit take longer to warm up during a cold chain break and pulp temperatures remain lower for a longer period of time (Dodd *et al*., 2007). This lower pulp temperature results in a reduction in respiration rate and subsequent water loss during the break and, thus, reduces the physiological activity of the fruit during this period, reducing the chance of in-storage ripening and external damage occurrence upon re-cooling in cold storage following the break in the cold chain.

A trend was noticeable towards increased pathological disorders as the DTR was excessively lengthened, through the combined effects of 1-MCP and 2°C storage. This illustrates the importance of identifying a desirable number of DTR and applying the suitable treatments. For example, storing fruit at 2°C as well as applying 1-MCP would result in a lengthy DTR (10-12 days), allowing time for latent infections to manifest, and thus would not be a desirable number of DTR as avocado fruit which take longer to ripen generally are more diseased when ripe (Hopkirk *et al.*, 1994). The effect of cold chain breaks on pathological infections, and hence fruit quality, is important and often overlooked. Although not found to be significant in this study, cold chain breaks and the associated condensation, can increase the occurrence of anthracnose and stem-end rot (Lemmer and Kruger, 2010), particularly in areas where high innoculum levels are present on the fruit, as the free water on the fruit surface provides a favourable environment for fungal growth.

4.2. Fruit quality following a delay in cooling

Once fruit is removed from the tree, mesocarp and possibly seed carbohydrate reserves are their only source of energy required for ripening and respiration. The climacteric ripening pattern exhibited by avocados requires large amounts of energy and if these energy reserves are depleted, to levels not sufficient for successful ripening, physiological disorders may result (Blakey and Bower, 2009). Thus, immediate cooling of fruit is necessary in order to minimise metabolic activity and maintain carbohydrate reserves, and to delay the onset of ripening, thereby achieving high quality fruit after cold storage. Overall, the 24 hour delay in

cooling resulted in increased fruit softening, mass loss and ECI during storage as well as a lower number of DTR when compared with the control (Table 7 and 8).

Similarly, a delay in cooling impacts on the ripening process and subsequent physiological activities of the fruit during cold storage. The use of 1-MCP and lower storage temperatures reduced the softening effect of the '24 hour delay' (Figure 18), which is in agreement with the findings of Blakey and Bower (2009).Although both lower temperature and 1-MCP treatments reduced the softening effect of the delay, they had a slightly smaller negating effect on softening during storage for the delay than for the break at 14 days (Figure 18). Clearly, the delay in cooling allowed for ripening to proceed; however, these treatments reduced further metabolic activity of the fruit and thus further softening during storage, particularly at 2^{o}C. Even though these treatments slow the ripening process, ripening does proceed during cold storage. When a break occurs later on during storage, the ripening process has progressed slightly and the softening effects of the breaks are more noticeable.

Although the delay in cooling impacts negatively on fruit softening during storage and subsequently the DTR, the results suggest that water loss is the main negative effect of a delay in cooling. Water loss is one of the main factors influencing deterioration of export avocado fruit (Milne, 1998), and although water loss is required for ripening (Wills *et al*., 2007), this water loss should be minimised before and during cold storage so that the ripening process can be delayed. A delay in the cooling of fruit results in a higher amount of water loss from the fruit because the field heat is not removed from the fruit immediately after harvest, resulting in steep vapour pressure gradient between the fruit and the surrounding air; thus water vapour moves down this gradient and is lost from the fruit rapidly (Wills *et al*., 2007).

Donkin and Cutting (1994) proved that the severity of external cold damage is related to the amount of water loss from the fruit, while other recent studies have confirmed the importance of minimising water loss from the fruit during cold storage (Bower and Jackson, 2003; Bower and Magwaza, 2004; Bower, 2005b; Blakey and Bower, 2009). The results of the delay in cooling confirm the important correlation between water loss and fruit quality, particularly occurrence of ECI. Waxing can reduce this water loss (Table 7) as well as ECI (Figure 21), particularly early in the season. These results suggest that a delay in cooling or a break in the cold chain are especially damaging to the external appearance of early-season fruit, where moisture loss from less mature fruit is higher, while breaks in the cold chain have less of an

effect on external quality later in the season, when fruit are more mature and likely to lose less moisture post-harvest. Unfortunately, growers will, on occasion, leave fruit in the packhouse for a certain period before cooling the fruit, to allow for some water loss in an attempt to reduce the cell turgidity and thus reduce the risk of lenticel damage. However, Donkin and Cutting (1994) suggested that fruit turgidity be maintained from the time of harvest, as this results in waxing being more effective in minimising water loss.

5. CONCLUSION

Proper maintenance of the cold chain is essential for preserving fruit quality, and is critical for the export of high quality fruit to distant markets. Cooling avocados immediately after harvest and maintaining the cold chain, are of great importance to maintain fruit quality; a scenario that can be better achieved when storage temperatures lower (2^oC) than currently adopted storage temperatures (averaging 5.5^oC through the season) are implemented. The most critical negative effect of a delay in cooling is the water loss during this delay, as this loss tends to increase ECI of 'Fuerte' avocados, particularly early in the season. The break at 14 days had a marked effect on fruit softening and water loss during storage, decreasing the DTR, particularly for fruit stored at 5.5^oC.

It can be concluded that the use of 1-MCP at 5.5^oC reduces the effects of cold chain breaks on fruit softening during storage; however, by lowering the storage temperature from 5^oC to 2^oC, the softening effects of a break in the cold chain are completely negated without using 1-MCP. Based on the positive results achieved at temperatures lower than currently used in the industry, particularly when delaying the onset of the cold chain or when breaking the cold chain, it is recommended that exporters consider lowering the storage temperature to 2^oC as a potential alternative to 1-MCP use at the currently standard 5.5^oC storage temperatures.

Table 6: Statistically significant interactions in the ANOVA for the various quality parameters measured after 28 days of cold storage. Percentage fruit softening, percentage mass loss, days-to-ripening (DTR) and external chilling injury (ECI) were significantly affected by treatments or interactions, while no significant treatment effects were found for the internal quality parameters after 28 days of cold storage.

Treatment/Interaction	% Fruit Softening	% Mass Loss	DTR	ECI
Harvest date	**	**	**	**
Break	**	**	**	*
Temp	**	**	**	**
1-MCP	**		**	
Wax	**	**	**	
Harvest date.Break	**	*	**	
Harvest date.Temp	**			**
Break.Temp	**			
Harvest date.1-MCP	**		**	
Break.1-MCP	*			
Temp.1-MCP	**	*		
Harvest date.Wax		*		**
Break.Wax				
Temp.Wax				*
1-MCP.Wax				
Harvest date.Break.Temp	**			
Harvest date.Break.1-MCP	**			
Harvest date.Temp.1-MCP				
Break.Temp.1-MCP	**		*	
Harvest date.Break.Wax		**		
Harvest date.Temp.Wax	*		**	**
Break.Temp.Wax				
Harvest date.1-MCP.Wax	**			
Break.1-MCP.Wax	*			
Temp.1-MCP.Wax				
Harvest date.Break.Temp.1-MCP	**		*	
Harvest date.Break.Temp.Wax	*			
Harvest date.Break.1-MCP.Wax			*	
Harvest date.Temp.1-MCP.Wax	*			
Break.Temp.1-MCP.Wax			*	
Harvest date.Break.Temp.1-MCP.Wax	**		*	
* = Significant (confidence level of 95%)				
** = Highly Significant (confidence level of 99%)				

Table 7: Effect of cold chain breaks on percentage fruit softening and percentage fresh mass loss of 'Fuerte' avocado fruit upon removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

				Fruit softening (%)			Mass loss (%)		
Treatments				control	24 hr Delay	Break @ 14	control	24 hr Delay	Break @ 14
Early	2°C	1-MCP	waxed	7.13 a	3.74 b	5.74 a	4.466 a	4.13 a	4.326 a
			Non-waxed	7.05 a	7.61 a	8.32 a	3.941 a	5.626 b	4.454 a
		No 1-MCP	waxed	6.7 a	4.16 a	7.22 a	3.797 a	3.701 a	4.658 a
			Non-waxed	6.41 a	8.3 a	6.83 a	3.572 a	4.972 b	4.118 a
	5.5°C	1-MCP	waxed	7.14 a	6.39 a	8.9 a	4.842 a	5.424 a	5.171 a
			Non-waxed	8.78 a	6.92 a	8.84 a	4.803 a	6.219 b	5.766 a
		No 1-MCP	waxed	11.12 a	10.06 a	16.94 b	4.964 a	6.055 a	5.194 a
			Non-waxed	11.3 a	10.84 a	14.17 a	5.202 a	6.728 b	6.154 a
Mid	2°C	1-MCP	waxed	2.54 a	5.3 a	6.76 b	2.066 a	2.432 a	3.174 a
			Non-waxed	4.89 a	6.59 a	5.56 a	3.608 a	4.0 a	3.661 a
		No 1-MCP	waxed	5.26 a	5.01 a	5.48 a	2.334 a	2.606 a	2.323 a
			Non-waxed	2.65 a	4.47 a	5.39 a	3.448 a	4.207 a	3.282 a
	5.5°C	1-MCP	waxed	3.07 a	7.95 b	3.0 a	2.834 a	3.251 a	2.782 a
			Non-waxed	3.99 a	10.39 b	6.09 a	3.625 a	4.566 a	4.223 a
		No 1-MCP	waxed	9.36 a	13.71 b	6.65 a	2.801 a	3.474 a	5.669 b
			Non-waxed	7.94 a	13.23 b	8.15 a	3.917 a	5.286 b	4.159 a
Late	2°C	1-MCP	waxed	4.85 a	5.21 a	4.45 a	2.29 a	4.263 b	2.675 a
			Non-waxed	5.37 a	4.82 a	5.44 a	3.009 a	4.138 a	3.221 a
		No 1-MCP	waxed	4.54 a	6.5 a	5.62 a	2.41 a	4.282 b	2.735 a
			Non-waxed	7.84 a	5.68 a	4.2 b	2.764 a	4.058 b	3.085 a
	5.5°C	1-MCP	waxed	6.21 a	11.46 b	5.49 a	3.429 a	5.461 b	3.16 a
			Non-waxed	7.82 a	7.23 a	5.3 a	4.652 a	4.835 a	4.849 a
		No 1-MCP	waxed	10.61 a	8.24 a	16.82 b	3.121 a	4.253 b	3.91 a
			Non-waxed	10.68 a	20.72 b	21.95 b	4.1 a	5.345 b	5.036 a
LSD across =				3.347			1.1318		

* Each point represents the mean of 10 fruit.

Different letters indicate significant differences between the cold chain breaks and the control for that treatment combination.

Table 8: Effect of cold chain breaks on days-to-ripening (DTR) at 20±2°C of 'Fuerte' avocado fruit after removal from cold storage as well as external chilling injury (ECI)upon removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated, waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

				DTR (days)			ECI (0-10)		
Treatments				control	24 hr Delay	Break @ 14	control	24 hr Delay	Break @ 14
Early	2°C	1-MCP	waxed	11.4 a	11.7 a	11.9 a	0.3 a	0.0 a	0.2 a
			Non-waxed	9.0 a	11 b	9.2 a	0.5 a	1.5 b	2.3 b
		No 1-MCP	waxed	6.6 a	7.1 a	4.3 b	0.6 a	0.6 a	0.4 a
			Non-waxed	4.7 a	6.6 b	3.1 a	0.9 a	1.4 a	0.9 a
	5.5°C	1-MCP	waxed	7.9 a	8.9 a	8.4 a	0.5 a	0.1 a	0.3 a
			Non-waxed	8.9 a	9.6 a	8.2 a	0.0 a	0.1 a	0.1 a
		No 1-MCP	waxed	3.1 a	3.5 a	2.6 a	0.1 a	1.2 b	0.0 a
			Non-waxed	2.7 a	3.0 a	2.4 a	0.1 a	0.0 a	0.1 a
Mid	2°C	1-MCP	waxed	14.0 a	10.8 b	9.4 b	0.0 a	0.2 a	1.2 b
			Non-waxed	11.6 a	7.9 b	11.2 a	0.0 a	0.0 a	0.0 a
		No 1-MCP	waxed	7.4 a	4.8 b	8.5 a	0.0 a	0.4 a	0.4 a
			Non-waxed	9.4 a	3.6 b	5.7 b	0.2 a	0.0 a	0.4 a
	5.5°C	1-MCP	waxed	11.3 a	5.9 b	11.1 a	0.0 a	0.0 a	0.0 a
			Non-waxed	9.8 a	4.3 b	7.1 b	0.0 a	0.0 a	0.0 a
		No 1-MCP	waxed	5.1 a	1.9 b	5.5 a	0.0 a	0.3 a	0.0 a
			Non-waxed	2.5 a	1.6 a	2.8 a	0.4 a	0.0 a	0.0 a
Late	2°C	1-MCP	waxed	9.3 a	8.3 a	10.2 a	0.2 a	0.3 a	0.5 a
			Non-waxed	8.4 a	7.4 a	9.0 a	0.2 a	0.1 a	0.1 a
		No 1-MCP	waxed	7.1 a	5.5 a	6.6 a	0.0 a	0.4 a	0.4 a
			Non-waxed	6.5 a	3.8 b	5.9 a	0.0 a	0.0 a	0.2 a
	5.5°C	1-MCP	waxed	7.1 a	4.2 b	8.3 a	0.3 a	0.6 a	0.3 a
			Non-waxed	5.9 a	4.5 a	6.0 a	0.0 a	0.1 a	0.4 a
		No 1-MCP	waxed	2.6 a	3.5 a	3.1 a	0.0 a	0.3 a	0.6 a
			Non-waxed	2.6 a	1.6 a	2.2 a	0.2 a	0.1 a	0.4 a
LSD across =				1.8783			0.7586		

* Each point represents the mean of 10 fruit.

Different letters indicate significant differences between the cold chain breaks and the control for that treatment combination.

Table 9: Effect of cold chain breaks on anthracnose (0-5) and stem-end rot (0-5) development at 20±2°C in 'Fuerte' avocado fruit after removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates combined. *

			Anthracnose (0-5)			Stem-end rot (0-5)		
Treatments			Control	24 hour delay	Break @ 14	Control	24 hour delay	Break @ 14
2°C	1-MCP	waxed	0.03 a	0.07 a	0.00 a	0.00 a	0.00 a	0.07 a
		Non-waxed	0.03 a	0.03 a	0.03 a	0.07 a	0.07 a	0.00 a
	No 1-MCP	waxed	0.00 a	0.00 a	0.00 a	0.03 a	0.00 a	0.00 a
		Non-waxed	0.00 a	0.00 a	0.00 a	0.07 a	0.00 a	0.03 a
5.5°C	1-MCP	waxed	0.03 a	0.00 a	0.00 a	0.00 a	0.00 a	0.03 a
		Non-waxed	0.13 a	0.00 b	0.03 b	0.1 a	0.00 a	0.00 a
	No 1-MCP	waxed	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
		Non-waxed	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
LSD			0.0348			0.0784		
* Each point represents the mean of 30 fruit.								
Different letters indicate significant differences between the cold chain breaks and the control for that treatment combination.								

Table 10: Effect of cold chain breaks on anthracnose (0-5) and stem-end rot (0-5) development at 20±2°C in 'Fuerte' avocado fruit after removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates combined. *

			Vascular browning (0-5)			Mesocarp discolouration (0-5)			Sound fruit (%)		
Treatments			Control	24 hour delay	Break @ 14	Control	24 hour delay	Break @ 14	Control	24 hour delay	Break @ 14
2°C	1-MCP	waxed	0.00 a	0.03 a	0.00 a	0.00 a	0.00 a	0.00 a	96.7 a	96.7 a	93.3 a
		Non-waxed	0.13 a	0.03 a	0.00 b	0.07 a	0.00 a	0.00 a	86.7 a	93.3 a	96.7 a
	No 1-MCP	waxed	0.03 a	0.00 a	0.00 a	0.07 a	0.00 a	0.00 a	90.0 b	100 a	100 a
		Non-waxed	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a	93.3 a	100 a	96.7 a
5.5°C	1-MCP	waxed	0.00 a	0.00 a	0.10 a	0.00 a	0.00 a	0.00 a	96.7 a	100 a	93.3 a
		Non-waxed	0.03 a	0.00 a	0.00 a	0.10 a	0.00 b	0.00 b	90.0 b	100 a	96.7 a
	No 1-MCP	waxed	0.03 a	0.00 a	0.00 a	0.00 a	0.03 a	0.00 a	96.7 a	96.7 a	100 a
		Non-waxed	0.00 a	0.00 a	0.00 a	0.00 a	0.03 a	0.00 a	100 a	96.7 a	100 a
LSD			0.1031			0.0821			9.535		

* Each point represents the mean of 30 fruit.

Sound fruit are ripe and free from disorders and diseases.

Different letters indicate significant differences between the cold chain breaks and the control for that treatment combination.

CHAPTER 4

The effects of ultra-low temperature storage and 1-MCP on fruit quality characteristics of 'Fuerte' avocados after 56 days

Andre Lütge, Isa Bertling and John Bower

Holding avocados fruit at shipping temperatures lower than the currently utilized 5.5°C over the common 28 day storage period, have, in the last decade, repeatedly been shown to deliver a good quality product to the overseas market. The high internal fruit quality, has prompted the investigation into storing fruit at 2°C for an extended period of 56 days. 'Fuerte' avocados were harvested at different maturity stages(moisture contents of 74%, 68% and 63%) and subjected to treatments of 1-MCP and waxing and stored at 2°C or 5.5°C for 56 days. Fruit softening, mass loss, days-to-ripening, external quality and internal quality were recorded. Storing fruit at 5.5°C for 56 days resulted in significant fruit softening during storage, even when 1-MCP was used; additionally significantly more external chilling injury in mid- and late-season fruit was observed than at 2°C. Treatment of waxed fruit with 1-MCP followed by storage at 2°C resulted in the best fruit quality and shelf-life, particularly in mid-season fruit which had negligible external chilling injury and 100% sound fruit. Early-season fruit suffered significant external chilling injury at 2°C and late-season fruit had high pathological disorders and internal disorders at this storage temperature. Although mid-season fruit could be successfully stored at 2°C for 56 days, the use of a 56 day storage period is not recommended as a practical storage period, due to the high risk of external damage, particularly if maturity levels are not optimal and fruit are not of the highest quality.

1. INTRODUCTION

Although the possibility of storing avocado fruit at lower than currently used storage temperatures (1°C and 2°C) have been investigated for various cultivars a for storage period of 28 days, very little information is available on extended storage of 56 days at these temperatures. For cold storage of up to 28 days, these ultra-low temperatures have been relatively successful (Bower and Magwaza, 2004; Van Rooyen and Bower, 2007; Kok *et al.*, 2010; Lütge *et al.*, 2010), reducing premature softening and moisture loss in greenskins. Lower temperatures than currently utilized have also been repeatedly shown to result in better fruit quality for various commercial cultivars when stored for 28 days (Bower and Jackson, 2003; Van Rooyen, 2009; Van Rooyen and Bower, 2006). These positive results from ultra-low storage temperatures have prompted this investigation into the possibility of storing fruit for an extended period of 56 days.

Following strikes at the Cape Town port in 2010, necessitating extended cold storage prior to the export of avocados during this period, an investigation on the fruit quality under extended cold storage was initiated. The main concern was the external quality of the fruit stored at 2°C, as the time-temperature interaction has been shown to have a major effect on external chilling injury (ECI) (Vorster *et al*., 1990). Lemmer *et al*. (2006) found that 'Fuerte' avocados could be stored for 2 months when combining 1-MCP treatment and CA, using a step down temperature regime with fruit stored at 7°C for the first month and 6°C for the second month. This indicates the potential for extended cold storage and requires further investigation into the possibility of success at temperatures lower than 6°C under RA. If successful, such extended storage would allow for a much higher price for late-season fruit and provide increased flexibility in marketing, as fruit could be withheld from the market during time of over-supply.

The objective of this study was to determine the possibility of storing 'Fuerte' avocados at 2°C for 56 days, and to ascertain the best possible combination of storage temperature, 1-MCP and waxing to maintain high fruit quality throughout such an extended storage.

2. MATERIALS AND METHODS

2.1. Fruit material

Export grade 'Fuerte' avocado fruit were obtained from Cooling Estate, located in Wartburg (29°27'S, 30°40' E, KwaZulu-Natal, South Africa). Fruit were collected on 25/06/2010 (early-season), 12/08/2010 (mid-season) and 16/09/2010 (late-season) in order to obtain differing maturity levels, namely 74%, 68% and 63%moisture, respectively. Fruit were harvested from the same block. All waxed fruit were of uniform 'count 16' size (204.4g-365.8g), while the non-waxed fruit were packed before passing through the packline and were, in general, slightly smaller in size (192.3g-351.4g). Post-harvest operations such as grading and sizing, 1-MCP treatment, waxing and forced-air cooling were carried out at the packhouse as well as the weighing, firmness measurement and labelling of individual fruit before being placed into storage or treated with 1-MCP so as to minimise any breaks later in the cold chain. The 1-MCP treatment was applied at the registered rate (500 ppb) for 16 hours in cold storage at 5.5°C, whilst the untreated fruit were stored under regular atmosphere at the same temperature for the same period. All fruit were transported in a non-refrigerated vehicle but were enclosed in a canopy and transported within 40 minutes to the laboratories of Horticultural Science at the University of KwaZulu-Natal (UKZN) and immediately prepared for simulated shipping. This did constitute a short cold chain break for all fruit, however, this was completed in a very short period of time and was not deemed to be a significant cold chain break. For each harvest date, ten addition fruit were collected from the same orchard to estimate the moisture content. Moisture content was measured by slicing a longitudinal strip of the entire avocado, of approximately 3mm in width, and weighing this sample before and after freeze drying the material. Fruit were randomly placed into export shipping cartons, each containing 10 fruit. Each carton was randomly assigned to one of 24 treatment combinations, including treatments of temperature (2°C and 5.5°C), 1-MCP (treated and untreated) and waxing (waxed with Avoshine® and non-waxed) over three harvest dates. Fruit were placed into cold storage containers in regular atmosphere (RA) for 56 days, at air delivery temperatures of 2°C (±1°C) or 5.5°C (±1°C). To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used.

