ENHANCEMENT OF 'HASS' AVOCADO SHELF LIFE USING ULTRA-LOW TEMPERATURE SHIPPING OR 1-MCP TREATMENT AND COLD CHAIN MANAGEMENT

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DECLARATION

I, Richard Dean Kok, declare that the research reported in this thesis, except where otherwise indicated, is my original work. This thesis has not been submitted for any degree or examination at any other university.

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We certify that the above statement is correct.

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This thesis has been compiled as separate chapters for publication in scientific journals, some overlap of data may occur.

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"I can do all things through Christ which strengtheneth me" Philippians 4:13

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DEDICATION

I dedicate this thesis to my parents. My mother, Mrs. Kim Kok. Through her I have witnessed strength and faith beyond measure through the toughest of times. My father, Mr. Leo Kok. He is the pillar of strength in my life and has shown me firsthand what it is to be a man and to put family first, he is and always will be my role model.

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ABSTRACT

Avocados are becoming an increasingly important crop in South Africa, where the main producing areas include Limpopo, Mpumalanga and KwaZulu-Natal provinces. The South African avocado industry faces considerable challenges including increasing competition exporting avocados, particularly to the European market. The processes involved to export avocados has markedly improved over the past two decades, however there is always room for improvement and it is necessary to remain competitive on a global scale. Issues such as fruit being partially soft on arrival, quality defects and cold chain management breakdown are still present. It is necessary to investigate new aspects of cold storage such as extending the storage period and understanding the physiological aspects involved. To improvement such issues, an investigation was conducted on ultra-low temperature shipping (1°C) as well as the use of 1-MCP; the implementation of deliberate cold chain breaks to achieve a better understanding as to the quality influences involved; an extended storage period of 56 days to assess the quality issues and benefits involved; as well as investigating the physiological aspects involved with all above treatments on 'Hass' avocados.

An initial study saw early-, mid- and late-season 'Hass' avocados stored at 1°C or 5.5°C for 28 days. Additional treatments included fruit treated and not treated with 1-MCP as well as waxed and unwaxed fruit. Storage at 1°C was comparable with 1-MCP treatment for both fruit softening in storage and extending the ripening period. Storage at 5.5°C resulted in partial in-transit ripening, if 1-MCP was not used. Early-season fruit incurred the most external chilling injury but overall levels were minimal and not concerning. Mid-season fruit were the most sound in terms of quality. It is suggested that 1°C can be used as a viable economic alternative to 1-MCP for long distance shipping of 'Hass' up to 28 days.

The cold chain break trial included a 24 hour delay before cold storage, a deliberate 8 hour break at day 14 of cold storage where fruit were removed from cold storage and a control of 28 days cold storage where no break was involved. Early-, mid- and late-season 'Hass' avocados were stored at 1°C or 5.5°C for 28 days. Additional treatments included fruit treated and not treated with 1-MCP as well as waxed and unwaxed fruit. It was found that cold chain breaks do influence the amount of water loss, fruit softening and days taken to ripen. Storage at 1°C did not entirely negate the effects of cold chain breaks compared with 5.5°C, but did result in fruit which were harder at the end of storage and took longer to ripen. The use of 1-MCP also had advantageous effects with respect to significantly lengthening the

ripening period, even when a cold chain break occurred, compared with fruit not treated with 1-MCP. As results of the study differed in some respects to those of previous studies, it is recommended that further work be conducted to determine what fruit or pre-harvest factors affect the fruit physiological changes which take place when cold chain breaks occur.

Having the option to make use of an extended storage period would be of benefit to the industry if delays occur and fruit have to be maintained under cold storage. Extended storage of South African avocados, especially at the end of the season would also allow for the option of strategically holding back fruit from the export market in order to extend the supply period. It would not only benefit export options, but would also be highly beneficial to local pre-packers, as it would reduce the need to import fruit from the Northern hemisphere production areas during the South African off-season. Early-, mid- and late-season 'Hass' avocados were stored at 1°C or 5.5°C for 56 days. Additional treatments included fruit treated and not treated with 1-MCP as well as waxed and unwaxed fruit. The combination of 1°C with the use of 1-MCP resulted in a good shelf life as well as maintenance of internal quality and integrity. External chilling injury is of concern for early-season fruit, however, mid- and late-season fruit did not incur extensive damage. It is, therefore, advised that fruit placed in extended storage are marketed through the 'Ready ripe' program to mask any chilling injury on the 'Hass' fruit.

Avocados are renown as a "healthy food" due to their nutritional value as well as containing relatively high concentrations of antioxidants. The fruit also contain high amounts of C7 sugars which can act as antioxidants. Additionally, C7 sugars and other antioxidants play important roles in fruit quality. Therefore, it is important to understand how varying storage conditions and treatments affect the levels of these physiological parameters. Treatments of cold chain break/delay included a deliberate 8 hour break at day 14 of cold storage where fruit were removed from cold storage, a 24 hour delay before cold storage and a control of 28 days where no break was involved. A 56 day extended storage period was also used. Early-, mid- and late-season 'Hass' avocados were stored at 1°C or 5.5°C for 28 days. Additional treatments included fruit treated and not treated with 1-MCP as well as waxed and unwaxed fruit. The use of 1-MCP maintained higher levels of antioxidants, ascorbic acid and C7 sugars for both the 28 day and the 56 day storage periods. The 24 hour delay had a tendency to increase consumption of anti-oxidant and sugar reserves. The use of 1°C resulted in antioxidant and ascorbic acid levels decreasing while maintaining higher sugar levels. Overall, high stress imposed on fruit decreased reserves resulting in poor quality fruit. The use of 1°C and 1-MCP treatments maintained fruit quality.

GENERAL INTRODUCTION

The South African avocado industry has progressively grown in recent years and currently is ranked fourth in the world in terms of world trade. Currently, the domestic market occupies 54% of the total avocado production volume, where 46% of the total avocado production volume is exported to the European market. It was reported that 12 million 4 kg cartons (48 000 tons) were exported to the European market in the 2010 season (Pers. com., 2011). The major markets within Europe are the United Kingdom and France, followed by Germany. Growing markets are the Scandinavian countries and eastern Europe. Considerable quantities of fruit are shipped through the Netherlands with the country itself being a small market. In terms of cultivar mix, approximately 55% were ‘Hass’ and 45% were greenskins, with ‘Fuerte’ making up more than 50% of the greenskins (Pers. com., 2011). World market trends indicate an increasing demand for ‘Hass’.