2.2 Data collection

Storage Data

Visual, external observations of fruit quality were made prior to cold storage, during break periods, when fruit were removed from cold storage and during ripening. Before entering cold storage, fruit mass, fruit softness, CO_2 evolution, ethylene evolution and overall fruit condition were measured. Fruit were visually rated for shrivel, sunburn and external damage before storage to be able to accurately distinguish between ECI and pre-storage damages. After 28 days, fruit were removed from cold storage and fruit softening, mass loss, CO_2 evolution and ethylene evolution were calculated. For the ripening period, the fruit were allowed to ripen in a laboratory at room temperature (18-22^{o}C).

2.3 Fruit softness

A hand-held densimeter (Bareiss, Oberdischingen, Germany) with a 5 mm tip was used to measure fruit firmness (ripeness) on a scale of 85-90 (hard, unripe; ≡ 8.06 N) to <60 (soft, ready to eat; ≡ 5.05 N). Four equally spaced readings were taken around the circumference of each fruit and the average reading recorded. Ripening time was calculated as the number of days from harvest to the 'eating soft' stage, which corresponded to an average densimeter reading of less than 60 (5.05 N). Fruit softening during storage was calculated as the difference in fruit firmness before and after cold storage, and expressed as a percentage of the initial firmness of each fruit (% fruit softening = {[softness before storage – softness after storage] / softness before storage} x 100).

2.4 Mass loss

During cold storage, water loss is the main contributor to mass loss, because of the reduced respiration rate and metabolic activity (thus the low usage of dry matter energy reserves) at low temperatures (Bower and Jackson, 2003). Therefore, it was assumed that mass loss is equivalent to water loss for the purpose of this study. Fruit mass loss was calculated as the difference in fruit mass before and after cold storage, and expressed as a percentage of the initial mass of each fruit. Individual fruit were initially weighed at the packhouse prior to any cold chain break treatment and again after removal from cold storage, allowing sufficient time for condensation to evaporate so as not to influence measurements.

2.5 Internal Quality

Upon ripening, all ten replications of each treatment were cut longitudinally and assessed for anthracnose, stem end rot, vascular browning and mesocarp discolouration according to Lütge *et al*., 2010. Internal assessments were made on a scale of 0 (no visible symptoms) to 5 (extremely severe, area completely infected or discoloured). Sound fruit were classified as fruit which ripened to the eating ripe stage with a rating of 0 for all internal disorders and body rots (i.e. free from any disorders and diseases).

2.6 External Quality

The severity of ECI was rated on a scale of 0 (no blemishes) to 10 (fruit surface area entirely blemished). The fruit were visually assessed for ECI once the fruit had reached room temperature, allowing for sufficient time for symptoms of chilling injury to be expressed.

3. RESULTS

3.1. Fruit Softening

Fruit softening during storage was significantly reduced by storing fruit at 2°C (P<0.001), by the use of 1-MCP (P<0.001) and by waxing (P=0.012) (Table 8). Harvest date also had a significant effect on fruit softening (P<0.001), with mid- and late-season fruit softening significantly more than early-season fruit, particularly at a storage temperature of 5.5°C (P<0.001; Table 9). There was no significant difference between the harvest dates for fruit stored at 2°C, with these fruit softening 7-10% over the 56 day storage period, which is comparable with '5.5°C, no 1-MCP' fruit after 28 days (Table 3, Chapter 2).

The interaction between temperature and 1-MCP was statistically significant (P<0.001). At 5.5°C, fruit not treated with 1-MCP softened by an average of 39% over the three harvest dates. Upon removal from storage these fruit had impressions of the aeration holes from the bottom of the cartons. The use of 1-MCP significantly reduced fruit softening at 5.5°C, however, these fruit were still significantly softer than fruit stored at 2°C, irrespective of 1-MCP treatment (Figure 22). The use of 1-MCP at 2°C had only a tendency to reduce fruit softening.

The interaction between harvest date, temperature, 1-MCP and waxing was statistically significant (P=0.026).Waxing significantly reduced fruit softening of 1-MCP fruit at 5.5°C storage, in the early- and mid-season, but not the late-season, nor in untreated fruit stored at 5.5°C or any fruit stored at 2°C (Table 9).

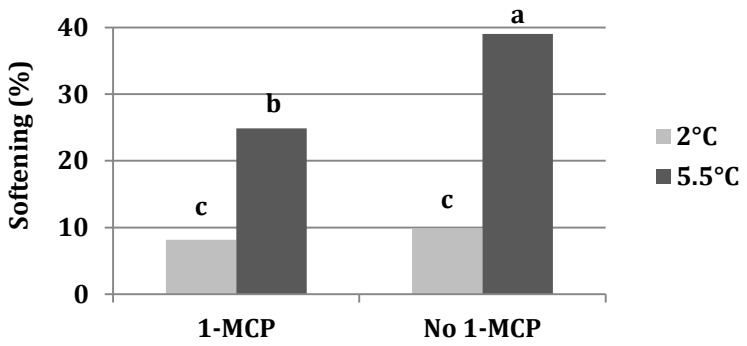

Figure 22: Percentage fruit softening for 1-MCP treated and untreated 'Fuerte' avocados following 56 days of cold storage at 2°C or 5.5°C.Different letters indicate significant differences between treatments (P≤0.05). LSD = 2.083.

3.2. Mass Loss

Mass loss (predominantly water loss) during storage was significantly reduced by storing fruit at 2°C ($P<0.001$) and by waxing ($P<0.001$) (Table 8). Harvest date also had a significant effect on mass loss ($P<0.001$), as did the interaction between harvest date and temperature ($P<0.001$; Figure 23). Significantly less mass was lost from fruit stored at 2°C as the season progressed, from 7% in the early-season fruit to 5% in the late-season fruit. Fruit stored at 5.5°C lost significantly more mass than fruit stored at 2°C, throughout the season, with the highest mass loss occurring in early-season fruit (Figure 23). Similarly, waxing significantly reduced mass loss in all harvest dates ($P=0.005$) and at both storage temperatures ($P=0.005$), with the lowest mass loss occurring in late-season, waxed fruit stored at 2°C with 1-MCP having no significant effect (Table 9).

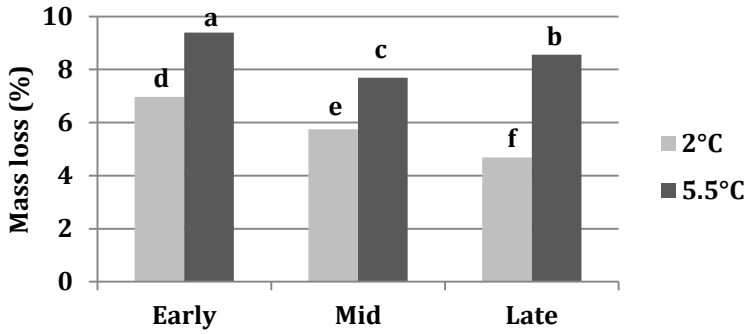

Figure 23: Percentage mass loss of 'Fuerte' avocados of different harvest dates after 56 days of cold storage at 2°C and 5.5°C. Different letters indicate significant differences between treatments ($P\leq0.05$). LSD = 0.647.

3.3. Days-to-ripening

Harvest date ($P<0.001$), temperature ($P<0.001$), 1-MCP ($P<0.001$) and waxing ($P<0.001$) affected DTR significantly (Table 7). In all three harvest dates, the use of 1-MCP ($P=0.001$) as well as waxing ($P=0.019$) increased DTR significantly, with the number of DTR decreasing as the season progressed (Table 9). The interaction between temperature, 1-MCP and waxing was highly significant (Figure 24). At 5.5°C, non-treated fruit ripened soon after removal from storage, indicating that softening had been initiated during cold storage. The number of DTR was increased, at this storage temperature, by the use of 1-MCP and waxing, resulting in 4 DTR. Conversely, an acceptable DTR was achieved for 1-MCP treated fruit stored at 2°C, with or without waxing, as well as waxed fruit stored at 2°C without 1-MCP treatment (Figure 24); such fruit had a mean of 7 and 9 DTR. The previously proposed best

treatment combination (Chapter 2), achieved approximately 7 DTR after both 28 days and 56 days of storage, throughout the season (Table 9).

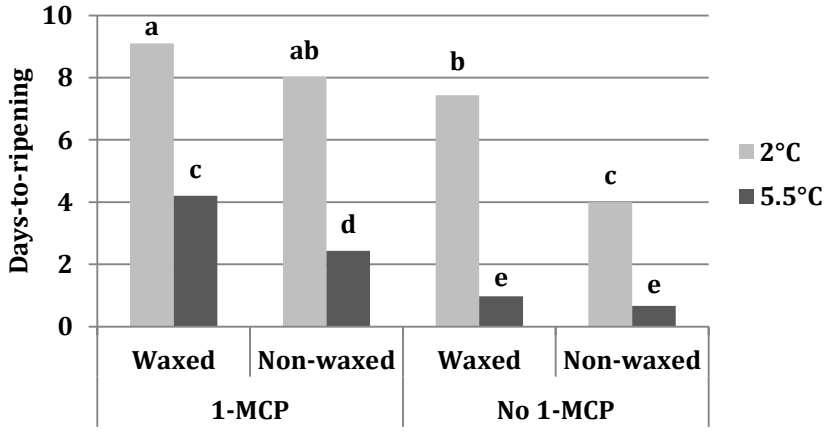

Figure 24: Effect of 1-MCP and waxing of 'Fuerte' avocados on days-to-ripeningfollowing56 days of cold storage at 2°C or 5.5°C.Different letters indicate significant differences between treatments (P≤0.05). LSD = 1.112.

3.4. External Quality

The severity of ECI after 56 days of cold storage was generally higher than after 28 days, shown in the significant increase in ECI from a 0.188 rating after 28 days to 1.87 after 56 days, on a scale from no blemishes (0) to entirely blemished (10). The main effect of temperature (P<0.001) was statistically significant, but varied significantly between the harvest dates (P<0.001). The two storage temperatures resulted in opposing trends as the season progressed (Figure 25). The ECI at 2°C was significantly higher in early-season fruit than in mid- and late-season fruit, while the ECI at 5.5°C increased significantly from early- to late-season fruit (Figure 25). Thus, early-season fruit suffered significantly more ECI at 2°C than at 5.5°C; mid- and late-season fruit had significantly higher ECI when stored at 5.5°C than at 2°C (Figure 25).

The use of 1-MCP tended to reduce ECI in the mid- and late-season fruit(P=0.041) but only significantly when stored at 5.5°C, and not significantly at 2°C (P<0.001; Table 9). Further, the mean ECI for all harvest dates of fruit stored at 2°C and not treated with 1-MCP did not have significantly higher ECI than 1-MCP fruit stored at 5.5°C (Figure 26).

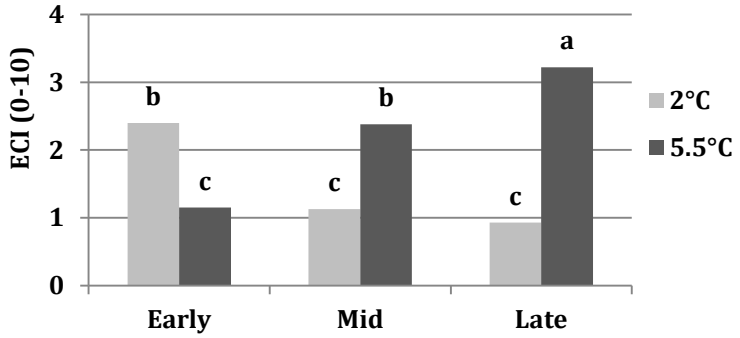

Figure 25: External chilling injury (ECI) of 'Fuerte' avocados of different harvest dates following 56 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.754.

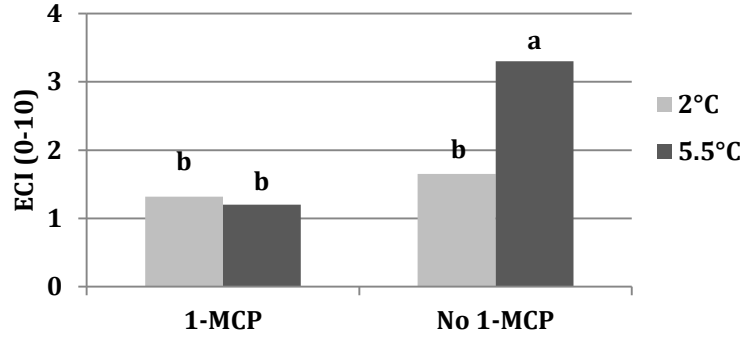

Figure 26: External chilling injury (ECI) of 1-MCP treated and untreated 'Fuerte' avocados following56 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.616.

Waxing had a statistically significant effect on ECI (Table 8; P=0.036); however, the effect varied with harvest date (Table 9; P=0.046). Waxing significantly reduced the ECI only in mid-season fruit (Table 9). Waxed fruit had slightly more ECI than non-waxed fruit, due to a markedly lower ECI for non-waxed late-season fruit stored at 2°C (Table 9).

3.5. Internal Quality

Even though there were few incidences of poor internal quality, a higher incidence of internal disorders and body rots were observed after 56 days than after 28 days cold storage; several treatments had a significant effect on internal quality after 56 days of storage (Table 10). Harvest date (P=0.005), waxing (P=0.033) and their interaction (P=0.011) affected severity of anthracnose. Late-season fruit had a significantly higher severity of anthracnose than early- and mid-season fruit; waxed fruit had a significantly higher anthracnose severity than non-waxed fruit in the late-season (Table 11). No fruit in the mid-season showed any symptoms of anthracnose infection.

Stem-end rot severity displayed a similar trend to anthracnose severity with harvest date (P=0.022), temperature (P=0.035) and their interaction (P=0.004) affecting stem-end rot severity. Late-season fruit had a higher severity of this disorder than early- and mid-season fruit, particularly fruit stored at 2^{o}C, which had a higher severity than fruit stored at 5.5^{o}C in the late-season (Table 11). No fruit in the mid-season showed symptoms of stem-end rot.

The occurrence of vascular browning was affected by temperature (P<0.001), 1-MCP (P<0.001) and their interaction (P<0.001), as well as the interaction between harvest date and 1-MCP (P=0.023). Cold storage at 5.5^{o}C resulted in a significantly higher severity of vascular browning than storage at 2^{o}C. Fruit not treated with 1-MCP showed significantly higher severity of vascular browning than treated fruit, particularly when stored at 5.5^{o}C (Table 10). The use of 1-MCP was able to significantly reduce the severity of vascular browning in early- and mid-season fruit, but not in late-season fruit (Table 11).

Like vascular browning, mesocarp discolouration severity was affected by harvest date (P<0.001), temperature (P<0.001) and their interaction (P<0.001). Late-season fruit had a significantly higher severity than early- and mid-season fruit, particularly fruit stored at 5.5^{o}C (Table 11). At 5.5^{o}C, fruit had a significantly higher severity of mesocarp discolouration than at 2^{o}C in mid- and late-season fruit, while not significantly higher in early-season fruit (Table 11). The use of 1-MCP (P<0.001) and the interaction between temperature and 1-MCP (P<0.001) also had significant effects on mesocarp discolouration severity. The use of 1-MCP reduced the severity significantly at 5.5^{o}C; however, the 2^{o}C storage temperature without the use of 1-MCP, still had a tendency to a lower severity of mesocarp discolouration (Table 11).

The highest percentage of sound fruit occurred in mid-season fruit while the lowest occurred in late-season fruit ($P<0.001$). A storage temperature of $2^{o}C$, resulted in a significantly higher percentage of sound fruit than storage at $5.5^{o}C$ ($P<0.001$); 1-MCP increased ($P<0.001$) the percentage of sound fruit through the season, and particularly when 1-MCP was used at $5.5^{o}C$ ($P<0.001$). The percentage of sound fruit at $2^{o}C$ was not affected by the use of 1-MCP, and resulted in approximately 80% sound fruit (Table 11). However, when 1-MCP was not used, waxing resulted in a lower percentage of sound fruit at $2^{o}C$ than omitting waxing ($P=0.042$).

4. DISCUSSION

'Fuerte' avocado fruit cannot be stored successfully at 5.5°C over an extended period, even with the use of 1-MCP, as such storage resulted in significant softening during storage (Figure 22), indicative of fruit which reached the climacteric phase during cold storage. Further, mass loss at 5.5°C was significantly higher than at 2°C throughout the season (Figure 23) and, thus, ECI following storage at 5.5°C was significantly higher than at 2°C in the mid- and late-season (Figure 25), as excessive softening during storage and subsequent ECI increases as the maturity of the fruit increased (Donkin and Cutting, 1994). Bezuidenhout (1983) found that subjecting 'Fuerte' fruit to excessive cold just prior to the climacteric, favours chilling injury and pulp spot development. The higher severity of internal disorders following storage at 5.5°C than at 2°C, particularly late in the season, can be explained by the timing of cold storage in relation to the climacteric peak and the increased sensitivity to chilling injury when ethylene is present in the storage atmosphere (Lee and Young, 1984). The only fruit stored at 5.5°C with low ECI were waxed, 1-MCP treated fruit, although these fruit softened significant (approximately 20%) during storage and ripened on average 4 days after removal from storage (Table 9), thus might only be accepted for the ripe-and-ready market given the fairly short ripening times (Figure 27).

The most important question, commercially, is whether the storage temperature of 2°C can provide good quality fruit after 56 day storage. Compared with the standard 28 day storage period, fruit experienced more fruit softening and mass loss, more ECI, greater severities of various internal disorders and diseases and a shorter shelf-life. This is to be expected as the length of cold storage has a significant effect on overall fruit quality (Saltveit and Morris, 1990). However, fruit stored at 2°C appear to have the potential to maintain a better quality than at 5.5°C over this extended period.

The treatment combination of '2°C, no 1-MCP and waxing' achieved a similar DTR after 56 days of cold storage (Figure 24) as the same treatment combination after 28 days of storage (Chapter 2, Table 3), indicating that the metabolic activity of the fruit is shut down effectively and, even after the extended storage period, fruit ripen in a similar pattern as fruit stored for half this time. However, over 56 days of storage, the use of 1-MCP appears to be necessary to maintain fruit quality over this extended storage period. The use of 1-MCP on waxed fruit stored at 2°C reduced the percentage softening to an average of approximately 8% through the season, and increased the DTR to approximately 9 days. Although this

extension in DTR may increase the chance of body rots (Eksteen and Truter, 1985), 1-MCP treated fruit at this storage temperature had a significantly reduced severity of mesocarp discolouration, a slightly lower severity of vascular browning, a slightly lower ECI and a higher percentage of sound fruit, particularly in early- and mid-season fruit (Table 9 and 11), ultimately suggesting that 1-MCP is a necessary treatment over this extended storage period.

Obviously, external damage at 2^{o}C over a lengthy storage period is a major concern. Early-season fruit should not be shipped at 2^{o}C for this extended period as the risk of external damage is too high, especially for a greenskin like 'Fuerte', as such fruit do not change rind colour to mask external damage. However, mid-season fruit had very little external damage when 1-MCP and waxing was used. Mid-season fruit are most likely to be stored successfully for 56 days, as these fruit suffered negligible levels ECI and ripened with 100% sound fruit (Figure 27). Although the greatest economic gains would be achieved by successfully storing late-season fruit for 56 days, the highest incidences of body rots and the lowest percentages of sound fruit were recorded, rendering such storage period extension not feasible.

5. CONCLUSION

Results indicate that mid-season, 1-MCP treated, waxed fruit can be stored at 2^{o}C for 56 days. Early-season fruit suffered significant ECI and late-season fruit, although potentially the most valuable, suffered significantly higher severities of anthracnose and stem-end rot. Unlike under 28 day storage, 1-MCP is needed if fruit are to be stored for 56 days, as this treatment, in conjunction with the 2^{o}C storage temperature, reduces the percentage fruit softening and results in better internal quality than that of untreated fruit. Waxing reduces the amount of water loss of firm fruit stored successfully without high levels of fruit softening; however, when fruit softening occurs during storage, waxing reduces the percentage of sound fruit. The use of a 56 day storage period is currently not recommended as a practical storage period. Further studies on the optimum maturity level for such an extended storage period should be conducted as fruit maturity was shown to play a large role in final fruit quality. Pre-harvest fruit quality is of prime importance to avoid damage, particularly with the added stress of ultra-low temperature storage.

Table 7: Statistically significant interactions in the ANOVA for the various quality parameters measured after 56 days of cold storage.