The South African avocado industry is largely dependent on export and all the major export markets are distant, resulting in complex and often problematic logistics (Kremer-Köhne, 1998). With the fruit having to be shipped to Europe, it means that the fruit may need to be in cold storage for up to 30 days (Bower and Cutting, 1988). Because of the highly climacteric nature of the fruit and rapid ripening characteristics great care needs to be taken to ensure fruit do not undergo excessive ripening during this shipping period. The delay of ripening while maintaining fruit quality of avocado fruit is achieved by lowering storage shipping temperatures. Currently, avocado fruit are shipped at an average temperature of 5.5°C however, early-season fruit are commonly shipped at 7.5°C and as the season progresses the shipping temperature can be as 3.5°C for the late-season fruit (Vorster *et al*., 1990). Low temperature shipping is used 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. Storage temperatures below the currently implemented average temperature of 5.5°C could negate the need for chemicals such as 1-MCP, but there are fears that temperatures below 5.5°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°C (Bower and Magwaza, 2004; Van Rooyen and Bower, 2006; Van Rooyen and Bower, 2002), and ‘Hass’ when stored at 1°C (Van Rooyen, 2009; Kok *et al*., 2010). Internal fruit quality seems to have improved under 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; Kok *et al*., 2010; 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; Kok *et al*., 2010; 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.

With increasing production potential and growing competition in Europe, the South African industry is currently seeking new international markets. The main market in question is the USA. By gaining access to this new market an extension and alternative to Europe will be created with the potential to enhance security and profitability of the South African industry. However, phytosanitary restrictions will probably apply. In considering mitigating treatments, it was found that the use of radiation treatments were unsuccessful in that fruit quality was severely compromised (du Rand *et al*., 2010). However, ultra-low temperature shipping at 1°C for the ‘Hass’ cultivar has been found to be successful in maintaining fruit quality (Van Rooyen, 2009; Kok *et al*., 2010) and would also be sufficient as a phytosanitary treatment.

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). There are evident problems associated with the export of avocado fruit, where the issues of chilling injury, cold chain breaks and increased pressure from the market to reduce the use of agro-chemicals has lead to an important investigation into the use of alternative shipping methods and the subsequent effects.

It is hypothesised that reducing the shipping temperature from the current standard protocol of 5.5°C to 1°C for 'Hass' avocados is possible with minimal chilling injury risk and effectively rendering the use of 1-MCP unnecessary due to significantly reducing fruit softening within storage, as well as effectively negating the detrimental effects of cold chain breaks.

The objectives set for this study include:

1) To ascertain whether a lower storage and shipping temperature of 1°Cfor ‘Hass’ as opposed to current industry protocol of 5.5^{o}C is comparable to the use of 1-MCP during simulated shipping.
2) To ascertain the effects of cold chain breaks on the final fruit quality for ‘Hass’ by investigating both observed quality attributes as well as physiological changes during shipping and subsequent fruit ripening.
3) To ascertain the influence of varying storage conditions with respect to the use of ultra-low temperature, 1-MCP, cold chain breaks and extended storage on the pool of anti-oxidants and C7 sugars in ‘Hass’ avocado fruit.
4) To ascertain whether an extended storage period of 56 days would be a credible option commercially, especially with the use of an ultra-low temperature of 1°C.

CHAPTER 1

LITERATURE REVIEW

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1. Avocado

The avocado (*Persea americana* Mill.) is a member of the family Lauraceae (Wills *et al.*, 1982). The avocado was classified as a member of the genus *Persea* by Miller in 1754, and, although there are over 50 species within the genus, the avocado is the only species of commercial importance. There are three distinct ecological races of avocado which have been named after their presumed origin, namely Mexican, Guatemalan and West Indian, the botanical classification of these races *Persea americana* var. *drymifolia*, *Persea americana* var. *guatemalensis*, and *Persea americana* var. *americana*, respectively (Chandler, 1957). The centre of origin of the avocado is thought to be the Meso-America region which classifies the fruit as subtropical. However, the different ecological races are found in climatically varied habitats and hence exhibit distinct characteristics. The Mexican race originated from the Mexican highlands, and even though this area is situated near the equator, the trees grow at an altitude of 1500-3000 m which reduces the temperature of the growing environment. The associated altitude of the growing habitat of this race has lead to an important trait of cold tolerance. The fruit are characterised by being small in size and having smooth, thin skins. The fruit are also very susceptible to anthracnose (Bergh, 1975). The Guatemalan race stems from the highlands of central Guatemala with an altitude of 1000-2000 m. These fruit are intolerant of high heat and low humidity, however they do not have the same degree of cold tolerance as the Mexican race due to the slightly warmer temperature found at the lower altitude. These fruit nevertheless have many good commercial characteristics which results in this race being included in breeding programmes. The fruit are characterised by being larger and having thick, rough skins (Storey *et al.*, 1986). The West Indian race stems from the lowlands of Central America and is most suited to hot, humid climates. The commercial characteristics of the fruit are poor with variable sizes and a skin which is slightly smoother and thinner to that of the Guatemalan race. Due to this there are few sought after commercially viable cultivars occurring from this race (Bergh, 1975; Storey *et al.*, 1986).

Many of the commercially important cultivars are selections found to be natural hybrids between the races, and thus exhibit traits of more than one race.

It has been thought that the introduction of avocados to South Africa occurred in the late 19^{th} century when West Indian race seedlings were planted near the Durban region (Ludman, 1930). Commercially important cultivars were later imported from California which were of Mexican and Guatemalan origins, including hybrids of the two, which suited the South African climate better (Malan, 1957).

1.1. The 'Hass' cultivar

The 'Hass' cultivar was named after R.G. Hass in California. The cultivar originates from a chance seedling of unknown parentage, and was subsequently patented on the 27th April 1935. It was later found that the 'Hass' cultivar is a hybrid combination of predominantly Guatemalan (85%) with traces of Mexican germplasm (15%) (Bergh and Ellstrand, 1986; Bender and Whiley, 2002). The fruit has characteristics of being small with a mass of 140-400g and is oval in shape with no neck. It has a thick and leathery skin with a course texture. Initially the fruit is green in colour (Figure 1.1) on the tree but changes to a purple-black when ripening occurs (Figure 1.2) off the tree. It is known to be a mid- to late-season cultivar. 'Hass' is one of the most popular avocado cultivars and considered to be the world standard, with production statistics which show 'Hass' accounting for 100% of avocado production in Peru; 98% in New Zealand; 95% in California; 95% in Mexico; 93% in Chile; 75% in Australia; 75% in Spain; 36% in South Africa; and 32% in Israel (Anonymous, 2009). The reason for this popularity is mainly due to outstanding postharvest characteristics. The thick exocarp leads to better storage and shipping capability, external blemishes tends to be masked by the dark colour upon ripening, internal quality is superior to many other cultivars, with fewer disorders evident and taste is highly acceptable to most consumers.

Figure 1.1: Typical shape and colour of an unripe 'Hass' fruit

Figure 1.2: Typical colour associated with a ripe 'Hass' fruit

2. Ripening

As a result of softening of the avocado fruit, mainly associated with ripening, an increased chance of physical and pathological damage to the fruit occurs (Brady, 1987). To prevent fruit softening during the long shipping periods to Europe low temperatures are used, but physiological damage can occur due to the avocados fruit's sensitivity to cold temperatures. An understanding of avocado ripening and the complex physiological processes involved is necessary in order to improve practices for increased quality and shelf life after shipping (Bower and Cutting, 1988).