Treatment/Interaction	% Fruit Softening	% Mass Loss	DTR	ECI	Anthracnose Severity	Stem-end rot Severity	Vascular Browning Severity	Mesocarp Discolouration Severity	% Sound Fruit
Harvest date	**	**	**		*	*		**	**
Temp	**	**	**	**		*	**	**	**
1-MCP	**		**	**			**	**	**
Wax	*	**	**	*	*				
Harvest date.Temp	**	**		**		*		**	
Harvest date.1-MCP			**	*			*		**
Temp.1-MCP	**			**			**	**	**
Harvest date.Wax		**	*	*	*				
Temp.Wax		**	*						
1-MCP.Wax									
Harvest date.Temp.1-MCP									
Harvest date.Temp.Wax									
Harvest date.1-MCP.Wax									
Temp.1-MCP.Wax			**						*
Harvest date.Temp.1-MCP.Wax	*								
* = Significant (confidence level of 95%)									
** = Highly Significant (confidence level of 99%)									

Table 8: Percentage fruit softening, percentage fresh mass loss and external chilling injury (ECI) of 'Fuerte' avocado fruit at the removal from cold storage as well as days-to-ripening (DTR) at 20±2°C after removal from cold storage. Fruit were stored for 56 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates combined. *

Treatments			Fruit Softening (%)	Mass Loss (%)	ECI (0-10)	DTR (days)
2°C	1-MCP	waxed	7.93 d	5.396 d	1.17 bc	9.1 a
		Non-waxed	8.42 d	6.363 c	1.47 bc	8.03 ab
	No 1-MCP	waxed	9.53 d	5.052 d	1.3 bc	7.43 b
		Non-waxed	10.16 d	6.38 c	2.0 b	4.0 c
5.5°C	1-MCP	waxed	21.92 c	7.09 bc	1.1 c	4.2 c
		Non-waxed	27.82 b	9.65 a	1.3 bc	2.43 d
	No 1-MCP	waxed	38.77 a	7.776 b	2.97 a	0.97 e
		Non-waxed	39.29 a	9.672 a	3.63 a	0.67 e
LSD			2.946	0.7475	0.871	1.112

* Each point represents the mean of 30 fruit.
Different letters indicate significant differences within each column.

Table 9: Seasonal changes in percentage fruit softening, percentage fresh mass loss and external chilling injury (ECI) of 'Fuerte' avocado fruit upon removal from cold storage, as well as days-to-ripening (DTR) at 20±2°C after removal from cold storage. Fruit were stored for 56 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

Treatment				Fruit Softening (%)	Mass Loss (%)	ECI (0-10)	DTR (days)
Early	2°C	1-MCP	waxed	8.7 g	6.605 fgh	1.9 defghi	10.9 a
			Non-waxed	9.62 g	7.245 ef	2.6 cde	10.5 a
		No 1-MCP	waxed	11.42 fg	6.636 fgh	2.3 defg	6.5 d
			Non-waxed	10.08 g	7.361 ef	2.8 bcde	5.5 e
	5.5°C	1-MCP	waxed	15.9 ef	8.489 cde	0.4 ij	6.6 d
			Non-waxed	21.36 d	10.23 a	0.8 ghij	3.8 fg
		No 1-MCP	waxed	34.32 b	8.952 bc	1.6 efghij	1.5 hij
			Non-waxed	31.92 bc	9.882 ab	1.8 defghi	1.7 hi
Mid	2°C	1-MCP	waxed	8.78 g	5.071 ij	0.2 j	8.7 b
			Non-waxed	8.09 g	6.458 fgh	1.4 efghij	6.8 cd
		No 1-MCP	waxed	8.85 g	4.304 j	0.6 ij	9.4 b
			Non-waxed	11.15 fg	7.144 f	2.3 defg	3.4 fg
	5.5°C	1-MCP	waxed	19.51 de	5.623 ghi	0.7 hij	4.2 f
			Non-waxed	33.72 b	8.812 bcd	0.7 hij	1.5 hij
		No 1-MCP	waxed	40.92 a	6.836 fg	3.2 bcd	0.8 ijk
			Non-waxed	42.68 a	9.49 abc	4.9 a	0.3k
Late	2°C	1-MCP	waxed	6.32 g	4.513 ij	1.4 efghij	7.7
			Non-waxed	7.54 g	5.386 hij	0.4 ij	6.8 cd
		No 1-MCP	waxed	8.31 g	4.217 j	1.0 fghij	6.4 de
			Non-waxed	9.24 g	4.635 ij	0.9 fghij	3.1 g
	5.5°C	1-MCP	waxed	30.36 bc	7.158 f	2.2 defgh	1.8 h
			Non-waxed	28.38 c	9.908 ab	2.4 def	2 h
		No 1-MCP	waxed	41.08 a	7.54 def	4.1 abc	0.6 jk
			Non-waxed	43.28 a	9.645 abc	4.2 ab	0.0 k
LSD				5.102	1.275	1.508	1.927

* Each point represents the mean of 10 fruit.

Different letters indicate significant differences within each column.

Table 10: Internal quality of 'Fuerte' avocado fruit upon reaching eating ripeness at 20±2°C. Fruit were stored for 56 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates are combined. *

Treatments			Anthracnose (0-5)	Stem-end rot (0-5)	Vascular browning (0-5)	Mesocarp discolouration (0-5)	Sound fruit (%)
2°C	1-MCP	waxed	0.20 a	0.17 a	0.03 b	0.00 d	80.0 ab
		Non-waxed	0.20 a	0.20 a	0.07 b	0.03 cd	80.0 ab
	No 1-MCP	waxed	0.20 a	0.13 a	0.10 b	0.30 b	70.0 bc
		Non-waxed	0.00 a	0.03 a	0.00 b	0.03 cd	93.3 a
5.5°C	1-MCP	waxed	0.20 a	0.10 a	0.07 b	0.37 bc	60.0 c
		Non-waxed	0.00 a	0.00 a	0.03 b	0.40 b	70.0 bc
	No 1-MCP	waxed	0.13 a	0.00 a	0.43 a	1.37 a	33.3 d
		Non-waxed	0.00 a	0.00 a	0.60 a	1.23 a	26.7 d
LSD			0.245	0.203	0.222	0.356	19.31

* Each point represents the mean of 30 fruit.

Sound fruit are ripe and free from disorders and diseases.

Different letters indicate significant differences within each column.

Table 11: Seasonal changes in internal quality of 'Fuerte' avocado fruit upon reaching eating ripeness at 20±2°C. Fruit were stored for 56 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate) and results from harvest dates are combined. *

Treatments				Anthracnose (0-5)	Stem-end rot (0-5)	Vascular browning (0-5)	Mesocarp discolour-ation (0-5)	Sound fruit (%)
Early	2°C	1-MCP	waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
			Non-waxed	0.4 b	0.2 abc	0.1 cd	0.1 f	80 abc
		No 1-MCP	waxed	0.0 b	0.0 c	0.2 cd	0.3 ef	70 abcd
			Non-waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
	5.5°C	1-MCP	waxed	0.4 b	0.3 abc	0.0 d	0.1 f	70 abcd
			Non-waxed	0.0 b	0.0 c	0.1 cd	0.1 f	80 abc
		No 1-MCP	waxed	0.0 b	0.0 c	0.4 bc	0.8 cde	50 cdef
			Non-waxed	0.0 b	0.0 c	0.7 ab	0.5 def	40 defg
Mid	2°C	1-MCP	waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
			Non-waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
		No 1-MCP	waxed	0.0 b	0.0 c	0.0 d	0.1 f	90 ab
			Non-waxed	0.0 b	0.0 c	0.0 d	0.1 f	90 ab
	5.5°C	1-MCP	waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
			Non-waxed	0.0 b	0.0 c	0.0 d	0.0 f	100 a
		No 1-MCP	waxed	0.0 b	0.0 c	0.8 a	0.9 cde	40 defg
			Non-waxed	0.0 b	0.0 c	0.8 a	1.4 bc	20 fg
Late	2°C	1-MCP	waxed	0.6 a	0.5 a	0.1 cd	0.0 f	40 defg
			Non-waxed	0.2 b	0.4 ab	0.1 cd	0.0 f	60 bcde
		No 1-MCP	waxed	0.6 a	0.4 ab	0.1 cd	0.5 def	50 cdef
			Non-waxed	0.0 b	0.1 bc	0.0 d	0.0 f	90 ab
	5.5°C	1-MCP	waxed	0.2 b	0.0 c	0.2 cd	1.0 cd	10 g
			Non-waxed	0.0 b	0.0 c	0.0 d	1.1 cd	30 efg
		No 1-MCP	waxed	0.4 b	0.0 c	0.1 cd	2.4 a	10 g
			Non-waxed	0.0 b	0.0 c	0.3 cd	1.8 ab	20 fg
	LSD			0.4244	0.3517	0.3847	0.6166	33.44

* Each point represents the mean of 10 fruit.

Sound fruit are ripe and free from disorders and diseases.

Different letters indicate significant differences within each column.

Figure 27: 'Fuerte' avocados one day after removal from cold storage. Fruit were stored at 2°C for 56 days, treated with 1-MCP and waxed. Fruit in the top photograph were stored at 2°C and had a mean fruit softening of 8.78%, a mean ECI rating of 0.2/10 and had 100% sound fruit, while fruit in the bottom photograph were stored at 5.5°C and had a mean fruit softening of 19.51 %, a mean ECI rating of 0.7/10 and had 100% sound fruit.

CHAPTER 5

Investigation into the anti-oxidants of 'Fuerte' avocados following ultra-low temperature storage for 28 and 56 days

Andre Lütge, Isa Bertling and John Bower

Anti-oxidants are produced in cells to protect cellular structures against free radicals and other damaging compounds, particularly reactive oxygen species, which are formed during oxidative stress. It has been reported that numerous anti-oxidant systems exist in avocado fruit. Recently, several studies have identified some of the functions, and the importance of, the two main C7 sugars in avocados, mannoheptulose and perseitol. However, information on the effect of different storage treatments and temperatures as well as cold chain breaks and extended storage periods on the anti-oxidant levels in the exocarp of avocados is limited, particularly for 'Fuerte' avocados. Therefore, the objective of the study was to determine the effects of different storage treatments, cold chain breaks and storage period on the anti-oxidants in the exocarp and C7 sugars in the mesocarp of 'Fuerte' avocados. 'Fuerte' avocados were harvested at three different maturity stages reflecting early-, mid- and late-season fruit, with moisture contents of 74%, 68% and 63%, respectively. Fruit were subjected to treatments of 1-MCP, waxing and cold chain breaks and stored at 2^{o}C and 5.5^{o}C. The total anti-oxidant capacity and ascorbic acid concentration in the exocarp as well as the concentrations of mannoheptulose and perseitol in the mesocarp were analysed after removal of fruit from cold storage. Anti-oxidant concentrations in the exocarp, as well as mannoheptulose and perseitol concentrations in the mesocarp, were highest in early-season fruit and declined significantly as the season progressed. Results suggest a link between anti-oxidant concentrations in the exocarp and the rate of metabolic activity during cold storage. Although trends were visible for different treatments, the level of C7 sugars found in the mesocarp was not able to conclusively indicate differences between storage treatments, storage duration or breaks in the cold chain. To successfully differentiate between treatment effects and the correlation between anti-oxidant levels, C7 sugar concentrations and fruit quality, measurements are recommended throughout the storage period, on large numbers of fruit to minimise the high variation found in these parameters.

1. INTRODUCTION

Numerous anti-oxidant systems exist in avocado fruit and in varying levels, depending on the stage of maturity (Slater *et al.*, 1975) and stage of avocado fruit development (Tesfay *et al*., 2010a). Anti-oxidants are produced in cells to protect cellular structures against reactive and damaging compounds, particularly reactive oxygen species (ROS), which are formed during oxidative stress (Fang *et al.*, 2002). Anti-oxidants function to scavenge ROS, and maintain a balance between anti-oxidants and ROS so that normal cell metabolism can proceed; however, under stress conditions, this balance can be easily disturbed and result in an accumulation of ROS (Foyer and Noctor, 2005). An accumulation of ROS, to a damaging concentration, results in oxidative stress which can cause membrane damage via peroxidation of membrane lipids, protein denaturation and DNA mutation (Borg and Schaich, 1988).

Ascorbic acid (AsA) is an important "broad spectrum" anti-oxidant found in avocados. It helps regenerate tocopherol, another important anti-oxidant compound that limits membrane damage (Tesfay *et al*., 2010a; Thomas *et al*., 1992). Tesfay *et al*. (2010a) reported that the total anti-oxidant capacity (TAOC) and AsA concentration was highest in the exocarp and seed of 'Hass' avocados and lowest in the mesocarp, thus these parameters were chosen as measurements to depict the anti-oxidant levels in the exocarp.In post-harvest avocado physiology, C7 sugars are believed to perform various important roles. Mannoheptulose has been proposed as a possible source of energy (Liu *et al*., 1999), the main anti-oxidant in the mesocarp (Tesfay *et al*., 2010a) and a major source of carbon (Blakey *et al*., 2011). Perseitol functions as a storage carbohydrate and as an important anti-oxidant in the mesocarp of avocados, albeit with a lower ability to scavenge ROS than mannoheptulose (Tesfay *et al*., 2010a). Although the exact roles of these sugars have not been elucidated, indications are that the pool of C7 sugars in the mesocarp of avocados plays an important role in the postharvest quality of avocados (Bertling and Bower, 2006; Liu *et al*., 1999; Tesfay *et al*., 2010a).

The altering of storage temperature and duration may lead to changes in carbohydrate storage and usage and, ultimately, affect final fruit quality (Eaks, 1990; Spalding, 1976). However, information on the effect of different storage treatments and temperatures as well as cold chain breaks and extended storage periods on the anti-oxidant levels in the exocarp of avocados is limited, particularly for 'Fuerte' avocados. Thus the objective of the study was to determine the effects of different storage treatments, cold chain breaks and storage period on the anti-oxidant concentrations in the exocarp and of C7 sugars in the mesocarp.

2. MATERIALS AND METHODS

2.1 Fruit material

Export grade 'Fuerte' avocado fruit were obtained from Cooling Estate, located in Wartburg (29°27'S, 30°40' E, KwaZulu-Natal, South Africa). The fruit were collected on 25/06/2010 (early-season, 74% moisture), 12/08/2010 (mid-season, 68% moisture) and 16/09/2010 (late-season, 63% moisture) in order to obtain differing maturity levels. The fruit were harvested from the same block. Waxed fruit were of uniform 'count 16' size (204.4g-365.8g), while the non-waxed fruit were packed before passing through the packline and were, in general, slightly smaller in size (192.3g-351.4g). Post-harvest operations such as grading and sizing, 1-MCP treatment, waxing and forced-air cooling were carried out at the packhouse as well as the weighing, firmness measurement and labelling of individual fruit before being placed into storage or treated with 1-MCP so as to minimise any breaks later in the cold chain. The 1-MCP treatment was applied at the registered rate (500 ppb) for 16 hours in cold storage at 5.5°C, whilst the untreated fruit were stored under regular atmosphere at the same temperature for the same period. All fruit were transported in a non-refrigerated vehicle but were enclosed in a canopy and transported within 40 minutes to the laboratories of Horticultural Science at the University of KwaZulu-Natal (UKZN) and immediately prepared for simulated shipping. This did constitute a short cold chain break for all fruit, however, this was completed in a very short period of time and was not deemed to be a significant cold chain break. For each harvest date, ten addition fruit were collected from the same orchard to estimate the moisture content. Moisture content was measured by slicing a longitudinal strip of the entire avocado, of approximately 3mm in width, and weighing this sample before and after freeze drying the material. Fruit were labelled and placed into cold storage containers in regular atmosphere (RA) for 28 and 56 days, at air delivery temperatures of either 2°C (±1°C) or 5.5°C (±1°C). To monitor the internal temperature and relative humidity of the storage containers, HOBO® H8 data loggers were used.

2.2 Treatments

Fruit were randomly placed into export shipping cartons, each containing 10 fruit. Each carton was randomly assigned to one of the 24 treatment combinations, including treatments of temperature (2°C or 5.5°C), 1-MCP (treated or untreated) and waxing (waxed with Avoshine® or non-waxed) over three harvest dates. Cold chain breaks included a 24 hour

delay in cooling and a break after 14 days of cold storage, where fruit were placed in the laboratory for eight hours at 20±2^{o}C and then returned to cold storage.

2.3 Post-storage sampling from fruit tissue

Upon removal from storage, a core sample of the mesocarp and an exocarp sample of three randomly selected fruit for each treatment combination were collected. The mesocarp core samples were taken using a 10 mm core borer and the exocarp samples were peeled from approximately one quarter of the fruit, using a potato peeler according to Blakey *et al*., (2010). The sampled areas were sealed with warm petroleum jelly according to Blakey *et al*., 2010, to prevent oxidation in an attempt to achieve normal ripening and to ensure that DTR and internal quality could be measured using all 10 fruit as replications. The samples were flash-frozen in liquid nitrogen, freeze-dried, ground and subsequently stored at -20^{o}C until further physiological analysis.

2.4 Anti-oxidant analysis

Anti-oxidant levels were determined as 'total anti-oxidant capacity' (TAOC) using the FRAP assay as described by Benzie and Strain (1996) with slight modifications. This method involves the reduction of a ferric-tripyridyltriazine complex to its ferrous form, which is visible in the blue colour change, in the presence of anti-oxidants in the. This colour change enables the quantification of the combined anti-oxidant capacity of the anti-oxidant molecules present in the 100mg mesocarp tissue sample. Prior to measurement, a fresh FRAP reagent solution (300 mM/L sodium acetate buffer pH3.6, 10mM/L TPTZ in 40 mM/L HCl and 20 mM/L $FeCl_3.6H_2O$) was prepared in a ratio of 10:1:1. A 30 µL aliquot of the sample was mixed with 900 µL of the FRAP reagent solution and absorbance readings taken at 593nm after 10 minutes. Results were expressed as mg $FeSO_4.7H_2O$ g^{-1} DW equivalents.

2.5 AsA analysis

Ascorbic acid concentrations were determined according to Böhm *et al.* (2006) with slight modifications, using the colour reaction with 2,4-dinitrophenylhydrazine (DNPH). Briefly, 100 mg of exocarp sample was mixed with 5 mL 0.56 M metaphosphoric acid, vigorously shaken, centrifuged at 2988 x g for 5 minutes and the supernatant transferred into a volumetric flask. This procedure was repeated twice and the combined extracts made up to 20

mL using 0.56 M meta-phosphoric acid. Subsequently,200 µL of this extract were mixed with 300 µL 0.3M trichloroacetic acid, centrifuged at 17212 x g for 10 minutes. Subsamples of the supernatant (300 µL aliquots)were mixed with 100 µL 2,4-dinitrophenylhydrazine reagent (0.013 M in 30 % perchloric acid), and heated to 60°C for 1 hour and subsequently cooled in an ice bath for5 minutes. Thereafter, 400 mL 15.75 M sulphuric acid were added to the sampleand the absorbance read at 520 nm after 20 minutes. Absorbance readings from tissue extracts at 520nm were compared with values obtained from an L-ascorbic acid standard curve, and expressed in mg AsA g^{-1} DW.

2.6 Sugar analysis

Sugar concentrations were measured according to Liu *et al*. (2002). An isocratic HPLC system (LC – 20AT, Shimadzu Corporation, Kyoto, Japan) was used, equipped with a refractive index detector (RID – 10A, Shimadzu Corporation, Kyoto, Japan) and a Rezex RCM Monosaccharide column (300 mm x 7.8 mm) (8 micron pore size, Phenomenex®, Torrance, CA, USA). The mobile phase consisted of double-distilled water at a flow rate of 0.6 mL/min. Individual sugar concentrations were determined by comparison with mannoheptulose and perseitol sugar standards (Glycoteam GmbH, Hamburg, Germany).

2.7. Statistical analysis

Statistical analyses were conducted using GenStat® version 12.1 (VSN International, Hemel Hempstead, UK). The data collected were statistically analysed in the form of a factorial design. Each treatment combination consisted of three samples, each constituting a single replication. Analysis of variance and Least Significant Difference (LSD) values were used to identify significantly different treatment combinations at a confidence level of 95%.

3. RESULTS

Baseline measurements were taken from ten fruit, for the three harvest dates, after packhouse treatments and one day of cold storage at 5.5°C (Table 12). These values were taken in order to give an indication of how the measured parameters differed as the season progressed, without the influence of the storage period. Values of TAOC, AsA and sugars decreased as the season progressed.

3.1. Total anti-oxidant capacity in the exocarp

3.1.1. 28 day storage

The TAOC of the exocarp was significantly affected by harvest date ($P<0.001$), as well as by the interaction between storage temperature and harvest date ($P=0.044$). Early-season fruit had a significantly higher TAOC than mid- and late-season fruit (Table 13). The TAOC of fruit not treated with 1-MCP and stored at 5.5°C was significantly lower than that of 1-MCP treated fruit at 5.5°C and fruit stored at 2°C (Figure 28).

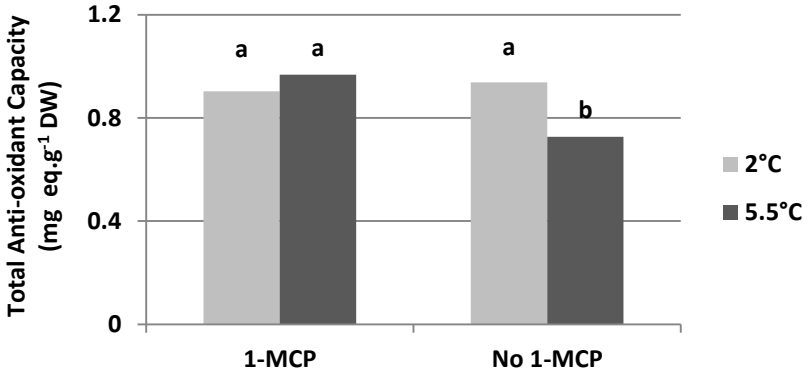

Figure 28: Mean TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW) extracted from the exocarp of 1-MCP treated and untreated 'Fuerte' avocados following 28 days of cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments ($P\leq0.05$). LSD = 0.1893.

3.1.2. Cold chain breaks and delays

The TAOC found in the exocarp was significantly affected by breaks ($P=0.008$), as well as by the interaction between harvest date and cold chain breaks ($P=0.021$). Both the delay in cooling and the break during storage resulted in significantly lower TAOC than the control, particularly in early-season fruit (Figure 29). Treatment effects on the delay in cooling and

cold chain break were inconsistent through the season and, due to the complexity of the treatment structure and the variation in TAOC between individual fruit no significant differences could be identified (Table 14).

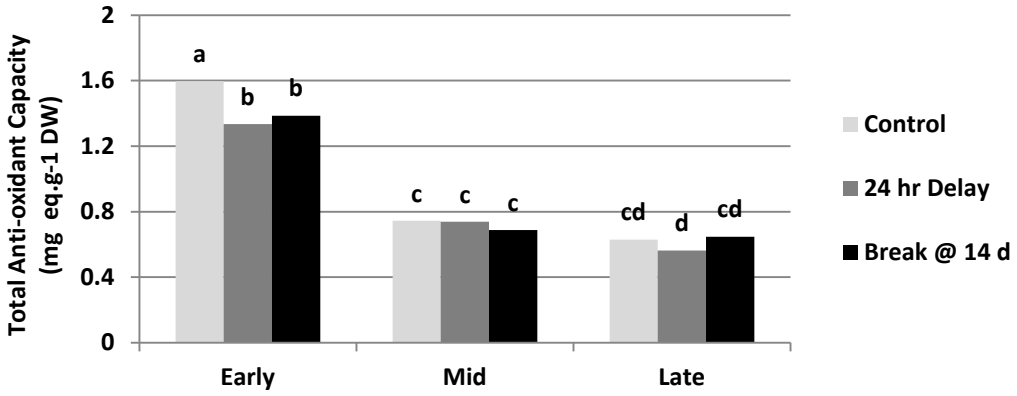

Figure 29: Effect of cold chain breaks on the mean TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW) extracted from the exocarp of 'Fuerte' avocados of different harvest dates following 28 days of cold storage. Different letters indicate significant differences between treatments ($P \leq 0.05$). LSD = 0.1423.

3.1.3. 56 day storage

The TAOC in the exocarp of fruit after 56 days of cold storage was generally lower than after 28 days, shown in the decreased grand mean TAOC from 0.88 mg $FeSO_4.7H_2O$ g^{-1} DW equivalents to 0.84 mg $FeSO_4.7H_2O$ g^{-1} DW equivalents after 56 day storage.

Similar to 28 day cold storage, early-season fruit had a significantly higher TAOC than mid- and late-season fruit ($P<0.001$; Table 15) after 56 days of cold storage. Lowering the storage temperature as well as waxing had a tendency to lower the TAOC compared with higher temperature storage and non-waxed fruit (Table 15). The use of 1-MCP resulted in a significantly lower TAOC early in the season ($P=0.010$), particularly of non-waxed fruit ($P<0.001$) (Table 15).

3.2. Ascorbic acid concentration in the exocarp

3.2.1. 28 day storage

The concentration of AsA found in the exocarp was affected by harvest date (P<0.001) and 1-MCP (P=0.039) as well as their interaction (P=0.009). Treatment of fruit with 1-MCP resulted in a significantly higher concentration of AsA, particularly in the mid- and late-season fruit (Figure 30); the exocarp AsA concentration declined with the progressing season (Figure 30). Storage at 5.5°C had a tendency to maintain AsA concentrations less effectively than 2°C throughout the storage period, particularly when fruit were not treated with 1-MCP (P=0.004; Figure 31). Waxing had a tendency to maintain higher concentrations of AsA through the storage period, particularly when waxed fruit stored at 2°C are compared with non-waxed fruit stored at 5.5°C and not treated with 1-MCP (P=0.001), which had the lowest concentrations of AsA in all harvest dates (Table 13).

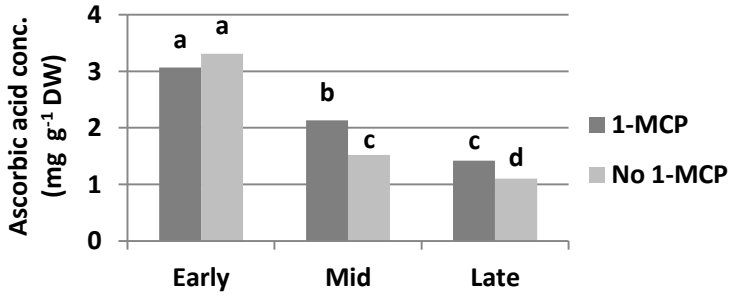

Figure 30: Mean ascorbic acid concentration (mg g^{-1} DW)extracted from the exocarp of 1-MCP treated and untreated 'Fuerte' avocados of different harvest dates following28 days cold storage. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.3784.

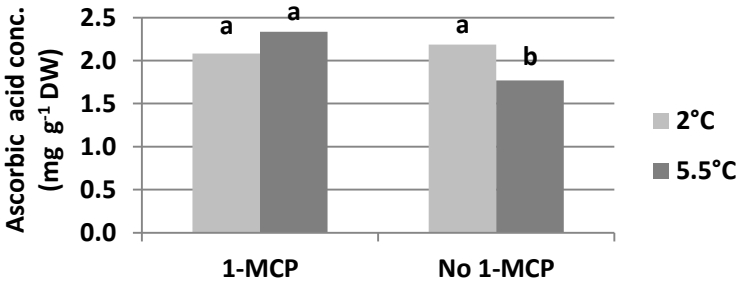

Figure 31: Ascorbic acid concentration (mg g^{-1} DW)extracted from the exocarp of 1-MCP treated and untreated 'Fuerte' avocados following 28 days cold storage at 2°C or 5.5°C. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.309.

3.2.2. Cold chain breaks and delays

The cold chain break and the delay in cooling had a tendency to reduce the concentration of AsA in the exocarp compared with the control, particularly early in the season. The delay in cold storage resulted in a significantly reduced AsA concentration in early-season fruit, while the break during storage resulted in a significantly reduced AsA concentration in early- and mid-season fruit (P=0.045; Figure 32). Lowering the storage temperature had a tendency to minimise the reduction in AsA concentration following breaks in the cold chain, compared with a storage temperature of 5.5^{o}C (Figure 33). The amount of AsA decline following a break during storage, was significantly reduced by waxing in early- and mid-season fruit (P=0.034; Table 14).

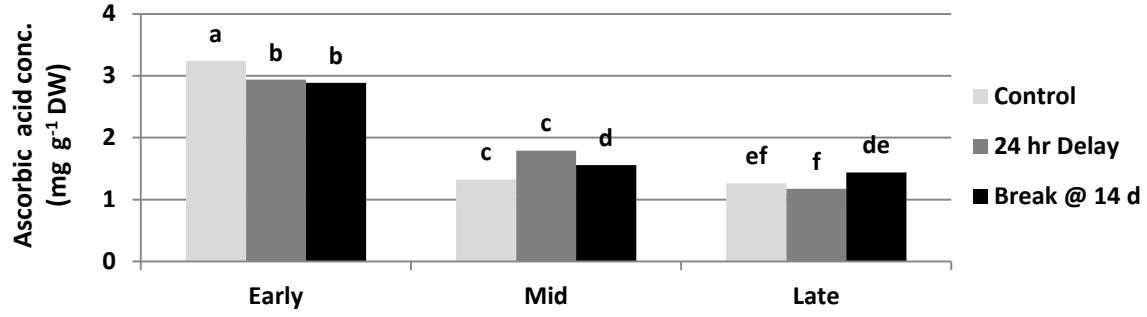

Figure 32: The effect of a delay in cooling and a cold chain break on the ascorbic acid concentration (mg g^{-1} DW)extracted from the exocarp of 'Fuerte' avocado of different harvest dates following 28 days of cold storage. Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.2924.

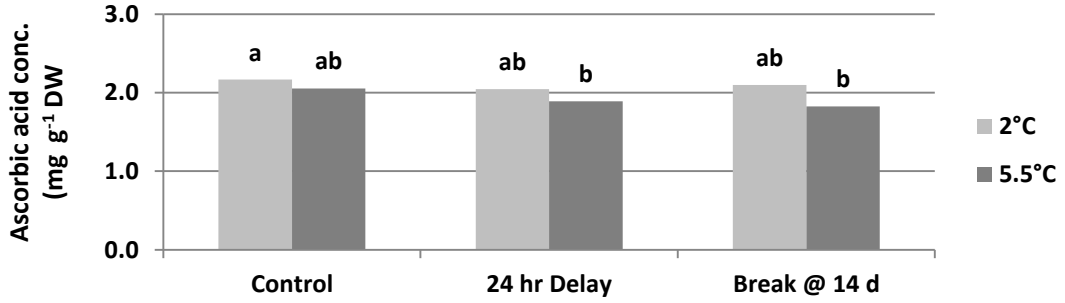

Figure 33: The effect of a delay in cooling and a cold chain break on the ascorbic acid concentration (mg g^{-1} DW)extracted from the exocarp of 'Fuerte' avocados following 28 days of cold storage at 2^{o}C or 5.5^{o}C.Different letters indicate significant differences between treatments (P≤0.05). LSD = 0.2387.

3.2.3. 56 day storage

The concentration of AsA remaining in the exocarp of fruit after 56 days of cold storage was generally lower than after 28 days, shown in the significant decrease in the grand mean AsA concentration from 2.09 mg/ml after 28 days to 1.76 mg/ml after 56 days.

The concentration of AsA found in the exocarp was significantly affected by harvest date ($P<0.001$) and the interaction between harvest date and temperature ($P=0.035$) over the 56 day storage period. As found following 28 day storage, early-season fruit had a significantly higher concentration of AsA than mid- and late-season fruit, at either storage temperature. Early-season fruit stored at 5.5°C had a significantly lower rind concentration of AsA than those stored at 2°C (Figure 34).

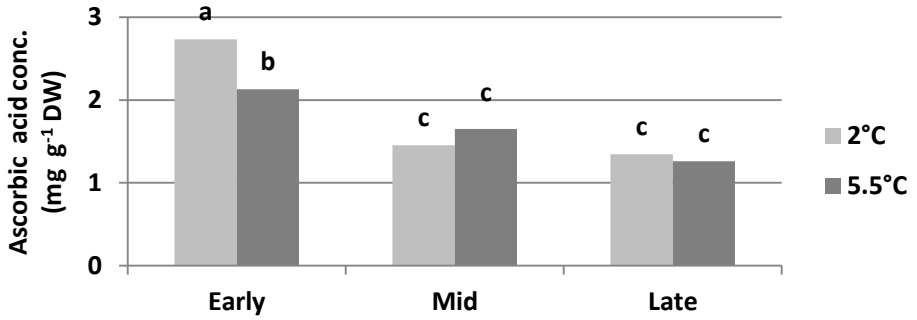

Figure 34: Ascorbic acid concentration (mg g^{-1} DW)extracted from the exocarp of 'Fuerte' avocado of different harvest dates following56 days cold storage. Different letters indicate significant differences between treatments ($P\leq0.05$). LSD = 0.431.

3.3. Mesocarp Sugar concentration

Mannoheptulose and perseitol concentrations after 28 days of cold storage had a tendency to decrease in the mesocarp tissue as the season progressed (Table 13). Reducing the storage temperature, 1-MCP treatment and waxing had a tendency to maintain mannoheptulose concentrations (Figure 35).

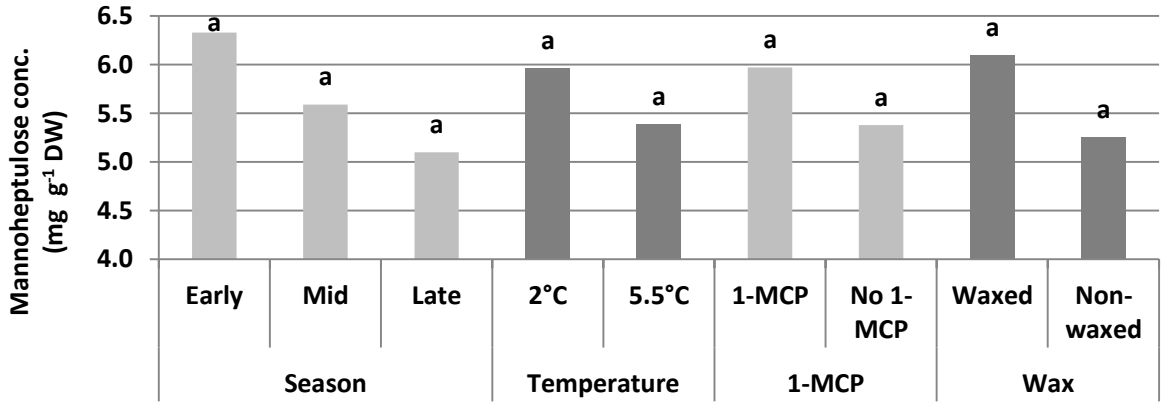

Figure 35: Mannoheptulose concentration (mg g^{-1} DW)of 'Fuerte' avocado mesocarp following 28 days cold storage. Different letters indicate significant differences for each treatment ($P \leq 0.05$). LSD (harvest date) = 1.62; LSD (temperature) = 1.323; LSD (1-MCP) = 1.323; LSD (wax) = 1.323.

The cold chain break during storage resulted in a significant reduction of mannoheptulose concentration (P=0.036); however, no treatments had a significant effect on either C7 sugar after such a break (data not shown). Similar trends to those found after 28 days of cold storage, were noticed for mannoheptulose and perseitol concentrations after 56 days, although levels of these sugars were generally lower after 56 than 28 days of storage (Table 13 and 15).

4. DISCUSSION

4.1. Anti-oxidants in the exocarp

The TAOC and AsA concentration in the exocarp showed similar trends through the season (Table 12), both decreasing as the season progressed, as was the case with these two measurements in the seed and exocarp found by Tesfay *et al*. (2010a). Anti-oxidants were found to be highest early in the season (Table 12), when fruit are most susceptible to ECI (Toerien, 1986). Early-season, non-waxed fruit stored at 2^{o}C resulted in a slightly higher TAOC and higher levels of AsA than waxed fruit at this temperature (Table 13), suggesting that the increased water loss associated with non-waxed fruit resulted in an increased stress level therefore the enhanced production of anti-oxidants. This physiological response of the fruit is presumably intended to balance the presence of anti-oxidants and harmful ROS, enabling the fruit to scavenge harmful radicals and reduce the effects of cold stress. Results suggest that the TAOC of the exocarp increases as the post-harvest stress is increased, to counter the dramatic increase in ROS production under stress conditions (Mittler, 2002).

Reducing storage temperatures, 1-MCP use and waxing all tended to maintain higher levels of anti-oxidants in the exocarp. It would be expected that higher levels of stress (i.e., lower storage temperature) result in increased production of ROS, therefore a greater usage of anti-oxidants to counteract these species. However, it appears that the anti-oxidant concentrations did not decline to the same degree in the lower as high temperature storage. This suggests that the lower storage temperature did not result in an increase in ROS levels. A further explanation could be that the usage of anti-oxidants is reduced at this lower temperature resulting in a significantly higher TAOC (Figure 28) and AsA concentration (Figure 31) than at a storage temperature of 5.5^{o}C without the use of 1-MCP and a storage temperature of 5.5^{o}C, respectively. This could be explained by the reduced metabolic activity of the fruit under these treatments, which possibly reduces the use of anti-oxidants and enzyme activity in cold storage. Such treatment combinations favoured fruit softening during storage and include a higher storage temperature without the use of 1-MCP (Figure 28 and 31), mid- and late-season fruit not treated with 1-MCP (Figure 30 and 34) and the cold chain breaks when fruit were stored at 5.5^{o}C (Figure 29 and 33). Conversely, fruit which received treatments that minimized metabolic activity during storage, tended to maintain higher levels of TAOC and AsA, however, upon removal from storage, and subsequent increase in metabolic activity of

the fruit, these anti-oxidants possibly decrease in concentration as they attempt to scavenge the harmful compounds caused during stress.

The break during storage resulted in a significantly lower TAOC and AsA concentration in early-season fruit (Figure 29 and 32), possibly because these values were higher in the early-season fruit and thus treatments could have a significant effect as opposed to late-season fruit where anti-oxidant levels were low. However, lowering the storage temperature (Figure 33) and waxing, particularly at 2^{o}C (Table 14), were able to minimise the reduction in AsA concentrations following a break in the cold chain, confirming the effect of storage temperature on negating effects of a cold chain break (chapter 3).

Due to the single anti-oxidant measurement after storage, it cannot be determined if anti-oxidants in early-season fruit were successfully reducing the level of ECI. Bertling *et al*. (2007) noted that AsA variations between samples were too high to distinguish significant differences between certain tissues. The fairly low occurrences of ECI found in this study, also makes it difficult to conclude a definite correlation.

4.2. Sugars in the mesocarp

The pool of C7 sugars is finite and as it is related to post-harvest quality (Tesfay *et al.*, 2010b), needs to be preserved during post-harvest handling and storage so that they can efficiently supply the required energy for ripening and the anti-oxidant benefits, firstly, to the fruit to minimise oxidative damage during cold storage, and secondly, to the consumer.

Several trends were detected, in agreement with previous findings (Bertling *et al*., 2007; Blakey *et al*., 2009; Tesfay *et al.*, 2010a; 2010b), with the most significant effect being the decline in sugars as the season progressed, as reported by Liu *et al*. (1999) and Meyer and Terry (2010) in 'Hass' avocados. Liu *et al*. (1999) as well as Landahl *et al.* (2009) also reported that mannoheptulose concentrations decreased during cold storage. Liu *et al*. (1999) proposed that this vital sugar is used as a carbon energy source for respiration during cold storage. Results suggest that reducing the storage temperature from 5.5^{o}C to 2^{o}C allowed for better preservation of the limited pool of C7 sugars found in the mesocarp (Figure 34). Further, treatments which delayed ripening during storage more effectively, tended to reduce the consumption of C7 sugars during storage (Figure 34). Similarly, Meyer and Terry (2010) found that significantly more mannohepulose was present and higher concentrations of perseitol were maintained with use of 1-MCP than without. This preservation of energy

reserves and anti-oxidant capacity of the mesocarp may be one of the primary reasons for the increase in shelf-life noted under these treatments (Chapter 2).

Tesfay *et al*. (2010b) proposed that perseitol is also the main storage carbohydrate which is easily converted to mannoheptulose, a compound identified as an energy source in the mesocarp, and importantly, the main transport sugar (Tesfay *et al.,* 2010b). Tesfay *et al.* (2010a) reported that the concentration of mannoheptulose in the mesocarp of 'Hass' avocados was higher than perseitol at the time of harvest, as was found in this study after one day of cold storage at 5.5^{o}C (Table 12). The mannoheptulose concentration detected after removal from cold storage was similar, and in some cases lower, than that of perseitol, suggesting that mannoheptulose, being the main transport sugar, may be utilised more easily than perseitol during cold storage. This is supported by the findings of Liu *et al*. (1999), who reported that perseitol concentrations did not decrease during cold storage but did decrease significantly during ripening, thus, as more energy is required during ripening, it appears that mannoheptulose is used up during storage and then perseitol is utilized, when mannoheptulose has been depleted. Thus, by the time measurements were taken, mannoheptulose concentrations were low and had a minimal effect on fruit quality.

The results were not as conclusive as anticipated, and results do not indicate that sugars in the mesocarp after storage correlate to treatment effects on fruit quality. This might be due to the stage of sampling (i.e., after cold storage) which may not have been ideal. Therefore, it is suggested that future studies monitor the decrease in sugars, particularly mannoheptulose, over the entire storage period, when levels of these sugars are still high and treatment effects can be identified. Further, fewer treatment combinations and increased replications would be beneficial in reducing the variation shown to be a problem in various studies on avocados.

5. CONCLUSION

Maintaining high levels of anti-oxidants in the exocarp will be vital in countering cold stress, particularly due to the likely stress involved in cold disinfestation treatments and the prevention of ECI under these conditions. The overall occurrence of external damage and internal quality defects were fairly low during this experiment, thus no conclusive results were obtained with respect to the ability of anti-oxidants in the exocarp and sugars in the mesocarp to reduce or prevent chilling damage and internal disorders, respectively. Further research into the effects of different storage treatments and temperatures on anti-oxidant levels in both the exocarp and mesocarp, are required. Particular focus on the continuous measurement of AsA and C7 sugars throughout the storage as well as the ripening period is required to elucidate how these anti-oxidants are used by the fruit to counter the damaging compounds following cold exposure.

Table 12: Mean total anti-oxidant capacity (TAOC) and concentrations of ascorbic acid(AsA) in the exocarp, and concentrations of mannoheptulose and perseitol in the mesocarp of 'Fuerte' avocados after packhouse treatment and one day of cold storage at 5.5°C (n=10). *

Harvest date	TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW)	AsA conc. (mg g^{-1} DW)	Mannoheptulose conc. (mg g^{-1} DW)	Perseitol conc. (mg g^{-1} DW)
Early	1.64	3.15	11.86	10.54
Mid	1.53	2.92	9.88	8.06
Late	0.63	1.78	7.04	5.62

* Each point represents the mean value for the same 10 fruit in each harvest date.