2.1. Ripening physiology

Ripening involves many progressive processes within the fruit which lead to changes in colour, taste, and texture, allowing the fruit to become edible (Wills *et al*., 1998). Unlike most other fruit, the avocado fruit will only begin the ripening process once it has been removed from the tree (Schroeder, 1953). This trait of the avocado fruit is not fully understood, but it has been proposed that it may be due to a ripening inhibitor, thought to be an anion, that moves to or from the fruit pedicel only once detached from the tree (Tingwa and Young, 1975). In recent studies it has been shown that the C7 sugars, particularly mannoheptulose, can be a ripening inhibitor where the levels of mannoheptulose need to decrease to a certain level before ripening is activated and therefore could be used as an indicator for ripening (Liu *et al*., 2002; Blakey *et al*., 2009b). Once the ripening process, a result of a number of complex physiological and physical changes, has begun it cannot be reversed, only slowed. Ripening involves many catabolic and anabolic processes, requiring large amounts of energy and prolonged membrane integrity (Bower and Cutting, 1988). Senescence will ultimately occur when catabolic processes exceed the anabolic processes, leaving the fruit in an over-ripe state and eventually result in tissue death (Wills *et al*., 1998).

2.1.1. Role of sugars

Carbohydrates are accumulated prior to harvest, and form a reserve which is used as an energy source for the respiratory process whilst the fruit is within storage and later during ripening (Kozlowski, 1992). Although avocado fruit accumulate lipids, Blanke (1991) showed that as the respiratory quotient in avocado is approximately 1, it is unlikely that lipids are the primary source of respiratory energy, and that carbohydrates fill this role. With respect to the avocado, it has been postulated that C7 sugars in particular are associated with not only the metabolic processes whilst the fruit is developing but are also involved in the respiratory processes linked with fruit maturation, postharvest quality and fruit ripening (Liu *et al*., 1999) as these carbohydrates form the major portion of total soluble carbohydrates. It has been suggested in numerous studies that changes in the carbohydrate reserves and use as well as fruit quality may be affected by varying storage temperatures and durations (Kikuta and Erickson, 1968; Spalding, 1976; Eaks, 1990; Luza *et al*., 1990). In a study conducted by Liu *et al.* (1999) it was found that the common sugars sucrose, fructose and glucose as well as D-mannoheptulose showed a significant decrease in the exocarp and mesocarp of the fruit when stored at 1°C or 5°C, which in turn suggests that it is these sugars which are used in the respiratory process. 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. It was suggested that the C7 sugars act as translocated forms of carbon, storage reserves and respiratory metabolites. Liu *et al.* (1999) indicated that when the fruit is removed from the tree and the sugar reserves become depleted, an inhibitory effect of the C7 sugars diminishes, acting as a trigger for the ripening process. Further work on C7 sugars has indicated that these compounds may provide protection against stresses due to their reducing power (Liu *et al*., 2002), however, no substantial evidence has been provided. It has been found, however, that an unusual C7 sugar alcohol, Volemitol, plays an important role in carbon assimilation, translocation and storage, provision of reducing power, and protection against various stresses in a certain *Primula* species (Hafliger *et al*., 1999), and this C7 sugar has reportedly been found within avocado fruit as well (Cowan, 2004).

2.1.2. Structural changes

The cell wall and membrane play important roles in the process of fruit ripening, in particular the plasma membrane (Bower and Cutting, 1988). Dallman *et al*. (1988) found that the plasma membranes and Golgi bodies showed an increase buoyant density within the ripening period, whereas the mitochondrial membranes and thylakoids showed no significant changes within the same period. In the case of avocados, lipid peroxidation is considered to be one of the earliest detectable processes in fruit ripening (Meir *et al*., 1991). Some of the ultrastructural changes include the degradation and eventual collapse of the cell wall, as well as the swelling of the rough endoplasmic reticulum (Platt-Aloia and Thompson, 1981).

2.1.3. Enzyme activity

Softening of the fruit is due to the degradation of cell wall integrity, with the main cause being increased activity of certain enzymes. It was found by Scott *et al*. (1963) that cellulose is the major constituent of avocado cell walls, therefore it was thought that cellulase would play a major role in the softening of cell walls. This enzyme increases rapidly with the evolution of ethylene and therefore control of ethylene production will impede cellulase activity (Pesis *et al*., 1978). In later studies by Hatfield and Nevins (1986) the role of cellulase was further understood in that the enzyme is a (1-α4)-β-D-glucanase which hydrolyse only (1-4)-β-glycosyl linkages, and does not appear to solubilise the cellulose polymers found in mature avocado cell walls. This lead to the conclusion that the cause of softening and breakdown of cell walls cannot be due to cellulase alone. It has been proposed that cellulose fibrils are hydrolysed, which concurs with microscopic observations of changes in cellulase fibres. Hydrogen bonding to other polysaccharides in the cell wall may be altered during ripening, disturbing the cell wall matrix and thus making the polygalacturans in the cell wall more accessible for enzymatic breakdown (Hatfield and Nevins, 1986). Overall it is concluded that cellulase appears to be responsible for early stages of fruit softening, which is controlled in part by ethylene, and polygalacturonase is responsible for final fruit softening (Bower and Cutting, 1988).

Currently it is considered that the cell walls of the avocado fruit are comprised of several compounds, such as cellulose, hemicellulose, and pectin. There are several ripening enzymes which have been characterised in the avocado fruit. These include cellulase (EC 3.2.1.4; β-1,4-endoglucanase), endo-polygalacturonase (PG; EC 3.2.1.15), pectin methyl esterase (PME; EC 3.2.1.11), β-1,4-xylosidase, β-1,4-xylanase and β-galactosidase. Fruit softening is brought about by these enzymes operating in conjunction hydrolysing the cell wall components (Blakey *et al*., 2009b)

Enzyme activity has been shown to increase rapidly after cold storage (Blakey *et al*., 2010), with a probability of enzyme activity occurring, to a certain extent, during cold storage. Activity of enzymes, such as cellulase, needs to be minimised in order to reduce cell wall degradation and subsequent fruit softening during cold storage. The most conventional and practical method of reducing any enzyme activity is to reduce the temperature. As it has been found that fruit softening occurs, to a certain extent, at 5.5°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.

2.1.4. Role of ethylene

The avocado fruit is a climacteric fruit, ripening rapidly and has a short shelf life (Wills *et al.*, 1998). Climacteric fruit show a distinct rise in the respiration rate when ripening has commenced, followed by a decline (Figure 1.3). Ethylene plays a significant role in this process and leads to the following definition of the climacteric by Rhodes (1981) 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.”