Table 13: Seasonal changes in total anti-oxidant capacity (TAOC) and ascorbic acid (AsA) concentration in the exocarp, and mannoheptulose and perseitol concentrations in the mesocarp of 'Fuerte' avocado fruit upon removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

Treatment				TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW)	AsA (mg g^{-1} DW)	Mannoheptulose (mg g^{-1} DW)	Perseitol (mg g^{-1} DW)
Early	2°C	1-MCP	waxed	1.38 bc	2.86 bcde	8.38 a	6.03 cde
			Non-waxed	1.73 abc	3.07 bcde	7.57 a	5.28 cde
		No 1-MCP	waxed	1.61 abc	3.57 ab	5.30 a	4.54 de
			Non-waxed	1.78 ab	3.83 a	5.13 a	13.75 ab
	5.5°C	1-MCP	waxed	1.89 a	3.16 abcd	7.30 a	5.34 cde
			Non-waxed	1.31 c	3.19 abcd	5.48 a	5.57 cde
		No 1-MCP	waxed	1.35 bc	3.30 abc	7.00 a	7.01 bcde
			Non-waxed	1.51 abc	2.54 defg	4.49 a	8.66 abcde
Mid	2°C	1-MCP	waxed	0.71 d	1.57 ijk	6.16 a	5.31 cde
			Non-waxed	0.53 de	2.38 efgh	5.42 a	2.11 e
		No 1-MCP	waxed	0.48 de	1.85 ghijk	5.26 a	4.64 de
			Non-waxed	0.72 d	1.36 ijkl	7.51 a	9.75 abcd
	5.5°C	1-MCP	waxed	0.74 d	1.88 ghij	4.52 a	6.17 cde
			Non-waxed	0.67 d	2.70 cdef	4.92 a	4.92 cde
		No 1-MCP	waxed	0.72 d	1.76 hijk	6.63 a	5.12 cde
			Non-waxed	0.28 de	1.10 kl	4.33 a	12.10 abc
Late	2°C	1-MCP	waxed	0.60 d	1.41 ijkl	5.63 a	5.48 cde
			Non-waxed	0.46 de	1.21 ijkl	4.84 a	9.70 abcd
		No 1-MCP	waxed	0.54 de	1.20 ijkl	5.76 a	7.44 abcde
			Non-waxed	0.50 de	1.29 ijkl	4.55 a	9.21 abcde
	5.5°C	1-MCP	waxed	0.52 de	1.12 jkl	6.66 a	14.47 a
			Non-waxed	0.68 d	1.96 fghi	4.77 a	6.24 cde
		No 1-MCP	waxed	0.38 de	1.16 jkl	4.59 a	6.49 cde
			Non-waxed	0.12 e	0.76 l	4.03 a	10.60 abcd
LSD				0.4638	0.7569	4.583	7.222

* Each point represents the mean of 3 fruit.

Different letters indicate significant differences within each column.

Table 14: The effect of cold chain breaks on total anti-oxidant capacity (TAOC) and ascorbic acid (AsA) concentrations in the exocarp of 'Fuerte' avocado fruit after removal from cold storage. Fruit were stored for 28 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated, waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

				TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW)			AsA (mg g^{-1} DW)		
Treatments				control	24 hr Delay	Break @ 14	control	24 hr Delay	Break @ 14
Early	2°C	1-MCP	waxed	1.378 a	0.983 a	1.317 a	2.856 a	2.585 a	3.112 a
			Non-waxed	1.731 a	0.871 b	1.516 a	3.456 a	2.485 b	2.468 b
		No 1-MCP	waxed	1.608 a	1.625 a	1.605 a	3.574 a	3.595 a	3.771 a
			Non-waxed	1.781 a	1.565 a	1.395 a	3.828 a	4.023 a	2.61 b
	5.5°C	1-MCP	waxed	1.887 a	1.139 b	1.016 b	3.18 a	1.992 b	2.686 a
			Non-waxed	1.307 a	1.372 a	1.185 a	3.189 a	2.725 a	3.189 a
		No 1-MCP	waxed	1.352 a	1.421 a	1.394 a	3.301 a	3.314 a	2.456 b
			Non-waxed	1.509 a	1.218 a	1.226 a	2.536 a	2.765 a	2.775 a
Mid	2°C	1-MCP	waxed	0.711 a	0.678 a	0.527 a	1.574 a	2.129 a	1.477 a
			Non-waxed	0.534 a	0.625 a	0.745 a	2.381 a	2.081 a	1.822 a
		No 1-MCP	waxed	0.480 a	0.647 a	0.654 a	1.854 a	2.044 a	2.502 a
			Non-waxed	0.719 a	0.613 a	0.294 b	1.364 a	1.184 a	1.102 a
	5.5°C	1-MCP	waxed	0.736 a	0.448 a	0.946 b	1.877 a	1.625 a	2.114 a
			Non-waxed	0.666 a	0.347 a	0.277 a	2.697 a	1.883 a	1.023 b
		No 1-MCP	waxed	0.715 a	0.712 a	0.624 a	1.761 a	1.386 a	1.383 a
			Non-waxed	0.284 a	0.722 b	0.275 a	1.104 a	2.004 b	1.047 a
Late	2°C	1-MCP	waxed	0.603 a	0.667 a	0.669 a	1.405 a	1.523 a	1.674 a
			Non-waxed	0.462 a	0.299 a	0.573 a	1.206 a	1.062 a	1.561 a
		No 1-MCP	waxed	0.543 a	0.455 a	0.424 a	1.199 a	1.125 a	1.112 a
			Non-waxed	0.496 a	0.132 a	0.450 a	1.294 a	0.716 a	1.939 a
	5.5°C	1-MCP	waxed	0.523 a	0.406 a	0.481 a	1.121 a	1.246 a	1.299 a
			Non-waxed	0.682 a	0.403 a	0.576 a	1.955 a	0.972 b	1.61 ab
		No 1-MCP	waxed	0.381 a	0.451 a	0.557 a	1.157 a	1.277 a	0.96 a
			Non-waxed	0.118 a	0.390 a	0.243 a	0.761 a	1.477 a	1.343 a
LSD across =				0.4024			0.827		

* Each point represents the mean of 3 fruit.

Different letters indicate significant differences between the cold chain breaks and the control for that treatment combination.

Table 15: Seasonal changes in total anti-oxidant capacity (TAOC) and ascorbic acid (AsA) concentration in the exocarp, and mannoheptulose and perseitol concentrations in the mesocarp of 'Fuerte' avocado fruit upon removal from cold storage. Fruit were stored for 56 days in regular atmosphere at 2°C and 5.5°C, treated with 1-MCP or untreated and waxed or non-waxed. Fruit were harvested thrice from a commercial orchard in Wartburg (cool, subtropical climate). *

Treatment				TAOC (mg $FeSO_4.7H_2O$ g^{-1} DW)	AsA (mg g^{-1} DW)	Manno-heptulose (mg g^{-1} DW)	Perseitol (mg g^{-1} DW)
Early	2°C	1-MCP	waxed	1.36 cd	2.34 bcd	-	-
			Non-waxed	1.38 cd	2.56 ab	-	-
		No 1-MCP	waxed	1.16 cde	2.74 ab	-	-
			Non-waxed	2.02 a	3.30 a	-	-
	5.5°C	1-MCP	waxed	1.48 bc	2.39 bc	-	-
			Non-waxed	1.01 def	1.62 cdefgh	-	-
		No 1-MCP	waxed	1.52 bc	2.27 bcde	-	-
			Non-waxed	1.88 ab	2.24 bcdef	-	-
Mid	2°C	1-MCP	waxed	0.56 gh	1.51 defgh	5.57 a	6.46 d
			Non-waxed	0.60 fgh	1.26 gh	4.47 ab	9.78 bcd
		No 1-MCP	waxed	0.52 gh	1.63 cdefgh	5.45 ab	8.45 d
			Non-waxed	0.52 gh	1.42 efgh	4.31 ab	9.76 bcd
	5.5°C	1-MCP	waxed	0.58 gh	1.41 efgh	2.87 bcd	24.34 a
			Non-waxed	1.04 de	2.21 bcdef	4.91 ab	9.07 cd
		No 1-MCP	waxed	0.83 efg	1.97 bcdefg	5.64 a	6.30 d
			Non-waxed	0.52 gh	1.02 h	3.8 abc	7.94 d
Late	2°C	1-MCP	waxed	0.48 gh	1.33 gh	1.16 cd	16.12 b
			Non-waxed	0.28 h	1.25 gh	1.36 cd	11.97 bcd
		No 1-MCP	waxed	0.45 gh	1.41 fgh	1.58 cd	11.08 bcd
			Non-waxed	0.30 h	1.39 fgh	1.35 cd	12.14 bcd
	5.5°C	1-MCP	waxed	0.61 fgh	1.28 gh	1.07 d	9.06 cd
			Non-waxed	0.21 h	1.10 gh	1.08 d	15.00 bc
		No 1-MCP	waxed	0.52 gh	1.54 cdefgh	3.79 abc	8.51 cd
			Non-waxed	0.22 h	1.11 gh	1.29 cd	6.38 d
LSD				0.4208	0.8619	2.697	6.528

* Each point represents the mean of 3 fruit.

Different letters indicate significant differences within each column.

Note: Early-season mesocarp samples (-) for the 56 day experiment were not analysed as the freeze-drier was interfered with by unauthorised students and samples had to be discarded, after the possibility for re-sampling.

CHAPTER 6
GENERAL DISCUSSION, OUTLOOK AND CONCLUSION

6.1 General Discussion

Cold storage is critical to the maintenance of high quality fresh produce during long distance transport; however, it results in stress (Wills *et al*., 2007). Stress can be divided into four main categories: metabolic stress, water stress, mechanical injury stress and microbial damage (Wills *et al*., 2007), of which the first two categories were most prominent in this study. Minimising physiological changes and metabolic activity of fruit, to maintain energy reserves and quality, is the main aim of cold storage and the treatments used in conjunction with cold storage. All treatments which reduced the metabolic activity of the fruit, seen in the decreased fruit softening and mass loss, also resulted in decreased usage of the main carbohydrates in the mesocarp, namely, mannoheptulose and perseitol. This metabolic activity can either be suppressed by modifying the atmosphere (CA or 1-MCP) or by lowering the storage temperature; however, this suppression of metabolic activity needs to be managed carefully as altering the normal metabolism of the fruit may lead to metabolic stress (Wills *et al*., 2007), either through normal metabolism (e.g. respiration-induced carbohydrate shortage) or abnormal metabolism (e.g. cold-induced imbalances in enzyme activity).

It appears that 1-MCP is an invaluable tool when 'ideal' shipping temperatures or cold chain maintenance are not optimal. If temperatures are not correctly adjusted according to fruit maturity or fruit suffer moisture loss as a result of cold chain breaks or delays, 1-MCP allows storage over the normal period and provides adequate fruit quality. Fruit treated with 1-MCP softened less during storage and took a longer time to ripen, as was expected. Most importantly, a storage temperature of 2^{o}C could be an effective alternative to the use of 1-MCP at a storage temperature of 5.5^{o}C.

Therefore, there is a definite potential for storing 'Fuerte' fruit at 2^{o}C. Storage at 2^{o}C for 28 days results in less fruit softening, less moisture loss, and provides a longer ripening time than storing fruit at 5.5^{o}C (Chapter 2), while maintaining levels of carbohydrate reserves in the mesocarp and anti-oxidants in the exocarp (Chapter 5), thus maintaining good internal and external quality,

respectively. Further, waxing of fruit stored at 2°C, minimises water loss and subsequent ECI suffered by the thin-skinned 'Fuerte' and provides a shelf-life similar to that of fruit stored at 5.5°C and treated with 1-MCP. Lowering the storage temperature to 2°C minimises physiological changes during storage effectively, and could be a valuable tool for marketers and exporters, particularly later in the season when "soft arrivals" are more likely and susceptibility to external damage is lower than early in the season.

Avocados are very susceptible to post-harvest quality losses and require extreme care when handling, packaging, storing and distributing this valuable commodity to the consumer. Although a number of treatments used to maintain post-harvest quality are technologically advanced (1-MCP and CA), one of the main solutions to improved quality is simple to implement at farm level. This study has highlighted the importance of cooling fruit immediately after harvest and ensuring that fruit do not stand in the packhouse, waiting to be packed and cooled, losing considerable amounts of moisture. Results indicate that a cold chain break during storage is detrimental to the quality and shelf-life of 'Fuerte' avocados, but also can cause problems associated with the condensation of water on the fruit, including higher microorganism growth, an increase in chilling injury (through evaporative cooling) and weakening of cartons, all of which will result in financial loss. Importantly, the detrimental effect of cold chain breaks on fruit softening is completely negated by lowering the storage temperature (Chapter 3), a treatment which tends to reduce the consumption of storage carbohydrates (Chapter 5) following the increased metabolic activity caused by these breaks.

Ultimately, 2°C has the potential to replace 1-MCP, but the subsequent external chilling injury, for a greenskin like 'Fuerte', needs to be minimised. Temperature pre-conditioning and fruit packaging appear to be the most practical and viable methods to reduce ECI, and may be substantially cheaper than the cost of using 1-MCP. Waxing reduces water loss and fruit softening during storage (Chapter 2), reduces the consumption of carbohydrate reserves (Chapter 5), and also minimises the effect of cold chain breaks on these parameters (Chapter 3) and improves the appearance of 'Fuerte' avocados. However, Bower and Magwaza (2004) found that polypropylene packaging was more effective than waxing in reducing ECI. If the combination of ECI mitigation and storage at 2°C can be achieved, the South African avocado industry may be able to successfully ship avocados without the need for 1-MCP, and could potentially realise

substantial savings in shipping costs. The other alternative for 'Fuerte' avocados is to continue using 1-MCP and storage temperatures of 5.5^{o}C,whereas, the ECI in non-greenskins, such as 'Hass', is immaterial after colour change, and thus storage temperatures of lower than present protocol could be used successfully with slightly less risk.

Only mid-season fruit had acceptable internal and external quality after cold storage at 2^{o}C for 56 days. The extended storage period magnified the important correlation between stressed fruit and poor final fruit quality in terms of internal and external quality. Fruit stored at 5.5^{o}C, softened significantly more during cold storage and subsequently suffered significantly higher levels of ECI; increasing in severity as the season progressed and fruit maturity increased. If a shipping consignment were to be delayed for a lengthy period, as was the case in 2010 due to strikes at the port, it is suggested that serious consideration be given to lowering the temperatures in the containers, given that fruit are not early-season fruit. Ultimately, a 56 day storage period is not recommended due to the high risk of external damage, but if required, 2^{o}C in conjunction with 1-MCP would be recommended.

6.2 Future Research

The use of mitigating techniques for ECI, particularly temperature pre-conditioning, in conjunction with storage temperatures of 2^{o}C, needs to be more thoroughly investigated. Research into new waxes needs to be conducted, given the recent banning of compounds found in previously successful waxes. Such research should also aim to settle the debate of the importance of waxes in the different cultivars and how waxes influence the gaseous exchange of various cultivars, given their different skin characteristics, and the effect of this gaseous exchange on fruit ripening and quality.

Lenticel damage is a form of external damage which is "plaguing" South African exporters on the European market (Nelson, 2005). Research into the severity of lenticel damage at ultra-low storage temperatures as well as the effect of delaying cooling over a range of different time periods would be beneficial to the industry to determine how best to manage this problem through the harvest season. Hydro-cooling of avocados needs to be investigated as this method of cooling removes field heat rapidly and causes minimal moisture loss from the fruit. Packhouses which already use post-harvest dips for the control of pathological disorder or use

water to lift fruit from picking lugs, to reduce lenticel damage through tipping fruit onto the packline, could incorporate these operations with simultaneous hydro-cooling and entry onto the packline, with minimal delays. In the future, investigations into the required rate of cooling, minimising pathogen spread in the water used for hydro-cooling and the feasibility of hydro-cooling in South Africa should be carried out.

Continued focus on maintaining the post-harvest levels of C7 sugars in the mesocarp as well as maintaining important anti-oxidants in the exocarp may provide important insights into the prevention of physiological disorders and the maintenance of high fruit quality. Treatments which reduce the consumption of these sugars appear to result in better fruit quality and shelf-life than treatments which allow for even low levels of metabolic activity during storage. Future studies should monitor the decrease in mannoheptulose and perseitol over the entire storage period, when levels of these sugars are still high and treatment effects can be identified. Fewer treatment combinations and increased replications would be beneficial in reducing the variation shown to be a problem in various studies on avocados. A further study on the effect of ultra-low temperature on the eating quality (flavour and aroma) is recommended, as suppressing the climacteric may affect the taste of avocados, as was reported for 1-MCP in other fruit (Wills *et al*., 2007).

The acceptance of cold sterilization as a phytosanitary control treatment by the USA, Japan and China is likely to only be realised in several years, due mainly to inter-governmental negotiations. During this time, a successful mitigating treatment or combination of treatments, to reduce the external damage of ‘Fuerte’ avocados following ultra-low temperature storage, needs to be presented, including large numbers of experimental units and fruit from numerous production regions and seasons to deal with the high variability found in avocados. If this is not achieved, ‘Fuerte’ avocados are unlikely to be sent to these new markets as ‘Hass’ would be the more likely exported cultivar, given its ability to mask external damage with its colour change during ripening.

6.3. Conclusion

'Fuerte' avocados can be successfully stored at 2^{o}C for 28 days without negatively impacting on the internal and external quality of the fruit, particularly when water loss during storage is reduced. This lower temperature negates the softening effects of cold chain breaks, and tends to maintain the levels of C7 sugars in the mesocarp and anti-oxidants in the exocarp, better than 1-MCP at 5.5^{o}C, seemingly reducing the metabolic stress placed on fruit stored at insufficiently low temperatures. Water loss plays a vital role in ECI and is enhanced by a cold chain break or delay which results in decreased fruit quality and should be avoided. A storage period of 56 days is not recommended; however, a storage temperature of 2^{o}C provides better fruit quality than 5.5^{o}C, and 1-MCP helps carry fruit through this extended storage period without detrimental effects on fruit quality.

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APPENDICES

Appendix 1 – ANOVA table for fruit softening (Chapter 2)

Variate: **% fruit softening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	68.92	7.66	0.68	
Reps.*Units* stratum					
Harvest date	2	442.64	221.32	19.73	<.001
Temp	1	448.14	448.14	39.96	<.001
%1_MCP	1	271.85	271.85	24.24	<.001
wax	1	16.00	16.00	1.43	0.234
Harvest date.Temp	2	8.59	4.30	0.38	0.682
Harvest date.%1_MCP	2	18.98	9.49	0.85	0.431
Temp.%1_MCP	1	209.45	209.45	18.67	<.001
Harvest date.wax	2	25.30	12.65	1.13	0.326
Temp.wax	1	0.01	0.01	0.00	0.971
%1_MCP.wax	1	25.05	25.05	2.23	0.137
Harvest date.Temp.%1_MCP	2	13.52	6.76	0.60	0.548
Harvest date.Temp.wax	2	11.74	5.87	0.52	0.593
Harvest date.%1_MCP.wax	2	47.13	23.57	2.10	0.125
Temp.%1_MCP.wax	1	3.65	3.65	0.33	0.569
Harvest date.Temp.%1_MCP.wax	2	30.27	15.14	1.35	0.262
Residual	207	2321.71	11.22		
Total	239	3962.95			

Appendix 2 – ANOVA table for mass loss (Chapter 2)

Variate: **% Mass loss**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	3.9780	0.4420	0.83	
Reps.*Units* stratum					
Harvest date	2	90.6561	45.3281	85.51	<.001
Temp	1	46.7047	46.7047	88.11	<.001
%1_MCP	1	0.5367	0.5367	1.01	0.315
wax	1	22.1320	22.1320	41.75	<.001
Harvest date.Temp	2	6.5186	3.2593	6.15	0.003
Harvest date.%1_MCP	2	1.1743	0.5872	1.11	0.332
Temp.%1_MCP	1	0.3981	0.3981	0.75	0.387
Harvest date.wax	2	17.6850	8.8425	16.68	<.001
Temp.wax	1	0.7339	0.7339	1.38	0.241
%1_MCP.wax	1	0.0075	0.0075	0.01	0.905
Harvest date.Temp.%1_MCP	2	3.3427	1.6714	3.15	0.045
Harvest date.Temp.wax	2	2.6827	1.3414	2.53	0.082
Harvest date.%1_MCP.wax	2	0.8859	0.4429	0.84	0.435
Temp.%1_MCP.wax	1	0.3013	0.3013	0.57	0.452
Harvest date.Temp.%1_MCP.wax	2	0.4264	0.2132	0.40	0.669
Residual	207	109.7228	0.5301		
Total	239	307.8867			

Appendix 3 – ANOVA table for DTR (Chapter 2)

Variate: **Days-to-ripening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	78.121	8.680	1.75	
Reps.*Units* stratum					
Harvest date	2	321.600	160.800	32.37	<.001
Temp	1	537.004	537.004	108.09	<.001
%1_MCP	1	1228.537	1228.537	247.29	<.001
wax	1	49.504	49.504	9.96	0.002
Harvest date.Temp	2	15.633	7.817	1.57	0.210
Harvest date.%1_MCP	2	75.100	37.550	7.56	<.001
Temp.%1_MCP	1	44.204	44.204	8.90	0.003
Harvest date.wax	2	2.033	1.017	0.20	0.815
Temp.wax	1	0.938	0.938	0.19	0.664
%1_MCP.wax	1	6.337	6.337	1.28	0.260
Harvest date.Temp.%1_MCP	2	5.033	2.517	0.51	0.603
Harvest date.Temp.wax	2	46.300	23.150	4.66	0.010
Harvest date.%1_MCP.wax	2	11.100	5.550	1.12	0.329
Temp.%1_MCP.wax	1	17.604	17.604	3.54	0.061
Harvest date.Temp.%1_MCP.wax	2	25.733	12.867	2.59	0.077
Residual	207	1028.379	4.968		
Total	239	3493.163			

Appendix 4 – ANOVA table for ECI (Chapter 2)

Variate: **External Chilling Injury (0-10)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	3.7708	0.4190	0.81	
Reps.*Units* stratum					
Harvest date	2	4.2750	2.1375	4.12	0.018
Temp	1	0.7042	0.7042	1.36	0.245
%1_MCP	1	0.1042	0.1042	0.20	0.654
wax	1	0.1042	0.1042	0.20	0.654
Harvest date.Temp	2	2.5583	1.2792	2.47	0.087
Harvest date.%1_MCP	2	0.8583	0.4292	0.83	0.438
Temp.%1_MCP	1	0.1042	0.1042	0.20	0.654
Harvest date.wax	2	0.3583	0.1792	0.35	0.708
Temp.wax	1	0.3375	0.3375	0.65	0.421
%1_MCP.wax	1	1.2042	1.2042	2.32	0.129
Harvest date.Temp.%1_MCP	2	1.3083	0.6542	1.26	0.285
Harvest date.Temp.wax	2	0.9750	0.4875	0.94	0.392
Harvest date.%1_MCP.wax	2	0.0083	0.0042	0.01	0.992
Temp.%1_MCP.wax	1	0.5042	0.5042	0.97	0.325
Harvest date.Temp.%1_MCP.wax	2	0.0583	0.0292	0.06	0.945
Residual	207	107.3292	0.5185		
Total	239	124.5625			

Appendix 5 – ANOVA table for anthracnose incidence (Chapter 2)

Variate: **anthracnose (0-5)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	0.58750	0.06528	0.82	
Reps.*Units* stratum					
Harvest date	2	0.15833	0.07917	0.99	0.372
Temp	1	0.03750	0.03750	0.47	0.494
%1_MCP	1	0.20417	0.20417	2.56	0.111
wax	1	0.03750	0.03750	0.47	0.494
Harvest date.Temp	2	0.07500	0.03750	0.47	0.626
Harvest date.%1_MCP	2	0.15833	0.07917	0.99	0.372
Temp.%1_MCP	1	0.03750	0.03750	0.47	0.494
Harvest date.wax	2	0.32500	0.16250	2.04	0.133
Temp.wax	1	0.03750	0.03750	0.47	0.494
%1_MCP.wax	1	0.03750	0.03750	0.47	0.494
Harvest date.Temp.%1_MCP	2	0.07500	0.03750	0.47	0.626
Harvest date.Temp.wax	2	0.07500	0.03750	0.47	0.626
Harvest date.%1_MCP.wax	2	0.32500	0.16250	2.04	0.133
Temp.%1_MCP.wax	1	0.03750	0.03750	0.47	0.494
Harvest date.Temp.%1_MCP.wax	2	0.07500	0.03750	0.47	0.626
Residual	207	16.51250	0.07977		
Total	239	18.79583			

Appendix 6 – ANOVA table for stem-end rot incidence (Chapter 2)

Variate: stem_end_rot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	0.31667	0.03519	0.88	
Reps.*Units* stratum					
Harvest date	2	0.23333	0.11667	2.92	0.056
Temp	1	0.01667	0.01667	0.42	0.519
%1_MCP	1	0.01667	0.01667	0.42	0.519
wax	1	0.15000	0.15000	3.75	0.054
Harvest date.Temp	2	0.03333	0.01667	0.42	0.660
Harvest date.%1_MCP	2	0.03333	0.01667	0.42	0.660
Temp.%1_MCP	1	0.06667	0.06667	1.67	0.198
Harvest date.wax	2	0.10000	0.05000	1.25	0.289
Temp.wax	1	0.00000	0.00000	0.00	1.000
%1_MCP.wax	1	0.06667	0.06667	1.67	0.198
Harvest date.Temp.%1_MCP	2	0.13333	0.06667	1.67	0.192
Harvest date.Temp.wax	2	0.10000	0.05000	1.25	0.289
Harvest date.%1_MCP.wax	2	0.13333	0.06667	1.67	0.192
Temp.%1_MCP.wax	1	0.01667	0.01667	0.42	0.519
Harvest date.Temp.%1_MCP.wax	2	0.03333	0.01667	0.42	0.660
Residual	207	8.28333	0.04002		
Total	239	9.73333			

Appendix 7 – ANOVA table for mesocarp discolouration (Chapter 2)

Variate: Mesocarp_discolouration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	0.83750	0.09306	1.33	
Reps.*Units* stratum					
Harvest date	2	0.00833	0.00417	0.06	0.942
Temp	1	0.00417	0.00417	0.06	0.807
%1_MCP	1	0.03750	0.03750	0.54	0.465
wax	1	0.03750	0.03750	0.54	0.465
Harvest date.Temp	2	0.20833	0.10417	1.49	0.228
Harvest date.%1_MCP	2	0.17500	0.08750	1.25	0.288
Temp.%1_MCP	1	0.03750	0.03750	0.54	0.465
Harvest date.wax	2	0.17500	0.08750	1.25	0.288
Temp.wax	1	0.03750	0.03750	0.54	0.465
%1_MCP.wax	1	0.20417	0.20417	2.92	0.089
Harvest date.Temp.%1_MCP	2	0.17500	0.08750	1.25	0.288
Harvest date.Temp.wax	2	0.17500	0.08750	1.25	0.288
Harvest date.%1_MCP.wax	2	0.00833	0.00417	0.06	0.942
Temp.%1_MCP.wax	1	0.00417	0.00417	0.06	0.807
Harvest date.Temp.%1_MCP.wax	2	0.20833	0.10417	1.49	0.228
Residual	207	14.46250	0.06987		
Total	239	16.79583			

Appendix 8 – ANOVA table for vascular browning incidence (Chapter 2)

Variate: vascular_browning

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	0.67083	0.07454	0.94	
Reps.*Units* stratum					
Harvest date	2	0.05833	0.02917	0.37	0.693
Temp	1	0.03750	0.03750	0.47	0.493
%1_MCP	1	0.03750	0.03750	0.47	0.493
wax	1	0.03750	0.03750	0.47	0.493
Harvest date.Temp	2	0.17500	0.08750	1.10	0.334
Harvest date.%1_MCP	2	0.17500	0.08750	1.10	0.334
Temp.%1_MCP	1	0.03750	0.03750	0.47	0.493
Harvest date.wax	2	0.17500	0.08750	1.10	0.334
Temp.wax	1	0.03750	0.03750	0.47	0.493
%1_MCP.wax	1	0.20417	0.20417	2.57	0.110
Harvest date.Temp.%1_MCP	2	0.22500	0.11250	1.42	0.245
Harvest date.Temp.wax	2	0.22500	0.11250	1.42	0.245
Harvest date.%1_MCP.wax	2	0.05833	0.02917	0.37	0.693
Temp.%1_MCP.wax	1	0.03750	0.03750	0.47	0.493
Harvest date.Temp.%1_MCP.wax	2	0.17500	0.08750	1.10	0.334
Residual	207	16.42917	0.07937		
Total	239	18.79583			

Appendix 9 – ANOVA table for percentage sound fruit (Chapter 3)

Variate: **percentage sound fruit**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	9	7708.3	856.5	1.51	
Reps.*Units* stratum					
Harvest date	2	3250.0	1625.0	2.87	0.059
Temp	1	1041.7	1041.7	1.84	0.177
%1_MCP	1	375.0	375.0	0.66	0.417
wax	1	375.0	375.0	0.66	0.417
Harvest date.Temp	2	83.3	41.7	0.07	0.929
Harvest date.%1_MCP	2	250.0	125.0	0.22	0.802
Temp.%1_MCP	1	375.0	375.0	0.66	0.417
Harvest date.wax	2	2250.0	1125.0	1.99	0.140
Temp.wax	1	41.7	41.7	0.07	0.787
%1_MCP.wax	1	2041.7	2041.7	3.60	0.059
Harvest date.Temp.%1_MCP	2	1750.0	875.0	1.54	0.216
Harvest date.Temp.wax	2	1083.3	541.7	0.96	0.386
Harvest date.%1_MCP.wax	2	2583.3	1291.7	2.28	0.105
Temp.%1_MCP.wax	1	41.7	41.7	0.07	0.787
Harvest date.Temp.%1_MCP.wax	2	83.3	41.7	0.07	0.929
Residual	207	117291.7	566.6		
Total	239	140625.0			

Appendix 10 – ANOVA table for fruit softening (Chapter 3)

Variate: **% fruit softening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	42.46	4.72	0.32		
Reps.*Units* stratum						
Harvest date	2	574.97	287.49	19.79	<.001	
Break	2	261.94	130.97	9.02	<.001	
Temp	1	2874.43	2874.43	197.89	<.001	
%1_MCP	1	1343.58	1343.58	92.50	<.001	
wax	1	146.24	146.24	10.07	0.002	
Harvest date.Break	4	562.89	140.72	9.69	<.001	
Harvest date.Temp	2	266.57	133.28	9.18	<.001	
Break.Temp	2	158.48	79.24	5.46	0.004	
Harvest date.%1_MCP	2	187.73	93.87	6.46	0.002	
Break.%1_MCP	2	101.99	51.00	3.51	0.030	
Temp.%1_MCP	1	1296.18	1296.18	89.23	<.001	
Harvest date.wax	2	29.42	14.71	1.01	0.364	
Break.wax	2	42.94	21.47	1.48	0.229	
Temp.wax	1	13.90	13.90	0.96	0.328	
%1_MCP.wax	1	0.15	0.15	0.01	0.919	
Harvest date.Break.Temp	4	469.28	117.32	8.08	<.001	
Harvest date.Break.%1_MCP	4	211.75	52.94	3.64	0.006	
Harvest date.Temp.%1_MCP	2	51.26	25.63	1.76	0.172	
Break.Temp.%1_MCP	2	169.70	84.85	5.84	0.003	
Harvest date.Break.wax	4	42.44	10.61	0.73	0.571	
Harvest date.Temp.wax	2	110.62	55.31	3.81	0.023	
Break.Temp.wax	2	8.85	4.43	0.30	0.737	
Harvest date.%1_MCP.wax	2	258.51	129.26	8.90	<.001	
Break.%1_MCP.wax	2	89.83	44.92	3.09	0.046	
Temp.%1_MCP.wax	1	46.85	46.85	3.23	0.073	
Harvest date.Break.Temp.%1_MCP	4	262.63	65.66	4.52	0.001	
Harvest date.Break.Temp.wax	4	158.94	39.74	2.74	0.028	
Harvest date.Break.%1_MCP.wax	4	134.85	33.71	2.32	0.056	
Harvest date.Temp.%1_MCP.wax	2	130.12	65.06	4.48	0.012	
Break.Temp.%1_MCP.wax	2	75.57	37.78	2.60	0.075	
Harvest date.Break.Temp.%1_MCP.wax	4	233.93	58.48	4.03	0.003	
Residual	639	9281.95	14.53			
Total	719	19640.94				

Appendix 11 – ANOVA table for mass loss (Chapter 3)

Variate: **% Mass loss**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	27.054	3.006	1.81		
Reps.*Units* stratum						
Harvest date	2	276.102	138.051	83.11	<.001	
Break	2	113.289	56.644	34.10	<.001	
Temp	1	194.582	194.582	117.14	<.001	
%1_MCP	1	0.764	0.764	0.46	0.498	
wax	1	81.115	81.115	48.83	<.001	
Harvest date.Break	4	19.429	4.857	2.92	0.021	
Harvest date.Temp	2	6.238	3.119	1.88	0.154	
Break.Temp	2	2.967	1.484	0.89	0.410	
Harvest date.%1_MCP	2	4.052	2.026	1.22	0.296	
Break.%1_MCP	2	3.341	1.670	1.01	0.366	
Temp.%1_MCP	1	10.285	10.285	6.19	0.013	
Harvest date.wax	2	12.001	6.001	3.61	0.028	
Break.wax	2	4.428	2.214	1.33	0.264	
Temp.wax	1	1.397	1.397	0.84	0.359	
%1_MCP.wax	1	0.487	0.487	0.29	0.588	
Harvest date.Break.Temp	4	7.087	1.772	1.07	0.372	
Harvest date.Break.%1_MCP	4	0.772	0.193	0.12	0.977	
Harvest date.Temp.%1_MCP	2	6.573	3.286	1.98	0.139	
Break.Temp.%1_MCP	2	4.548	2.274	1.37	0.255	
Harvest date.Break.wax	4	35.469	8.867	5.34	<.001	
Harvest date.Temp.wax	2	8.123	4.062	2.45	0.088	
Break.Temp.wax	2	1.794	0.897	0.54	0.583	
Harvest date.%1_MCP.wax	2	1.295	0.648	0.39	0.677	
Break.%1_MCP.wax	2	6.099	3.049	1.84	0.160	
Temp.%1_MCP.wax	1	0.032	0.032	0.02	0.890	
Harvest date.Break.Temp.%1_MCP	4	11.441	2.860	1.72	0.143	
Harvest date.Break.Temp.wax	4	7.353	1.838	1.11	0.352	
Harvest date.Break.%1_MCP.wax	4	5.312	1.328	0.80	0.526	
Harvest date.Temp.%1_MCP.wax	2	3.542	1.771	1.07	0.345	
Break.Temp.%1_MCP.wax	2	5.801	2.900	1.75	0.175	
Harvest date.Break.Temp.%1_MCP.wax	4	11.901	2.975	1.79	0.129	
Residual	639	1061.442	1.661			
Total	719	1936.118				

Appendix 12 – ANOVA table for DTR (Chapter 3)

Variate: **Days-to-ripening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	127.144	14.127	3.09		
Reps.*Units* stratum						
Harvest date	2	259.744	129.872	28.39	<.001	
Break	2	245.686	122.843	26.85	<.001	
Temp	1	1366.756	1366.756	298.76	<.001	
%1_MCP	1	3537.800	3537.800	773.33	<.001	
wax	1	213.422	213.422	46.65	<.001	
Harvest date.Break	4	547.689	136.922	29.93	<.001	
Harvest date.Temp	2	20.278	10.139	2.22	0.110	
Break.Temp	2	20.786	10.393	2.27	0.104	
Harvest date.%1_MCP	2	176.400	88.200	19.28	<.001	
Break.%1_MCP	2	18.775	9.387	2.05	0.129	
Temp.%1_MCP	1	15.606	15.606	3.41	0.065	
Harvest date.wax	2	18.344	9.172	2.00	0.136	
Break.wax	2	10.186	5.093	1.11	0.329	
Temp.wax	1	0.939	0.939	0.21	0.651	
%1_MCP.wax	1	1.250	1.250	0.27	0.601	
Harvest date.Break.Temp	4	17.156	4.289	0.94	0.442	
Harvest date.Break.%1_MCP	4	48.500	12.125	2.65	0.032	
Harvest date.Temp.%1_MCP	2	0.744	0.372	0.08	0.922	
Break.Temp.%1_MCP	2	39.119	19.560	4.28	0.014	
Harvest date.Break.wax	4	7.622	1.906	0.42	0.797	
Harvest date.Temp.wax	2	60.678	30.339	6.63	0.001	
Break.Temp.wax	2	13.919	6.960	1.52	0.219	
Harvest date.%1_MCP.wax	2	2.633	1.317	0.29	0.750	
Break.%1_MCP.wax	2	5.508	2.754	0.60	0.548	
Temp.%1_MCP.wax	1	2.689	2.689	0.59	0.444	
Harvest date.Break.Temp.%1_MCP	4	58.456	14.614	3.19	0.013	
Harvest date.Break.Temp.wax	4	39.489	9.872	2.16	0.072	
Harvest date.Break.%1_MCP.wax	4	52.733	13.183	2.88	0.022	
Harvest date.Temp.%1_MCP.wax	2	6.978	3.489	0.76	0.467	
Break.Temp.%1_MCP.wax	2	30.886	15.443	3.38	0.035	
Harvest date.Break.Temp.%1_MCP.wax	4	55.222	13.806	3.02	0.018	
Residual	639	2923.256	4.575			
Total	719	9946.394				

Appendix 13 – ANOVA table for ECI (Chapter 3)

Variate: **External Chilling Injury (0-10)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	6.7833	0.7537	1.01		
Reps.*Units* stratum						
Harvest date	2	17.0528	8.5264	11.43	<.001	
Break	2	4.8028	2.4014	3.22	0.041	
Temp	1	9.3389	9.3389	12.52	<.001	
%1_MCP	1	0.0500	0.0500	0.07	0.796	
wax	1	0.0000	0.0000	0.00	1.000	
Harvest date.Break	4	1.4139	0.3535	0.47	0.755	
Harvest date.Temp	2	13.2528	6.6264	8.88	<.001	
Break.Temp	2	2.8028	1.4014	1.88	0.154	
Harvest date.%1_MCP	2	0.3250	0.1625	0.22	0.804	
Break.%1_MCP	2	2.3250	1.1625	1.56	0.211	
Temp.%1_MCP	1	0.2722	0.2722	0.36	0.546	
Harvest date.wax	2	8.1750	4.0875	5.48	0.004	
Break.wax	2	0.7583	0.3792	0.51	0.602	
Temp.wax	1	3.7556	3.7556	5.03	0.025	
%1_MCP.wax	1	0.0889	0.0889	0.12	0.730	
Harvest date.Break.Temp	4	2.5306	0.6326	0.85	0.495	
Harvest date.Break.%1_MCP	4	4.6250	1.1562	1.55	0.186	
Harvest date.Temp.%1_MCP	2	0.0361	0.0181	0.02	0.976	
Break.Temp.%1_MCP	2	0.9361	0.4681	0.63	0.534	
Harvest date.Break.wax	4	6.3917	1.5979	2.14	0.074	
Harvest date.Temp.wax	2	19.0861	9.5431	12.79	<.001	
Break.Temp.wax	2	1.5361	0.7681	1.03	0.358	
Harvest date.%1_MCP.wax	2	3.5361	1.7681	2.37	0.094	
Break.%1_MCP.wax	2	3.3361	1.6681	2.24	0.108	
Temp.%1_MCP.wax	1	0.2000	0.2000	0.27	0.605	
Harvest date.Break.Temp.%1_MCP	4	1.9306	0.4826	0.65	0.629	
Harvest date.Break.Temp.wax	4	3.5972	0.8993	1.21	0.307	
Harvest date.Break.%1_MCP.wax	4	3.0139	0.7535	1.01	0.402	
Harvest date.Temp.%1_MCP.wax	2	1.7583	0.8792	1.18	0.308	
Break.Temp.%1_MCP.wax	2	0.3250	0.1625	0.22	0.804	
Harvest date.Break.Temp.%1_MCP.wax	4	5.5417	1.3854	1.86	0.116	
Residual	639	476.8167	0.7462			
Total	719	606.3944				

Appendix 14 – ANOVA table for anthracnose incidence (Chapter 3)

Variate: **anthracnose (0-5)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	0.18889	0.02099	0.58		
Reps.*Units* stratum						
Harvest date	2	0.10833	0.05417	1.49	0.226	
Break	2	0.05833	0.02917	0.80	0.448	
Temp	1	0.00000	0.00000	0.00	1.000	
%1_MCP	1	0.20000	0.20000	5.51	0.019	
wax	1	0.02222	0.02222	0.61	0.434	
Harvest date.Break	4	0.08333	0.02083	0.57	0.682	
Harvest date.Temp	2	0.07500	0.03750	1.03	0.357	
Break.Temp	2	0.07500	0.03750	1.03	0.357	
Harvest date.%1_MCP	2	0.10833	0.05417	1.49	0.226	
Break.%1_MCP	2	0.05833	0.02917	0.80	0.448	
Temp.%1_MCP	1	0.00000	0.00000	0.00	1.000	
Harvest date.wax	2	0.21944	0.10972	3.02	0.049	
Break.wax	2	0.03611	0.01806	0.50	0.609	
Temp.wax	1	0.02222	0.02222	0.61	0.434	
%1_MCP.wax	1	0.02222	0.02222	0.61	0.434	
Harvest date.Break.Temp	4	0.05000	0.01250	0.34	0.848	
Harvest date.Break.%1_MCP	4	0.08333	0.02083	0.57	0.682	
Harvest date.Temp.%1_MCP	2	0.07500	0.03750	1.03	0.357	
Break.Temp.%1_MCP	2	0.07500	0.03750	1.03	0.357	
Harvest date.Break.wax	4	0.17222	0.04306	1.19	0.316	
Harvest date.Temp.wax	2	0.01944	0.00972	0.27	0.765	
Break.Temp.wax	2	0.01944	0.00972	0.27	0.765	
Harvest date.%1_MCP.wax	2	0.21944	0.10972	3.02	0.049	
Break.%1_MCP.wax	2	0.03611	0.01806	0.50	0.609	
Temp.%1_MCP.wax	1	0.02222	0.02222	0.61	0.434	
Harvest date.Break.Temp.%1_MCP	4	0.05000	0.01250	0.34	0.848	
Harvest date.Break.Temp.wax	4	0.13889	0.03472	0.96	0.431	
Harvest date.Break.%1_MCP.wax	4	0.17222	0.04306	1.19	0.316	
Harvest date.Temp.%1_MCP.wax	2	0.01944	0.00972	0.27	0.765	
Break.Temp.%1_MCP.wax	2	0.01944	0.00972	0.27	0.765	
Harvest date.Break.Temp.%1_MCP.wax	4	0.13889	0.03472	0.96	0.431	
Residual	639	23.21111	0.03632			
Total	719	25.80000				

Appendix 15 – ANOVA table for stem-end rot incidence (Chapter 3)