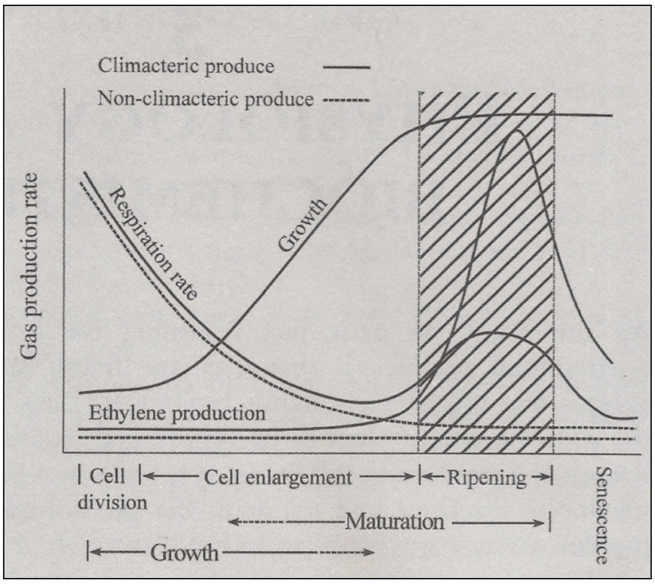

Figure1.3: Growth, respiration and ethylene production pattern of climacteric and non-climacteric plant organs (Wills *et al.*, 1998)

The exact role of ethylene in avocado ripening has been studied extensively with differing conclusions. Ethylene is commonly thought of as a trigger to the ripening process (Starrett and Laties, 1993). However, it has been shown that ethylene may not only be a trigger to ripening but is also required to be continuously present throughout the ripening period in order for successful ripening (Zauberman *et al.*, 1988). A detailed investigation by Bower and Cutting (1988) suggested that ethylene does play a significant role in the ripening process, but is neither the trigger nor the cause of ripening. Eaks (1966) showed that if ethylene is applied to produce postharvest, it does result in an earlier climacteric peak and subsequent earlier ripening. This is an important trait with respect to the export of climacteric fruit, such as avocados, as the management of respiration and ethylene levels is essential to delay the ripening process within the shipping period and deliberate applications to enforce ripening when fruit arrive at the market. A break within the cold chain may result in an increased respiration rate and initiate ethylene production, which would result in fruit softening within cold storage.

The initial steps involved in ethylene biosynthesis include the amino acid methionine being converted to S-adenosylmethionine (SAM) by methionine adenosyltransferase, followed by SAM being converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase. However, this step is the rate-limiting step in overall ethylene production. Finally ACC is converted into ethylene by ACC oxidase (Blumenfeld *et al.*, 1986).

$$\textit{Methionine} \xrightarrow{\text{methionine adenosyltransferase}} \textit{SAM} \xrightarrow{\text{ACC synthase}} \textit{ACC} \xrightarrow{\text{ACC oxidase}} \textit{Ethylene}$$

Blumenfeld *et al*. (1986) found that ACC is only present in small amounts when fruit are still attached to the tree, with an increase in ACC synthase activity when fruit are harvested. Thus, the ACC concentration in the fruit on the tree, which is limited by low ACC synthase activity, may be the limiting factor for the on-tree ripening and only after harvest (reasons for activation uncertain) is the ACC synthase activity increased, which elevates ethylene levels and the ripening process begins.

There is no clear evidence as to what the substrate for respiration in ripening avocado fruit is, however, it is believed that it is probably a carbohydrate rather than a lipid despite the high lipid content of the fruit, due to the ratio of carbon dioxide produced to oxygen consumed being a value of approximately 1 (i.e. the respiratory quotient) (Blanke, 1991). The predominance of C7 sugars implies that they may play a role, and it was found by Liu *et al.* (2002) that the respiratory climacteric is not initiated until a significant drop in C7 sugars occurs.

During the climacteric period in avocado fruit the production of ATP increases (Biale and Young, 1971). This is thought to be a defence mechanism initiated by the mitochondria due to an associated increase in membrane permeability allowing for harmful substances to be present around the mitochondria (Huang and Romani, 1991). Due to this increase in ATP the ADP/ATP balance is altered which is observed as the change from preclimacteric to climacteric (Bennett *et al*., 1987). It is possible that it is not the increase in energy demand which is the trigger, but rather the changes which occur in the cytosol or mitochondria (Blanke, 1991).

2.1.5. Role of plant growth regulators

Plant growth regulators (PGR's) influence fruit ripening, not only for avocados but also for many other commodities (Bower and Cutting, 1988). Ethylene plays a critical role in avocado fruit ripening (Seymore and Tucker, 1993). The production of ethylene may also be influenced by the presence or absence of particular PGR's. Events such as water stress or wounding of the fruit increases the production of ethylene, which may be linked to production of the stress hormone abscisic acid (ABA) (Cutting *et al*., 1986; Vendrell and Palomer, 1997; Blakey *et al*., 2009b). The PGR's which have been found to inhibit ripening include cytokinins, auxins and gibberellins (Rhodes, 1981).

2.1.6. Temperature effect on ripening

Temperature is a critical component in the postharvest life of fresh produce. Metabolic activity is of vital importance in not only the storage and shipping of fruit, but during the ripening process after shipping. Low temperatures are needed during the shipping of fruit in order to significantly reduce the metabolic activity, particularly the ripening enzymes and ethylene production which occurs only in a certain temperature range (Donkin, 1995), so that shelf life is extended. The control of ethylene is practiced in the commercial storage of many fruits as high concentrations during cold storage is undesirable (Hofman *et al*., 1995). After the cold storage shipping period ethylene treatment enhances ripening without injury (Zauberman *et al*., 1988). The temperature is also increased and controlled after the shipping period so that the enzyme activity is reactivated and so that the incidence of various disorders is controlled. It has been shown that body rots, stem-end rot, vascular browning and uneven ripening increases as the ripening temperature increases from 20°C to 30°C, whilst at 15°C the incidence of these was significantly decreased (Hopkirk *et al*., 1994). It is therefore essential to maintain a balance between optimal ethylene production and limiting 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. Water Relations

Water is the main constituent of the fruit and due to this the water content may play a crucial role in eventual quality aspects (Wills *et al*., 1998). Once the fruit is removed from the tree water loss cannot be replaced so conditions and practices need to be in place to ensure minimal water loss (Donkin, 1995).

3.1. Maturity

Kaiser *et al*. (1995) define physiological maturity in avocados as “that stage of development at which the fruit, once detached from the tree, will ripening 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). Postharvest fruit quality is highly reliant on fruit being harvested at the correct maturity (Milne, 1998).