Variate: stem_end_rot

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	0.31111	0.03457	1.44		
Reps.*Units* stratum						
Harvest date	2	0.16944	0.08472	3.54	0.030	
Break	2	0.07778	0.03889	1.63	0.198	
Temp	1	0.05000	0.05000	2.09	0.149	
%1_MCP	1	0.05000	0.05000	2.09	0.149	
wax	1	0.05000	0.05000	2.09	0.149	
Harvest date.Break	4	0.15556	0.03889	1.63	0.166	
Harvest date.Temp	2	0.05833	0.02917	1.22	0.296	
Break.Temp	2	0.00000	0.00000	0.00	1.000	
Harvest date.%1_MCP	2	0.05833	0.02917	1.22	0.296	
Break.%1_MCP	2	0.00000	0.00000	0.00	1.000	
Temp.%1_MCP	1	0.00556	0.00556	0.23	0.630	
Harvest date.wax	2	0.05833	0.02917	1.22	0.296	
Break.wax	2	0.13333	0.06667	2.79	0.062	
Temp.wax	1	0.00556	0.00556	0.23	0.630	
%1_MCP.wax	1	0.00556	0.00556	0.23	0.630	
Harvest date.Break.Temp	4	0.01667	0.00417	0.17	0.952	
Harvest date.Break.%1_MCP	4	0.11667	0.02917	1.22	0.301	
Harvest date.Temp.%1_MCP	2	0.03611	0.01806	0.75	0.471	
Break.Temp.%1_MCP	2	0.07778	0.03889	1.63	0.198	
Harvest date.Break.wax	4	0.18333	0.04583	1.92	0.106	
Harvest date.Temp.wax	2	0.13611	0.06806	2.84	0.059	
Break.Temp.wax	2	0.01111	0.00556	0.23	0.793	
Harvest date.%1_MCP.wax	2	0.00278	0.00139	0.06	0.944	
Break.%1_MCP.wax	2	0.14444	0.07222	3.02	0.050	
Temp.%1_MCP.wax	1	0.00556	0.00556	0.23	0.630	
Harvest date.Break.Temp.%1_MCP	4	0.15556	0.03889	1.63	0.166	
Harvest date.Break.Temp.wax	4	0.02222	0.00556	0.23	0.920	
Harvest date.Break.%1_MCP.wax	4	0.22222	0.05556	2.32	0.055	
Harvest date.Temp.%1_MCP.wax	2	0.03611	0.01806	0.75	0.471	
Break.Temp.%1_MCP.wax	2	0.04444	0.02222	0.93	0.396	
Harvest date.Break.Temp.%1_MCP.wax	4	0.03889	0.00972	0.41	0.804	
Residual	639	15.28889	0.02393			
Total	719	17.72778				

Appendix 16 – ANOVA table for mesocarp discolouration (Chapter 3)

Variate: Mesocarp_discolouration

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	0.34583	0.03843	1.47		
Reps.*Units* stratum						
Harvest date	2	0.00833	0.00417	0.16	0.853	
Break	2	0.10833	0.05417	2.07	0.128	
Temp	1	0.00139	0.00139	0.05	0.818	
%1_MCP	1	0.00139	0.00139	0.05	0.818	
wax	1	0.01250	0.01250	0.48	0.490	
Harvest date.Break	4	0.03333	0.00833	0.32	0.866	
Harvest date.Temp	2	0.05278	0.02639	1.01	0.366	
Break.Temp	2	0.01944	0.00972	0.37	0.690	
Harvest date.%1_MCP	2	0.11944	0.05972	2.28	0.103	
Break.%1_MCP	2	0.05278	0.02639	1.01	0.366	
Temp.%1_MCP	1	0.00139	0.00139	0.05	0.818	
Harvest date.wax	2	0.05833	0.02917	1.11	0.329	
Break.wax	2	0.02500	0.01250	0.48	0.621	
Temp.wax	1	0.01250	0.01250	0.48	0.490	
%1_MCP.wax	1	0.06806	0.06806	2.60	0.108	
Harvest date.Break.Temp	4	0.18889	0.04722	1.80	0.127	
Harvest date.Break.%1_MCP	4	0.08889	0.02222	0.85	0.495	
Harvest date.Temp.%1_MCP	2	0.05278	0.02639	1.01	0.366	
Break.Temp.%1_MCP	2	0.05278	0.02639	1.01	0.366	
Harvest date.Break.wax	4	0.11667	0.02917	1.11	0.350	
Harvest date.Temp.wax	2	0.05833	0.02917	1.11	0.329	
Break.Temp.wax	2	0.02500	0.01250	0.48	0.621	
Harvest date.%1_MCP.wax	2	0.00278	0.00139	0.05	0.948	
Break.%1_MCP.wax	2	0.13611	0.06806	2.60	0.075	
Temp.%1_MCP.wax	1	0.00139	0.00139	0.05	0.818	
Harvest date.Break.Temp.%1_MCP	4	0.15556	0.03889	1.48	0.206	
Harvest date.Break.Temp.wax	4	0.11667	0.02917	1.11	0.350	
Harvest date.Break.%1_MCP.wax	4	0.00556	0.00139	0.05	0.995	
Harvest date.Temp.%1_MCP.wax	2	0.06944	0.03472	1.32	0.267	
Break.Temp.%1_MCP.wax	2	0.00278	0.00139	0.05	0.948	
Harvest date.Break.Temp.%1_MCP.wax	4	0.13889	0.03472	1.32	0.259	
Residual	639	16.75417	0.02622			
Total	719	18.88750				

Appendix 17 – ANOVA table for vascular browning incidence (Chapter 3)

Variate: vascular_browning

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	0.57778	0.06420	1.55		
Reps.*Units* stratum						
Harvest date	2	0.05833	0.02917	0.71	0.494	
Break	2	0.05833	0.02917	0.71	0.494	
Temp	1	0.00556	0.00556	0.13	0.714	
%1_MCP	1	0.08889	0.08889	2.15	0.143	
wax	1	0.00000	0.00000	0.00	1.000	
Harvest date.Break	4	0.10833	0.02708	0.65	0.624	
Harvest date.Temp	2	0.00278	0.00139	0.03	0.967	
Break.Temp	2	0.08611	0.04306	1.04	0.354	
Harvest date.%1_MCP	2	0.11944	0.05972	1.44	0.237	
Break.%1_MCP	2	0.00278	0.00139	0.03	0.967	
Temp.%1_MCP	1	0.00556	0.00556	0.13	0.714	
Harvest date.wax	2	0.00833	0.00417	0.10	0.904	
Break.wax	2	0.07500	0.03750	0.91	0.404	
Temp.wax	1	0.05000	0.05000	1.21	0.272	
%1_MCP.wax	1	0.02222	0.02222	0.54	0.464	
Harvest date.Break.Temp	4	0.28056	0.07014	1.70	0.149	
Harvest date.Break.%1_MCP	4	0.16389	0.04097	0.99	0.412	
Harvest date.Temp.%1_MCP	2	0.05278	0.02639	0.64	0.529	
Break.Temp.%1_MCP	2	0.08611	0.04306	1.04	0.354	
Harvest date.Break.wax	4	0.24167	0.06042	1.46	0.212	
Harvest date.Temp.wax	2	0.17500	0.08750	2.12	0.121	
Break.Temp.wax	2	0.02500	0.01250	0.30	0.739	
Harvest date.%1_MCP.wax	2	0.00278	0.00139	0.03	0.967	
Break.%1_MCP.wax	2	0.21944	0.10972	2.65	0.071	
Temp.%1_MCP.wax	1	0.05000	0.05000	1.21	0.272	
Harvest date.Break.Temp.%1_MCP	4	0.28056	0.07014	1.70	0.149	
Harvest date.Break.Temp.wax	4	0.12500	0.03125	0.76	0.554	
Harvest date.Break.%1_MCP.wax	4	0.13056	0.03264	0.79	0.532	
Harvest date.Temp.%1_MCP.wax	2	0.15833	0.07917	1.91	0.148	
Break.Temp.%1_MCP.wax	2	0.02500	0.01250	0.30	0.739	
Harvest date.Break.Temp.%1_MCP.wax	4	0.09167	0.02292	0.55	0.696	
Residual	639	26.42222	0.04135			
Total	719	29.80000				

Appendix 18 – ANOVA table for percentage sound fruit (Chapter 3)

Variate: **percentage sound fruit**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	9	5013.9	557.1	1.58		
Reps.*Units* stratum						
Harvest date	2	3083.3	1541.7	4.36	0.013	
Temp	1	680.6	680.6	1.92	0.166	
%1_MCP	1	1125.0	1125.0	3.18	0.075	
wax	1	125.0	125.0	0.35	0.552	
Break	2	2333.3	1166.7	3.30	0.038	
Harvest date.Temp	2	361.1	180.6	0.51	0.600	
Harvest date.%1_MCP	2	1083.3	541.7	1.53	0.217	
Temp.%1_MCP	1	13.9	13.9	0.04	0.843	
Harvest date.wax	2	250.0	125.0	0.35	0.702	
Temp.wax	1	125.0	125.0	0.35	0.552	
%1_MCP.wax	1	347.2	347.2	0.98	0.322	
Harvest date.Break	4	1833.3	458.3	1.30	0.270	
Temp.Break	2	444.4	222.2	0.63	0.534	
%1_MCP.Break	2	333.3	166.7	0.47	0.624	
wax.Break	2	333.3	166.7	0.47	0.624	
Harvest date.Temp.%1_MCP	2	1027.8	513.9	1.45	0.235	
Harvest date.Temp.wax	2	1083.3	541.7	1.53	0.217	
Harvest date.%1_MCP.wax	2	694.4	347.2	0.98	0.375	
Temp.%1_MCP.wax	1	13.9	13.9	0.04	0.843	
Harvest date.Temp.Break	4	388.9	97.2	0.27	0.894	
Harvest date.%1_MCP.Break	4	333.3	83.3	0.24	0.918	
Temp.%1_MCP.Break	2	1444.4	722.2	2.04	0.131	
Harvest date.wax.Break	4	3166.7	791.7	2.24	0.063	
Temp.wax.Break	2	0.0	0.0	0.00	1.000	
%1_MCP.wax.Break	2	2111.1	1055.6	2.98	0.051	
Harvest date.Temp.%1_MCP.wax	2	194.4	97.2	0.27	0.760	
Harvest date.Temp.%1_MCP.Break	4	1888.9	472.2	1.34	0.255	
Harvest date.Temp.wax.Break	4	1166.7	291.7	0.82	0.510	
Harvest date.%1_MCP.wax.Break	4	2222.2	555.6	1.57	0.180	
Temp.%1_MCP.wax.Break	2	111.1	55.6	0.16	0.855	
Harvest date.Temp.%1_MCP.wax.Break	4	555.6	138.9	0.39	0.814	
Residual	639	225986.1	353.7			
Total	719	259875.0				

Appendix 19 – ANOVA table for fruit softening (Chapter 4)

Variate: **% fruit softening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	640.10	71.12	2.12	
Rep.*Units* stratum					
Harvest date	2	789.64	394.82	11.79	<.001
Temp	1	31578.60	31578.60	943.08	<.001
%1_MCP	1	3758.13	3758.13	112.24	<.001
wax	1	212.93	212.93	6.36	0.012
Harvest date.Temp	2	1566.08	783.04	23.39	<.001
Harvest date.%1_MCP	2	11.49	5.74	0.17	0.843
Temp.%1_MCP	1	2342.79	2342.79	69.97	<.001
Harvest date.wax	2	189.65	94.82	2.83	0.061
Temp.wax	1	105.39	105.39	3.15	0.078
%1_MCP.wax	1	102.65	102.65	3.07	0.081
Harvest date.Temp.%1_MCP	2	18.91	9.46	0.28	0.754
Harvest date.Temp.wax	2	171.56	85.78	2.56	0.080
Harvest date.%1_MCP.wax	2	156.47	78.23	2.34	0.099
Temp.%1_MCP.wax	1	114.40	114.40	3.42	0.066
Harvest date.Temp.%1_MCP.wax	2	247.68	123.84	3.70	0.026
Residual	207	6931.27	33.48		
Total	239	48937.74			

Appendix 20 – ANOVA table for mass loss (Chapter 4)

Variate: **% Mass loss**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	26.496	2.944	1.37	
Rep.*Units* stratum					
Harvest date	2	120.913	60.457	28.04	<.001
Temp	1	453.431	453.431	210.29	<.001
%1_MCP	1	0.546	0.546	0.25	0.615
wax	1	170.901	170.901	79.26	<.001
Harvest date.Temp	2	40.327	20.164	9.35	<.001
Harvest date.%1_MCP	2	4.708	2.354	1.09	0.338
Temp.%1_MCP	1	4.013	4.013	1.86	0.174
Harvest date.wax	2	23.457	11.728	5.44	0.005
Temp.wax	1	17.523	17.523	8.13	0.005
%1_MCP.wax	1	0.342	0.342	0.16	0.691
Harvest date.Temp.%1_MCP	2	2.543	1.272	0.59	0.555
Harvest date.Temp.wax	2	3.748	1.874	0.87	0.421
Harvest date.%1_MCP.wax	2	2.882	1.441	0.67	0.514
Temp.%1_MCP.wax	1	3.937	3.937	1.83	0.178
Harvest date.Temp.%1_MCP.wax	2	2.052	1.026	0.48	0.622
Residual	207	446.342	2.156		
Total	239	1324.163			

Appendix 21 – ANOVA table for DTR (Chapter 4)

Variate: **Days-to-ripening**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep.*Units* stratum	9	101.771	11.308	2.37	
Label.*Units* stratum					
Harvest date	2	221.858	110.929	23.23	<.001
Temp	1	1545.338	1545.338	323.60	<.001
%1_MCP	1	429.337	429.337	89.90	<.001
wax	1	161.704	161.704	33.86	<.001
Harvest date.Temp	2	2.725	1.363	0.29	0.752
Harvest date.%1_MCP	2	65.775	32.888	6.89	0.001
Temp.%1_MCP	1	1.837	1.837	0.38	0.536
Harvest date.wax	2	38.758	19.379	4.06	0.019
Temp.wax	1	22.204	22.204	4.65	0.032
%1_MCP.wax	1	3.038	3.038	0.64	0.426
Harvest date.Temp.%1_MCP	2	12.775	6.388	1.34	0.265
Harvest date.Temp.wax	2	25.258	12.629	2.64	0.073
Harvest date.%1_MCP.wax	2	21.475	10.737	2.25	0.108
Temp.%1_MCP.wax	1	55.104	55.104	11.54	<.001
Harvest date.Temp.%1_MCP.wax	2	13.908	6.954	1.46	0.235
Residual	207	988.529	4.776		
Total	239	3711.396			

Appendix 22 – ANOVA table for ECI (Chapter 4)

Variate: **External Chilling Injury (0-10)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	22.983	2.554	0.87	
Rep.*Units* stratum					
Harvest date	2	5.233	2.617	0.89	0.410
Temp	1	35.267	35.267	12.06	<.001
%1_MCP	1	88.817	88.817	30.37	<.001
wax	1	13.067	13.067	4.47	0.036
Harvest date.Temp	2	133.033	66.517	22.74	<.001
Harvest date.%1_MCP	2	19.033	9.517	3.25	0.041
Temp.%1_MCP	1	46.817	46.817	16.01	<.001
Harvest date.wax	2	18.233	9.117	3.12	0.046
Temp.wax	1	0.067	0.067	0.02	0.880
%1_MCP.wax	1	2.817	2.817	0.96	0.328
Harvest date.Temp.%1_MCP	2	9.033	4.517	1.54	0.216
Harvest date.Temp.wax	2	4.633	2.317	0.79	0.454
Harvest date.%1_MCP.wax	2	4.233	2.117	0.72	0.486
Temp.%1_MCP.wax	1	0.017	0.017	0.01	0.940
Harvest date.Temp.%1_MCP.wax	2	3.033	1.517	0.52	0.596
Residual	207	605.417	2.925		
Total	239	1011.733			

Appendix 23 – ANOVA table for anthracnose incidence (Chapter 4)

Variate: **anthracnose (0-5)**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	1.2333	0.1370	0.59	
Rep.*Units* stratum					
Harvest date	2	2.5333	1.2667	5.47	0.005
Temp	1	0.2667	0.2667	1.15	0.285
%1_MCP	1	0.2667	0.2667	1.15	0.285
wax	1	1.0667	1.0667	4.60	0.033
Harvest date.Temp	2	0.5333	0.2667	1.15	0.318
Harvest date.%1_MCP	2	0.5333	0.2667	1.15	0.318
Temp.%1_MCP	1	0.0667	0.0667	0.29	0.592
Harvest date.wax	2	2.1333	1.0667	4.60	0.011
Temp.wax	1	0.0667	0.0667	0.29	0.592
%1_MCP.wax	1	0.0667	0.0667	0.29	0.592
Harvest date.Temp.%1_MCP	2	0.1333	0.0667	0.29	0.750
Harvest date.Temp.wax	2	0.9333	0.4667	2.01	0.136
Harvest date.%1_MCP.wax	2	0.1333	0.0667	0.29	0.750
Temp.%1_MCP.wax	1	0.2667	0.2667	1.15	0.285
Harvest date.Temp.%1_MCP.wax	2	0.5333	0.2667	1.15	0.318
Residual	207	47.9667	0.2317		
Total	239	58.7333			

Appendix 24 – ANOVA table for stem-end rot incidence (Chapter 4)

Variate: **stem_end_rot**

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	9		1.1357	0.1262	0.79	
Rep.*Units* stratum						
Harvest date	2		1.2408	0.6204	3.90	0.022
Temp	1		0.7141	0.7141	4.49	0.035
%1_MCP	1		0.3444	0.3444	2.16	0.143
wax	1		0.1004	0.1004	0.63	0.428
Harvest date.Temp	2		1.7806	0.8903	5.60	0.004
Harvest date.%1_MCP	2		0.1774	0.0887	0.56	0.574
Temp.%1_MCP	1		0.0352	0.0352	0.22	0.638
Harvest date.wax	2		0.1030	0.0515	0.32	0.724
Temp.wax	1		0.0034	0.0034	0.02	0.883
%1_MCP.wax	1		0.0034	0.0034	0.02	0.883
Harvest date.Temp.%1_MCP	2		0.1682	0.0841	0.53	0.590
Harvest date.Temp.wax	2		0.5183	0.2592	1.63	0.199
Harvest date.%1_MCP.wax	2		0.0546	0.0273	0.17	0.842
Temp.%1_MCP.wax	1		0.2096	0.2096	1.32	0.252
Harvest date.Temp.%1_MCP.wax	2		0.1576	0.0788	0.50	0.610
Residual	206		32.7719	0.1591		
Total	238		39.4895			

Appendix 25 – ANOVA table for mesocarp discolouration (Chapter 4)

Variate: **Mesocarp_discolouration**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	6.1500	0.6833	1.40	
Rep.*Units* stratum					
Harvest date	2	17.8583	8.9292	18.26	<.001
Temp	1	33.7500	33.7500	69.00	<.001
%1_MCP	1	17.0667	17.0667	34.89	<.001
wax	1	0.4167	0.4167	0.85	0.357
Harvest date.Temp	2	15.3250	7.6625	15.67	<.001
Harvest date.%1_MCP	2	1.3083	0.6542	1.34	0.265
Temp.%1_MCP	1	8.8167	8.8167	18.03	<.001
Harvest date.wax	2	1.4583	0.7292	1.49	0.228
Temp.wax	1	0.0667	0.0667	0.14	0.712
%1_MCP.wax	1	0.8167	0.8167	1.67	0.198
Harvest date.Temp.%1_MCP	2	0.9083	0.4542	0.93	0.397
Harvest date.Temp.wax	2	0.2583	0.1292	0.26	0.768
Harvest date.%1_MCP.wax	2	1.9083	0.9542	1.95	0.145
Temp.%1_MCP.wax	1	0.0667	0.0667	0.14	0.712
Harvest date.Temp.%1_MCP.wax	2	0.3083	0.1542	0.32	0.730
Residual	207	101.2500	0.4891		
Total	239	207.7333			

Appendix 26 – ANOVA table for vascular browning incidence (Chapter 4)

Variate: **vascular_browning**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	1.0000	0.1111	0.58	
Rep.*Units* stratum					
Harvest date	2	0.3583	0.1792	0.94	0.392
Temp	1	3.2667	3.2667	17.16	<.001
%1_MCP	1	3.2667	3.2667	17.16	<.001
wax	1	0.0167	0.0167	0.09	0.768
Harvest date.Temp	2	1.0583	0.5292	2.78	0.064
Harvest date.%1_MCP	2	1.4583	0.7292	3.83	0.023
Temp.%1_MCP	1	3.2667	3.2667	17.16	<.001
Harvest date.wax	2	0.1083	0.0542	0.28	0.753
Temp.wax	1	0.1500	0.1500	0.79	0.376
%1_MCP.wax	1	0.0167	0.0167	0.09	0.768
Harvest date.Temp.%1_MCP	2	1.0583	0.5292	2.78	0.064
Harvest date.Temp.wax	2	0.1750	0.0875	0.46	0.632
Harvest date.%1_MCP.wax	2	0.1083	0.0542	0.28	0.753
Temp.%1_MCP.wax	1	0.4167	0.4167	2.19	0.141
Harvest date.Temp.%1_MCP.wax	2	0.2083	0.1042	0.55	0.579
Residual	207	39.4000	0.1903		
Total	239	55.3333			

Appendix 27 – ANOVA table for percentage sound fruit (Chapter 4)

Variate: **percentage sound fruit**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	9	20167.	2241.	1.56	
Rep.*Units* stratum					
Harvest date	2	79083.	39542.	27.48	<.001
Temp	1	66667.	66667.	46.33	<.001
%1_MCP	1	16667.	16667.	11.58	<.001
wax	1	2667.	2667.	1.85	0.175
Harvest date.Temp	2	2583.	1292.	0.90	0.409
Harvest date.%1_MCP	2	22583.	11292.	7.85	<.001
Temp.%1_MCP	1	20167.	20167.	14.02	<.001
Harvest date.wax	2	8083.	4042.	2.81	0.063
Temp.wax	1	1500.	1500.	1.04	0.308
%1_MCP.wax	1	167.	167.	0.12	0.734
Harvest date.Temp.%1_MCP	2	4083.	2042.	1.42	0.244
Harvest date.Temp.wax	2	250.	125.	0.09	0.917
Harvest date.%1_MCP.wax	2	1583.	792.	0.55	0.578
Temp.%1_MCP.wax	1	6000.	6000.	4.17	0.042
Harvest date.Temp.%1_MCP.wax	2	1750.	875.	0.61	0.545
Residual	207	297833.	1439.		
Total	239	551833.			