The maturity index used in the South African avocado industry is based on water content of the fruit (Eksteen, 2001). As water content declines and oil content increases, so does maturity. Water content is, however, easier to measure than oil. In avocado fruit, water needs to decline and the oils increase (while fruit is still on the tree) to a particular threshold level for the fruit to be considered physiologically mature, and normal ripening to occur after harvest. For the ‘Hass’ cultivar, the water content of the fruit which is acceptable for harvest is considered to be at most 77% (Ernst, 2007). However, it is well known that early-season fruit with a high water content are more susceptible to storage disorders such as chilling injury (Kosiyachinda and Young, 1976) than more mature fruit. It is generally considered that water content levels of early-season fruit are in the low 70% values (±73%), mid-season fruit in high 60% values (±69%), and late-season fruit in the mid 60% values (±66%) (Roets *et al*., 2010; Van Rooyen, 2009).

3.2. Water stress

The occurrence of water stress is a major concern within any fresh produce commodity. Water loss, even in small quantities, can result in a reduced appeal to the consumer as well as upset physiological processes within the fruit. Water stress can be manifested pre- or postharvest where both can affect the quality, ripening and shelf life negatively (Figure 1.4) (Wills *et al*., 1998).

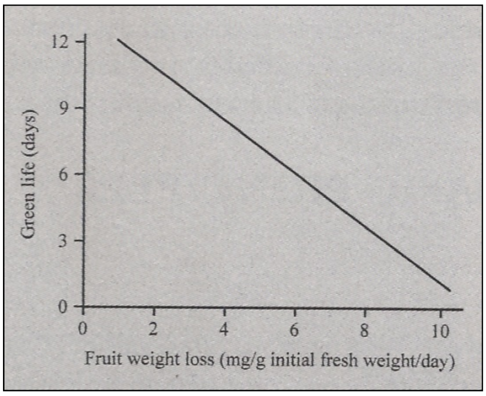

Figure 1.4: Relationship between weight loss (predominately water loss) and shelf life of mangoes (Wills *et al*., 1998)

3.2.1. Pre-harvest water stress

Fruit which are subject to water stress early in their development have an altered ethylene evolution pattern mainly because the biosynthetic pathway of ethylene is reliant on water for the biochemical reactions. Bower (1984) suggested that the synthesis of ethylene is reduced due to long term water stress altering the membrane functioning. This can result in uneven ripening and a marked compromise in quality. It was found that fruit with a lower water potential at the time of harvest have the tendency to ripen more rapidly after storage (Bower, 1984).

3.2.2. Postharvest water stress

Postharvest water loss is a critical factor that needs to be prevented at all possible points within the logistics chain. The issue of postharvest water loss is of major concern due to the fact that the water lost from the fruit cannot be replaced after the fruit is detached from the tree. As with pre-harvest water stress, high postharvest water loss also affects ethylene synthesis and the subsequent peak, leading to fruit ripening at a more rapid rate, whereas if the fruit loses less water, then the shelf life is extended and ethylene production reduced (Bower *et al*., 2003). Water loss does not only have an influence on the ripening of the fruit, but also plays a significant role in the sensitivity to chilling injury (Bower and Magwaza, 2004). Postharvest water loss can also result in internal quality defects (Arpaia *et al*. 1992) with enhanced potential for mesocarp discolouration and decay incidence and severity.

3.2.3. Water loss control

Bower and Magwaza (2004) found that in the case of avocado fruit, the degree of chilling injury was influenced by water loss during storage (Figure 1.5). The proposed solution in this study was to use waxing or micro-perforated bags to limit the amount of water lost from the fruit. The results showed the wax reduced water loss by 6.7% and the micro-perforated bag reduced the water loss by 14.7%. However, wax may alter internal fruit gaseous concentrations, which may also alter physiological activity.

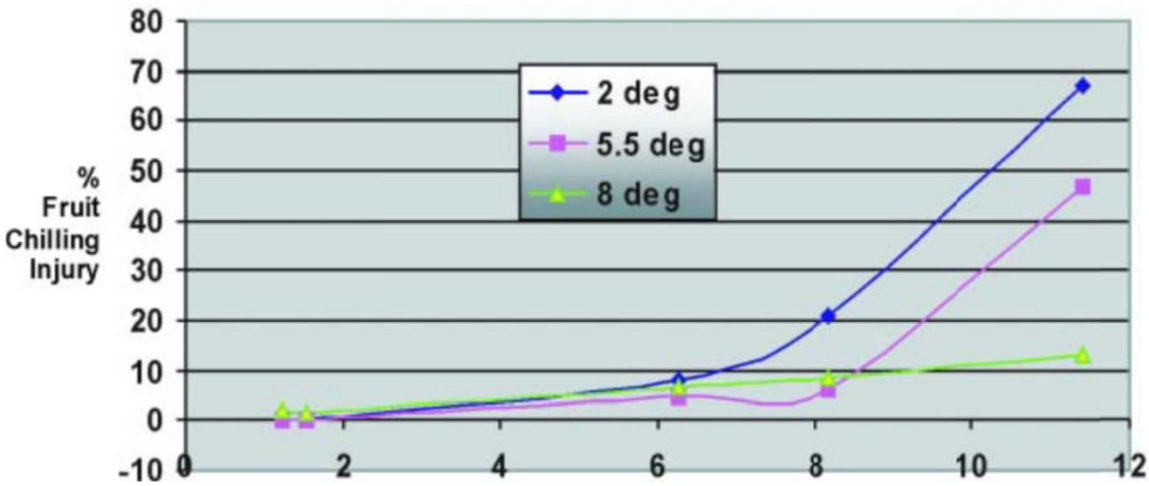

Figure 1.5: Effect of increasing mass loss and temperature on the incidence of fruit chilling injury for 'Fuerte' (Bower and Magwaza, 2004)

The time taken to remove field heat from the fruit as well as the relative humidity at which the fruit are stored affects the amount of water loss from the fruit. The fruit is generally stored at a relative humidity (RH) of 90% (Wills *et al*., 1989) but current protocol tends towards 95%. This is, however, an optimistic value because in actual shipping it is nearly impossible to maintain a RH value of 95%. It has been shown for several fruit that relative humidity is more important in cold storage than during cooling (Mitchell, 1992).

To reduce water loss during cooling, it is necessary to reduce the vapour pressure deficit (VPD), the differential in water content between the fruit and the surrounding air, as rapidly as possible. 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), can be used to explain why a high RH (85-95%), without free water being present, 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 (Mitchell, 1992). 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.

4. Postharvest Storage

Harvested fruit remains a living commodity and needs to be retained as such for as long as possible. The fruit will have continuous processes such as respiration and associated metabolic activity throughout its postharvest life. Ripening is also a major factor that needs to be addressed once the fruit has been harvested. In order to retain shelf life for as long as possible there are a number of current procedures in place to delay ripening, such as low temperatures, CA, and use of 1-MCP (Maré *et al*., 2002).

4.1. Controlled Atmosphere (CA) & 1-MCP

4.1.1. CA

Experience has shown that as a result of long shipping times to the traditional markets for South African fruit, refrigeration alone, at conventional threshold ‘non-damaging’ temperatures, is not sufficient to extend the storage life of the fruit (Bester, 1982; Bower and Cutting, 1988). This is primarily the reason why CA has been implemented. This system makes use of altering atmospheric conditions within the shipping containers and maintaining them. With the shipment of avocados common recommendations for CA conditions are 2-5% O_2 and 3-10% CO_2 (Thompson *et al*., 1998; Burdon *et al*., 2008). If CA is used correctly then the results are be highly beneficial due to an atmosphere of low oxygen and/or elevated carbon dioxide, which reduces fruit respiration and ethylene production rates. Controlled Atmosphere conditions will retard the loss of chlorophyll, biosynthesis of anthocyanins and carotenoids, and biosynthesis and oxidation of various phenolic compounds (Kader, 2003). However, the use of CA may not be as helpful as presumed. It has been found the even slight variations in the atmosphere composition can cause extensive losses. Work of Arpaia *et al.* (1990) and Burdon *et al.* (2008) showed that avocado fruit seem to be very sensitive to CO_2 levels above 5%. Retarded colour change (‘Hass’) and an increased incidence of rots was recorded. Further even 1 ppm of ethylene present in the atmosphere (2% O_2, 2.5% CO_2) may negate the positive effects of CA.

4.1.2. 1-MCP

The use of 1-methylcyclopropene (1-MCP), sold as Smartfresh®, in the commercial export industry for horticultural produce has been highly successful and is now one of the major forms of counteracting ripening in storage. In the South African context the use of 1-MCP has been found to be more effective than CA (Lemmer *et al.*, 2002). 1-MCP is applied in gaseous form at very low concentrations, and binds irreversibly with the ethylene binding sites within the fruit cells. By carefully controlling the application dosage, the proportion of ethylene binding sites rendered unavailable to ethylene can also be controlled, thus controlling the rate of ripening (Lemmer and Kruger, 2003). Even though 1-MCP does, in general, work well for the intended purpose of ensuring hard fruit on arrival in the market by decreasing the rate of ripening, it still does have some problems associated with the use of it. For best results it needs to be applied to the fruit within 72 hours of harvest otherwise it is not effective (Nelson, 2005). Further, a study conducted by Maré *et al*. (2002) showed that fruit stored in normal atmosphere had a higher percentage of 'sound fruit' than 1-MCP treated fruit, 91.3% vs 85%. Lemmer and Kruger (2003) also reported a significant amount of fruit that had anthracnose and stem end rot once ripened for 1-MCP treated fruit. Ripening after storage may also be uneven. The inhibition of ethylene action through the blocking of binding sites will delay ripening, and although it is thought that new receptor sites may form, this will be time consuming, and as a result fruit ripening could be variable and retarded and result in the fruit having to stay in storage before entering the market. This is particularly problematical for pre-packers wishing to ripen fruit before sale. It is therefore crucial that the concentration of 1-MCP is correct. If there is too much of the chemical present within the fruit then the fruit may be too slow to ripen or never ripen at all. It was found that the ripening time for 'Fuerte' and 'Hass' was effectively doubled with the use of 1-MCP and some fruit never ripen (Lemmer *et al.*, 2002). Correct application needs care, as the concentration rates of 1-MCP that need to be applied are in the parts per billion (Roets *et al*., 2010), so it is possible that a mistake can easily be made.

However, a major consideration in the choice of both CA and 1-MCP is the cost. Presently, the application cost is within the region of US$800-1500 per container. The CA needs to be maintained and regulated to ensure it is effective, and the required hardware (containers and control systems) is costly. The chemical and application costs for 1-MCP are similar.

4.2 Cooling methods

Cooling is an important factor relating to eventual fruit quality. There have been indications in studies that water loss, and therefore potential for chilling injury, occurs largely during the initial cooling phase of the fruit (Bower and Magwaza, 2004). The type of cooling systems used may influence how much water is lost by the fruit. Cooling of the fruit also results in the metabolic activity of the fruit decreasing, so the cooler the fruit are, the longer the shelf life will be, provided chilling injury is not induced.

4.2.1. Static air cooling

Static air cooling involves cold air being blown across the top of the produce and heat transfer from the product occurring by diffusion, with the air then being circulated back through the cold room heat exchanger to again be cooled (Figure 1.6). This cooling allows the fruit to be cooled and stored in the same room. However, this type of cooling is slow and may result in excessive water loss from the fruit. Because cooling rate is slow, air passes over the cooling coils for a long period of time and water is lost from the atmosphere due to condensation on the cold coils. The reduction in water vapour creates a higher vapour pressure gradient between fruit and the atmosphere in the cold room, thus greater water loss from fruit to atmosphere. (Mitchell, 1992; Donkin, 1995).

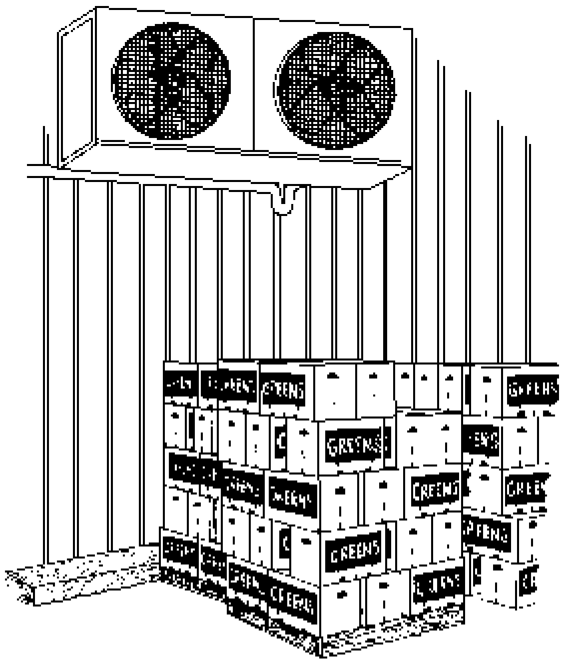

Figure 1.6: Static air cooling system (Boyette *et al.*, undated)

4.2.2. Forced air cooling

Forced air cooling involves the air being forced through the fruit pallets and passed the fruit (Figure 1.7) which allows for faster removal of heat and therefore quicker cooling compared with static air cooling (Figure 1.9). The disadvantage of this cooling method is that possible desiccation may be a factor if the RH is not high enough, due to high wind velocities associated with this system (Mitchell, 1992), as well as possible occurrence of external chilling injury on 'Fuerte' avocados (Slabbert and Toerien, 1984) due to an evaporative cooling effect.