Appendix 28 – ANOVA table for Total Anti-oxidant Capacity – 28 day storage (Chapter 5)

Variate: **Total Anti-oxidant Capacity**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.07424	0.03712	0.47	
Reps.*Units* stratum					
Harvest date	2	17.12251	8.56126	107.53	<.001
Temp	1	0.09784	0.09784	1.23	0.273
%1_MCP	1	0.19036	0.19036	2.39	0.129
wax	1	0.04948	0.04948	0.62	0.435
Harvest date.Temp	2	0.03616	0.01808	0.23	0.798
Harvest date.%1_MCP	2	0.08758	0.04379	0.55	0.581
Temp.%1_MCP	1	0.34034	0.34034	4.27	0.044
Harvest date.wax	2	0.05872	0.02936	0.37	0.694
Temp.wax	1	0.25471	0.25471	3.20	0.080
%1_MCP.wax	1	0.01003	0.01003	0.13	0.724
Harvest date.Temp.%1_MCP	2	0.02152	0.01076	0.14	0.874
Harvest date.Temp.wax	2	0.20446	0.10223	1.28	0.287
Harvest date.%1_MCP.wax	2	0.14756	0.07378	0.93	0.403
Temp.%1_MCP.wax	1	0.01767	0.01767	0.22	0.640
Harvest date.Temp.%1_MCP.wax	2	0.62326	0.31163	3.91	0.027
Residual	46	3.66254	0.07962		
Total	71	22.99899			

Appendix 29 – ANOVA table for Ascorbic acid conc. – 28 day storage (Chapter 5)

Variate: **Ascorbic acid conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.2232	0.1116	0.53	
Reps.*Units* stratum					
Harvest date	2	47.1284	23.5642	111.12	<.001
Temp	1	0.1203	0.1203	0.57	0.455
%1_MCP	1	0.9531	0.9531	4.49	0.039
wax	1	0.0367	0.0367	0.17	0.679
Harvest date.Temp	2	0.3968	0.1984	0.94	0.400
Harvest date.%1_MCP	2	2.2456	1.1228	5.29	0.009
Temp.%1_MCP	1	2.0000	2.0000	9.43	0.004
Harvest date.wax	2	0.1186	0.0593	0.28	0.757
Temp.wax	1	0.0837	0.0837	0.39	0.533
%1_MCP.wax	1	2.4888	2.4888	11.74	0.001
Harvest date.Temp.%1_MCP	2	0.2438	0.1219	0.57	0.567
Harvest date.Temp.wax	2	0.5810	0.2905	1.37	0.264
Harvest date.%1_MCP.wax	2	0.9384	0.4692	2.21	0.121
Temp.%1_MCP.wax	1	0.8027	0.8027	3.79	0.058
Harvest date.Temp.%1_MCP.wax	2	0.3382	0.1691	0.80	0.457
Residual	46	9.7552	0.2121		
Total	71	68.4544			

Appendix 30 – ANOVA table for Mannoheptulose conc. – 28 day storage (Chapter 5)

Variate: **Mannoheptulose conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	18.404	9.202	1.18	
Reps.*Units* stratum					
Harvest date	2	18.318	9.159	1.18	0.317
Temp	1	5.747	5.747	0.74	0.394
%1_MCP	1	6.255	6.255	0.80	0.374
wax	1	12.876	12.876	1.66	0.205
Harvest date.Temp	2	1.956	0.978	0.13	0.882
Harvest date.%1_MCP	2	17.209	8.605	1.11	0.339
Temp.%1_MCP	1	0.458	0.458	0.06	0.809
Harvest date.wax	2	5.221	2.611	0.34	0.717
Temp.wax	1	6.521	6.521	0.84	0.365
%1_MCP.wax	1	0.170	0.170	0.02	0.883
Harvest date.Temp.%1_MCP	2	8.985	4.493	0.58	0.565
Harvest date.Temp.wax	2	2.133	1.067	0.14	0.872
Harvest date.%1_MCP.wax	2	0.181	0.090	0.01	0.988
Temp.%1_MCP.wax	1	3.468	3.468	0.45	0.508
Harvest date.Temp.%1_MCP.wax	2	10.441	5.221	0.67	0.516
Residual	46	357.758	7.777		
Total	71	476.101			

Appendix 31 – ANOVA table for Perseitol conc. – 28 day storage (Chapter 5)

Variate: **Perseitol conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	18.81	9.41	0.49	
Reps.*Units* stratum					
Harvest date	2	74.60	37.30	1.93	0.156
Temp	1	11.18	11.18	0.58	0.451
%1_MCP	1	64.36	64.36	3.33	0.074
wax	1	49.23	49.23	2.55	0.117
Harvest date.Temp	2	21.49	10.74	0.56	0.577
Harvest date.%1_MCP	2	53.42	26.71	1.38	0.261
Temp.%1_MCP	1	8.31	8.31	0.43	0.515
Harvest date.wax	2	13.97	6.98	0.36	0.698
Temp.wax	1	20.70	20.70	1.07	0.306
%1_MCP.wax	1	178.52	178.52	9.25	0.004
Harvest date.Temp.%1_MCP	2	3.50	1.75	0.09	0.914
Harvest date.Temp.wax	2	39.33	19.66	1.02	0.369
Harvest date.%1_MCP.wax	2	9.14	4.57	0.24	0.790
Temp.%1_MCP.wax	1	4.77	4.77	0.25	0.622
Harvest date.Temp.%1_MCP.wax	2	104.77	52.38	2.71	0.077
Residual	46	888.12	19.31		
Total	71	1564.22			

Appendix 32 – ANOVA table for Total Anti-oxidant Capacity – Cold Chain Breaks (Chp 5)

Variate: **Total Anti-oxidant Capacity**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	2	0.02372	0.01186	0.19		
Reps.*Units* stratum						
Harvest date	2	37.02392	18.51196	297.90	<.001	
Temp	1	0.23709	0.23709	3.82	0.053	
%1_MCP	1	0.00182	0.00182	0.03	0.864	
wax	1	0.41681	0.41681	6.71	0.011	
Break	2	0.61444	0.30722	4.94	0.008	
Harvest date.Temp	2	0.05803	0.02902	0.47	0.628	
Harvest date.%1_MCP	2	0.89240	0.44620	7.18	0.001	
Temp.%1_MCP	1	0.08190	0.08190	1.32	0.253	
Harvest date.wax	2	0.20120	0.10060	1.62	0.202	
Temp.wax	1	0.10866	0.10866	1.75	0.188	
%1_MCP.wax	1	0.06758	0.06758	1.09	0.299	
Harvest date.Break	4	0.74137	0.18534	2.98	0.021	
Temp.Break	2	0.09783	0.04892	0.79	0.457	
%1_MCP.Break	2	0.61380	0.30690	4.94	0.008	
wax.Break	2	0.04322	0.02161	0.35	0.707	
Harvest date.Temp.%1_MCP	2	0.24248	0.12124	1.95	0.146	
Harvest date.Temp.wax	2	0.25935	0.12968	2.09	0.128	
Harvest date.%1_MCP.wax	2	0.02330	0.01165	0.19	0.829	
Temp.%1_MCP.wax	1	0.00802	0.00802	0.13	0.720	
Harvest date.Temp.Break	4	0.20381	0.05095	0.82	0.514	
Harvest date.%1_MCP.Break	4	0.18532	0.04633	0.75	0.563	
Temp.%1_MCP.Break	2	0.27657	0.13829	2.23	0.112	
Harvest date.wax.Break	4	0.21608	0.05402	0.87	0.484	
Temp.wax.Break	2	0.36025	0.18013	2.90	0.058	
%1_MCP.wax.Break	2	0.15976	0.07988	1.29	0.280	
Harvest date.Temp.%1_MCP.wax	2	0.19485	0.09743	1.57	0.212	
Harvest date.Temp.%1_MCP.Break	4	0.57264	0.14316	2.30	0.061	
Harvest date.Temp.wax.Break	4	0.19113	0.04778	0.77	0.547	
Harvest date.%1_MCP.wax.Break	4	0.23313	0.05828	0.94	0.444	
Temp.%1_MCP.wax.Break	2	0.06498	0.03249	0.52	0.594	
Harvest date.Temp.%1_MCP.wax.Break	4	0.87929	0.21982	3.54	0.009	
Residual	142	8.82395	0.06214			
Total	215	54.11869				

Appendix 33 – ANOVA table for Ascorbic acid conc. – Cold Chain Breaks (Chp 5)

Variate: **Ascorbic acid conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	2	0.6300	0.3150	1.20		
Reps.*Units* stratum						
Harvest date	2	116.3916	58.1958	221.70	<.001	
Temp	1	1.7604	1.7604	6.71	0.011	
%1_MCP	1	0.1065	0.1065	0.41	0.525	
wax	1	0.1080	0.1080	0.41	0.522	
Break	2	1.0257	0.5129	1.95	0.146	
Harvest date.Temp	2	0.8743	0.4371	1.67	0.193	
Harvest date.%1_MCP	2	5.1592	2.5796	9.83	<.001	
Temp.%1_MCP	1	1.2831	1.2831	4.89	0.029	
Harvest date.wax	2	0.5061	0.2530	0.96	0.384	
Temp.wax	1	0.4951	0.4951	1.89	0.172	
%1_MCP.wax	1	1.5962	1.5962	6.08	0.015	
Harvest date.Break	4	2.6318	0.6580	2.51	0.045	
Temp.Break	2	0.2441	0.1220	0.46	0.629	
%1_MCP.Break	2	2.1244	1.0622	4.05	0.020	
wax.Break	2	0.5515	0.2757	1.05	0.352	
Harvest date.Temp.%1_MCP	2	1.1369	0.5684	2.17	0.118	
Harvest date.Temp.wax	2	0.0145	0.0072	0.03	0.973	
Harvest date.%1_MCP.wax	2	1.5017	0.7509	2.86	0.061	
Temp.%1_MCP.wax	1	0.0061	0.0061	0.02	0.879	
Harvest date.Temp.Break	4	0.9419	0.2355	0.90	0.468	
Harvest date.%1_MCP.Break	4	2.0834	0.5209	1.98	0.100	
Temp.%1_MCP.Break	2	1.5158	0.7579	2.89	0.059	
Harvest date.wax.Break	4	2.8112	0.7028	2.68	0.034	
Temp.wax.Break	2	1.0562	0.5281	2.01	0.138	
%1_MCP.wax.Break	2	1.4369	0.7185	2.74	0.068	
Harvest date.Temp.%1_MCP.wax	2	2.4572	1.2286	4.68	0.011	
Harvest date.Temp.%1_MCP.Break	4	0.4641	0.1160	0.44	0.778	
Harvest date.Temp.wax.Break	4	3.6007	0.9002	3.43	0.010	
Harvest date.%1_MCP.wax.Break	4	0.4824	0.1206	0.46	0.765	
Temp.%1_MCP.wax.Break	2	1.0483	0.5242	2.00	0.140	
Harvest date.Temp.%1_MCP.wax.Break	4	1.9193	0.4798	1.83	0.127	
Residual	142	37.2753	0.2625			
Total	215	195.2398				

Appendix 34 – ANOVA table for Mannoheptulose conc. – Cold Chain Breaks (Chp 5)

Variate: **Mannoheptulose conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	2	61.25	30.63	2.84		
Reps.*Units* stratum						
Harvest date	2	29.24	14.62	1.36	0.261	
Temp	1	1.89	1.89	0.18	0.676	
%1_MCP	1	4.44	4.44	0.41	0.522	
wax	1	39.45	39.45	3.66	0.058	
Break	2	73.42	36.71	3.41	0.036	
Harvest date.Temp	2	1.71	0.85	0.08	0.924	
Harvest date.%1_MCP	2	246.24	123.12	11.43	<.001	
Temp.%1_MCP	1	28.94	28.94	2.69	0.103	
Harvest date.wax	2	28.79	14.39	1.34	0.266	
Temp.wax	1	44.00	44.00	4.08	0.045	
%1_MCP.wax	1	9.85	9.85	0.91	0.340	
Harvest date.Break	4	47.67	11.92	1.11	0.356	
Temp.Break	2	11.76	5.88	0.55	0.581	
%1_MCP.Break	2	50.67	25.33	2.35	0.099	
wax.Break	2	10.34	5.17	0.48	0.620	
Harvest date.Temp.%1_MCP	2	68.27	34.13	3.17	0.045	
Harvest date.Temp.wax	2	27.58	13.79	1.28	0.281	
Harvest date.%1_MCP.wax	2	53.00	26.50	2.46	0.089	
Temp.%1_MCP.wax	1	16.74	16.74	1.55	0.215	
Harvest date.Temp.Break	4	11.42	2.85	0.26	0.900	
Harvest date.%1_MCP.Break	4	99.46	24.86	2.31	0.061	
Temp.%1_MCP.Break	2	16.28	8.14	0.76	0.472	
Harvest date.wax.Break	4	23.15	5.79	0.54	0.709	
Temp.wax.Break	2	28.95	14.48	1.34	0.264	
%1_MCP.wax.Break	2	3.49	1.75	0.16	0.850	
Harvest date.Temp.%1_MCP.wax	2	104.16	52.08	4.83	0.009	
Harvest date.Temp.%1_MCP.Break	4	27.03	6.76	0.63	0.644	
Harvest date.Temp.wax.Break	4	30.25	7.56	0.70	0.592	
Harvest date.%1_MCP.wax.Break	4	39.23	9.81	0.91	0.460	
Temp.%1_MCP.wax.Break	2	29.71	14.85	1.38	0.255	
Harvest date.Temp.%1_MCP.wax.Break	4	71.50	17.88	1.66	0.163	
Residual	142	1529.79	10.77			
Total	215	2869.65				

Appendix 35 – ANOVA table for Perseitol conc. – Cold Chain Breaks (Chp 5)

Variate: **Perseitol conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.	
Reps stratum	2	13.77	6.89	0.38		
Reps.*Units* stratum						
Harvest date	2	243.59	121.79	6.64	0.002	
Temp	1	34.78	34.78	1.90	0.171	
%1_MCP	1	585.21	585.21	31.89	<.001	
wax	1	111.75	111.75	6.09	0.015	
Break	2	28.72	14.36	0.78	0.459	
Harvest date.Temp	2	43.02	21.51	1.17	0.313	
Harvest date.%1_MCP	2	71.57	35.79	1.95	0.146	
Temp.%1_MCP	1	51.72	51.72	2.82	0.095	
Harvest date.wax	2	31.64	15.82	0.86	0.424	
Temp.wax	1	112.16	112.16	6.11	0.015	
%1_MCP.wax	1	156.27	156.27	8.52	0.004	
Harvest date.Break	4	11.75	2.94	0.16	0.958	
Temp.Break	2	69.86	34.93	1.90	0.153	
%1_MCP.Break	2	98.09	49.05	2.67	0.073	
wax.Break	2	17.70	8.85	0.48	0.618	
Harvest date.Temp.%1_MCP	2	76.56	38.28	2.09	0.128	
Harvest date.Temp.wax	2	164.23	82.12	4.47	0.013	
Harvest date.%1_MCP.wax	2	116.42	58.21	3.17	0.045	
Temp.%1_MCP.wax	1	4.66	4.66	0.25	0.615	
Harvest date.Temp.Break	4	107.72	26.93	1.47	0.215	
Harvest date.%1_MCP.Break	4	66.76	16.69	0.91	0.460	
Temp.%1_MCP.Break	2	4.49	2.25	0.12	0.885	
Harvest date.wax.Break	4	33.97	8.49	0.46	0.763	
Temp.wax.Break	2	7.70	3.85	0.21	0.811	
%1_MCP.wax.Break	2	66.33	33.17	1.81	0.168	
Harvest date.Temp.%1_MCP.wax	2	458.55	229.28	12.49	<.001	
Harvest date.Temp.%1_MCP.Break	4	15.51	3.88	0.21	0.932	
Harvest date.Temp.wax.Break	4	13.40	3.35	0.18	0.947	
Harvest date.%1_MCP.wax.Break	4	59.62	14.91	0.81	0.519	
Temp.%1_MCP.wax.Break	2	10.62	5.31	0.29	0.749	
Harvest date.Temp.%1_MCP.wax.Break	4	25.69	6.42	0.35	0.844	
Residual	142	2605.75	18.35			
Total	215	5519.59				

Appendix 36 – ANOVA table for Total Anti-oxidant Capacity – 56 day storage (Chapter 5)

Variate: **Total Anti-oxidant Capacity**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.04308	0.02154	0.33	
Reps.*Units* stratum					
Harvest date	2	15.55796	7.77898	118.67	<.001
Temp	1	0.07646	0.07646	1.17	0.286
%1_MCP	1	0.09398	0.09398	1.43	0.237
wax	1	0.00099	0.00099	0.02	0.903
Harvest date.Temp	2	0.14654	0.07327	1.12	0.336
Harvest date.%1_MCP	2	0.66584	0.33292	5.08	0.010
Temp.%1_MCP	1	0.00684	0.00684	0.10	0.748
Harvest date.wax	2	0.63752	0.31876	4.86	0.012
Temp.wax	1	0.18891	0.18891	2.88	0.096
%1_MCP.wax	1	0.12240	0.12240	1.87	0.178
Harvest date.Temp.%1_MCP	2	0.08246	0.04123	0.63	0.538
Harvest date.Temp.wax	2	0.22545	0.11272	1.72	0.190
Harvest date.%1_MCP.wax	2	1.17299	0.58650	8.95	<.001
Temp.%1_MCP.wax	1	0.05877	0.05877	0.90	0.349
Harvest date.Temp.%1_MCP.wax	2	0.13750	0.06875	1.05	0.359
Residual	46	3.01538	0.06555		
Total	71	22.23306			

Appendix 37 – ANOVA table for Ascorbic acid conc. – 56 day storage (Chapter 5)

Variate: **Ascorbic acid conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	0.2540	0.1270	0.46	
Reps.*Units* stratum					
Harvest date	2	16.8786	8.4393	30.68	<.001
Temp	1	0.4896	0.4896	1.78	0.189
%1_MCP	1	0.3937	0.3937	1.43	0.238
wax	1	0.2305	0.2305	0.84	0.365
Harvest date.Temp	2	1.9777	0.9888	3.60	0.035
Harvest date.%1_MCP	2	0.7460	0.3730	1.36	0.268
Temp.%1_MCP	1	0.2820	0.2820	1.03	0.317
Harvest date.wax	2	0.1016	0.0508	0.18	0.832
Temp.wax	1	0.3953	0.3953	1.44	0.237
%1_MCP.wax	1	0.0810	0.0810	0.29	0.590
Harvest date.Temp.%1_MCP	2	0.1871	0.0935	0.34	0.714
Harvest date.Temp.wax	2	0.6936	0.3468	1.26	0.293
Harvest date.%1_MCP.wax	2	1.4520	0.7260	2.64	0.082
Temp.%1_MCP.wax	1	0.3592	0.3592	1.31	0.259
Harvest date.Temp.%1_MCP.wax	2	0.9294	0.4647	1.69	0.196
Residual	46	12.6521	0.2750		
Total	71	38.1033			

Appendix 38 – ANOVA table for Mannoheptulose conc. – 56 day storage (Chapter 5)

Variate: **Mannoheptulose conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	13.303	6.651	2.54	
Reps.*Units* stratum					
Harvest date	1	111.047	111.047	42.46	<.001
Temp	1	0.115	0.115	0.04	0.835
%1_MCP	1	4.152	4.152	1.59	0.217
wax	1	3.903	3.903	1.49	0.231
Harvest date.Temp	1	3.571	3.571	1.37	0.252
Harvest date.%1_MCP	1	0.724	0.724	0.28	0.603
Temp.%1_MCP	1	3.684	3.684	1.41	0.245
Harvest date.wax	1	0.041	0.041	0.02	0.901
Temp.wax	1	0.000	0.000	0.00	0.999
%1_MCP.wax	1	8.837	8.837	3.38	0.076
Harvest date.Temp.%1_MCP	1	0.064	0.064	0.02	0.877
Harvest date.Temp.wax	1	4.486	4.486	1.72	0.200
Harvest date.%1_MCP.wax	1	0.184	0.184	0.07	0.793
Temp.%1_MCP.wax	1	6.528	6.528	2.50	0.125
Harvest date.Temp.%1_MCP.wax	1	0.580	0.580	0.22	0.641
Residual	30	78.465	2.615		
Total	47	239.685			

Appendix 39 – ANOVA table for Perseitol conc. – 56 day storage (Chapter 5)

Variate: **Perseitol conc.**

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Reps stratum	2	55.63	27.82	1.81	
Reps.*Units* stratum					
Harvest date	1	12.51	12.51	0.82	0.374
Temp	1	0.14	0.14	0.01	0.925
%1_MCP	1	182.80	182.80	11.93	0.002
wax	1	12.89	12.89	0.84	0.366
Harvest date.Temp	1	122.61	122.61	8.00	0.008
Harvest date.%1_MCP	1	1.90	1.90	0.12	0.727
Temp.%1_MCP	1	121.47	121.47	7.93	0.009
Harvest date.wax	1	17.71	17.71	1.16	0.291
Temp.wax	1	24.22	24.22	1.58	0.218
%1_MCP.wax	1	27.21	27.21	1.78	0.193
Harvest date.Temp.%1_MCP	1	53.15	53.15	3.47	0.072
Harvest date.Temp.wax	1	118.71	118.71	7.75	0.009
Harvest date.%1_MCP.wax	1	59.09	59.09	3.86	0.059
Temp.%1_MCP.wax	1	5.99	5.99	0.39	0.536
Harvest date.Temp.%1_MCP.wax	1	194.47	194.47	12.69	0.001
Residual	30	459.79	15.33		
Total	47	1470.29			

Representative Photos of the External Chilling Injury Rating Scale used (0-10)