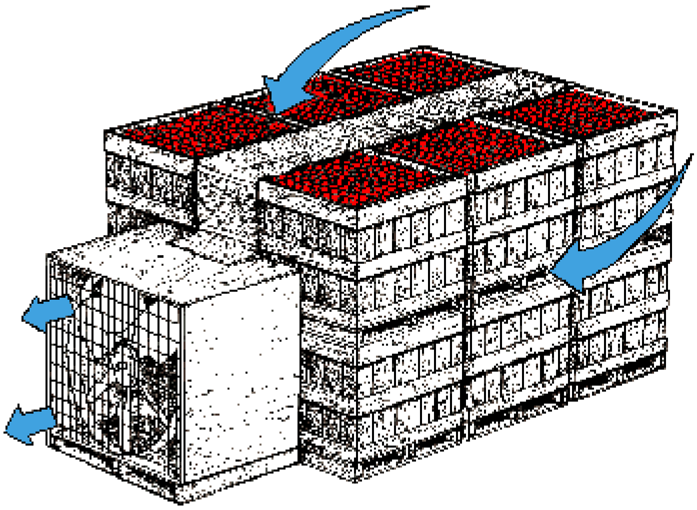

Figure 1.7: Forced air cooling system. Arrows denote air movement (Boyette *et al*., undated)

4.2.3. Hydrocooling

Hydrocooling makes use of cold water to remove heat from the avocado fruit. It involves the fruit being placed in bulk bins and then passed through a cold water shower (Figure 1.8). The advantage of this type of cooling is that there is a negation of water loss and it also allows for rapid cooling of the fruit (range from 10-15 minutes) (Figure 1.9). This system is said to be more efficient than forced air cooling (Wills *et al*., 1998). The disadvantages are that the fruit may experience a rise in temperature during the packing process once cooling has stopped (Mitchell, 1992) and fruit will need to be dried before packing.

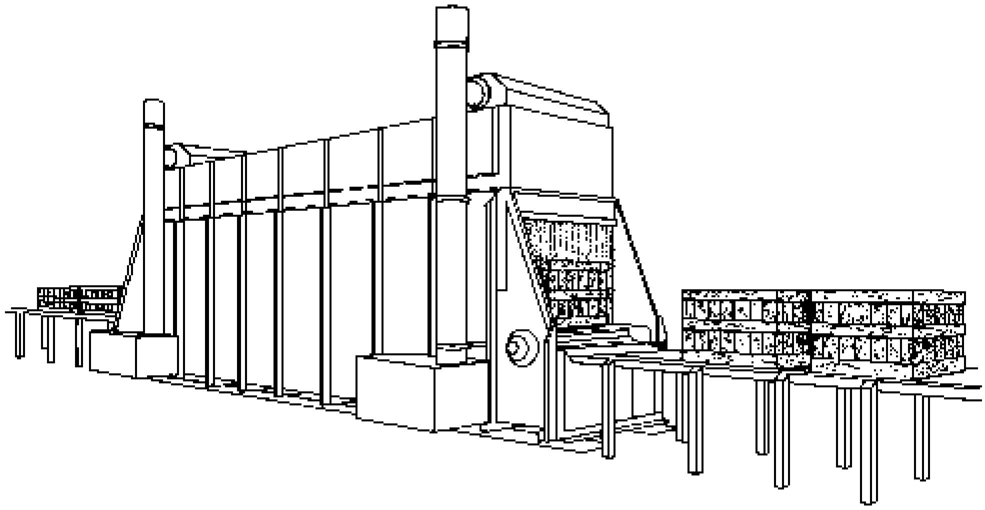

Figure 1.8: Hydrocooling system (Boyette *et al*., undated)

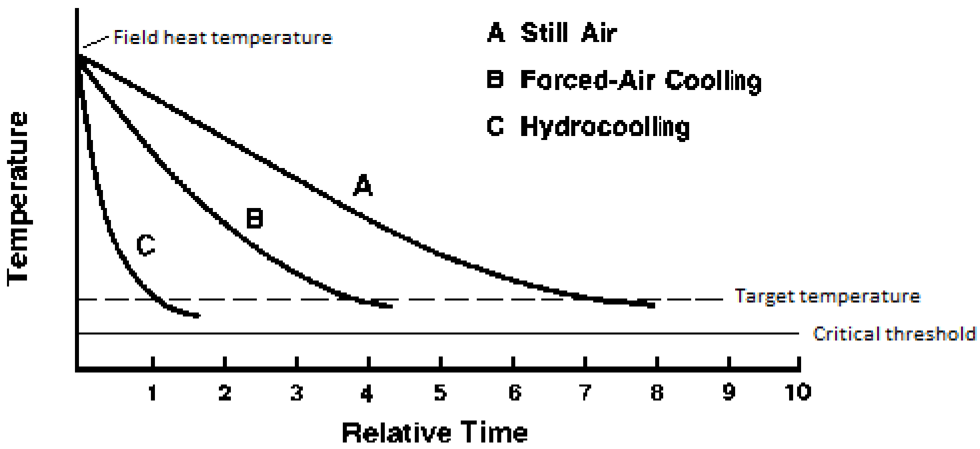

Figure 1.9: Time taken to reduce fruit temperature to a sufficient level for three different cooling systems (static air, forced air and hydrocooling) (Boyette *et al*., undated)

5. Chilling Injury

Chilling injury (CI) can be defined 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. A chilling temperature is any temperature below the critical threshold temperature (but above freezing) that causes injury.” (Lyons and Breidenbach, 1987).

5.1. Factors affecting chilling injury

The extent of CI attained by a fruit varies with species and among different cultivars of the same species. To compound the interaction effects further, environmental conditions prior to harvest greatly influence the susceptibility to chilling injury (Kader, 2002; Kader and Rolle, 2004). While a number of possible mitigating treatments exist, there is no single evident solution.

The degree of chilling injury incurred is heavily dependent on the temperature that the fruit or plant parts are exposed to. Along with this, the duration of exposure is important, as chilling injury is essentially a temperature to time interaction. The longer the fruit are exposed to a chilling temperature below its critical threshold, the greater the severity of chilling injury that will be incurred (Van Rooyen, 2006).

Greenskin avocado cultivars are much more susceptible to chilling injury, especially externally. ‘Hass’ is slightly more tolerant then greenskin avocados and can withstand cooler storage temperatures (Eaks, 1976). It was found by Swarts (1982) that the temperature sensitivity of avocados (particularly ‘Fuerte’) was heavily influenced by the number of hours that pre-harvest orchard temperatures fell below 17°C, which further complicates the potential for damage at any particular temperature.

The potential for chilling injury varies throughout the season (Swarts, 1980; Bower *et al*., 1986; Voster *et al*., 1987). Fruit from the early-season are more susceptible to chilling injury than fruit from the late-season (Smith and Lunt, 1984). Toerien (1986) proposed a "step down" acclimation procedure, but currently the export of South African avocados makes use of an integrated approach in which fruit maturity determines the degree of temperature reduction (Vorster *et al*.,1990; Donkin and Cutting, 1994). Early-season fruit are not as tolerant of low temperatures and therefore need to be shipped at slightly higher temperatures to avoid significant chilling injury.

The stresses associated with water loss during storage appear to be a contributing factor to chilling injury. Bower *et al*. (2003) showed that the higher the water loss after harvest, the higher the chilling injury, and that the threshold temperature for damage was higher if water loss was greater, showing that there is an interaction between water loss and chilling injury. Relative humidity levels in excess of 95% in cold storage resulted in a restriction of chilling injury due to a reduction in water loss by the fruit through transpiration (Adato and Gazit, 1974). Early-season fruit, which have a high moisture content and a low oil content, are more sensitive to lower storage temperatures and, therefore, more susceptible to CI, whereas late-season fruit have a lower moisture content and higher oil content and are, therefore, less susceptible to chilling injury at low storage temperatures (Van Rooyen and Bower, 2006).

Water stress is not just a postharvest issue. Pre-harvest water stress may also affect potential for postharvest chilling injury. This may be partially through the influence on uptake of calcium. Calcium is an important mineral that is required by the plant for structural integrity of cell membranes and cell walls. If this mineral is deficient within the plant's organs many postharvest physiological disorders may occur (Chaplin and Scott, 1980; Eaks, 1985; Cutting *et al*., 1992; Hofman *et al*., 2002). Any factor that results in a reduction in calcium uptake into the plant, and subsequent transport to the fruit, will increase the chance of calcium related physiological disorders. One of the major factors which affect calcium transport into and within the plant is water stress and low transpiration rates. This is due to phloem immobility of calcium as it can only move efficiently through the xylem with the transpirational flow (Marschner, 1995). Further reduction in calcium allocation to the fruit results from the simultaneous development of spring vegetative growth(flush) and early fruit, creating competition between developing leaves and fruit. Bower and Cutting (1988) demonstrated that the terminal bud is initially a stronger sink than the fruit due to higher auxin movement, and because of this stronger sink the terminal bud receives more calcium than the fruit. It is therefore necessary to apply any cultural practice possible to reduce the quality reducing effects to the fruit from spring flush, and find a balance between fruit growth and vegetative growth. Calcium is important to the fruit/plant, particularly in terms of chilling injury, as it plays an important role in cell division and cell development (Marschner, 1995), and brings about a stabilization effect in cell membranes (Battey, 1990). Calcium also affects the permeability of the membrane (Ferguson and Drobek, 1988), so if less calcium is available there is likely to be less electrolyte leakage (Simon, 1978). Calcium also plays a significant role in cell wall structure (Ferguson, 1984) where it is a normal component of the middle lamella (Conway *et al*., 1992). Cross linkages are formed when calcium ions bond to the pectin present within the middle lamella resulting in strength and stability (Burns and Pressey, 1987). The cross linkage bonds reduce the ability of enzymes to break down cell walls, resulting in slower softening (Conway *et al*., 1992).

Avocado fruit are more sensitive to chilling injury at the climacteric peak (Couey, 1982) and noticeably more tolerant before and after the climacteric peak, with the highest observed tolerance two days after the climacteric peak (Paull, 1990). This sensitivity may be related to the high metabolic activity occurring at certain periods within the fruit, particularly in the immediate pre-climacteric phase. Further, chilling damage may, in part, be due to the presence of ethylene in the storage atmosphere (Donkin, 1995).

5.2. Symptoms

Chilling injury may be found in many different forms, some obvious but others not so evident. Chilling injury is usually most damaging when the plant tissue is exposed to cold temperatures for extended periods of time, however, this temperature to time interaction limit varies for different species and cultivars. The common symptoms most apparent in avocado fruit are external injuries which are apparent on the exocarp. Blackened areas become visible and tissue eventually collapses, referred to as “pitting” (Saltveit and Morris, 1990; Van Rooyen, 2006). The damage first occurs in the inner cell layers of the exocarp, and then moves to the outer layers (Woolf, 1997). The next obvious visual sign of CI is the internal discolouration of the mesocarp which includes vascular browning (Van Rooyen, 2006). Less obvious CI symptoms include failure to ripen normally after storage, water soaking of tissues, cellular damage, and increased susceptibility to decay (Saltveit and Morris, 1990).

5.2.1 Mesocarp discolouration

Mesocarp discolouration is not always associated with vascular browning, but may include light grey discolouration to entire blackening of the mesocarp. Swarts (1984) found two common forms, namely grey pulp and pulp spot, which are considered independent of each other, yet may still occur within the same fruit. Grey pulp is a general grey discolouration or browning of the mesocarp, whereas pulp spot is seen as localised spherical grey spots in the mesocarp associated with the cut ends of vascular bundles.

The browning reactions within the fruit are the result of a two step process which begins with polyphenoloxidase (PPO) catalysing the oxidation of o-diphenols to the corresponding o-quinone. In the second step the o-quinones is irreversibly oxidised to brown melanin pigments when oxygen is present (Bower and Cutting, 1988).

$$o\text{-}diphenols \xrightarrow{\text{PPO}} o\text{-}quinones + H^+$$
$$o\text{-}quinones + O_2 \longrightarrow melanin$$

The symptoms become evident when cell damage occurs due to physical means or a breakdown in cell compartmentation which may be caused by, among other factors, chilling injury. The PPO is released from the thylakoid membranes, where it is bound in a latent form, and mixes in the cytoplasm with the phenolic substrate where browning then occurs (Bower and Cutting, 1988).

5.3 Physiology of chilling injury

The reduction of temperature during shipping and storage causes many physiological as well as biochemical reactions within fruit cells. There have been numerous investigations into the stress imposed on plant cells caused by chilling. These include effects on the structure and function of membranes, enzyme conformation, photosynthetic function, respiration rates, as well as termination of cytoplasmic streaming (Morris, 1982).

5.3.1. Cell membranes

When plant cells are subjected to chilling temperatures, changes to the mitochondria, tonoplasts and chloroplasts occur (Murata, 1990). These changes are not initially visually evident but are the precursors to visible chilling injury. Due to the changes, the ultra-structure of the cell is affected leading to the function of membranes being compromised. The membrane permeability increases which allows for water and solutes to be passed through the membrane at an increased rate (Murata, 1990). This increased permeability has been found to have an effect on membrane bound enzymes, altering their activation energy. If membrane permeability is increased, it increases the activation energy for membrane bound enzymes which leads to a suppressed reaction rate. Non-membrane bound enzymes are however not affected, instead membrane permeability creates an imbalances within critical systems such as mitochondrial respiration and glycolysis, as well as photosynthetic metabolism (Lyons, 1973).

5.3.2. Primary vs. secondary chilling injury

The cause of chilling injury symptoms is often the cause of confusion and controversy due to a notion that there is more than one event which is ultimately responsible. Raison and Orr (1990) stated that the process of chilling injury includes two stages, a primary event and a secondary event (Figure 1.10). The primary event is temperature dependent and is activated when the temperature drops below a particular critical temperature threshold. The primary event is thought to involve a membrane phase transition which initiates several secondary events which involve metabolic dysfunctions. The process is reversible if the exposure to chilling has not been too great for too long and cell integrity has not been compromised. The chilling temperatures create an imbalance in metabolism within the cells, but a rise in temperature in the reversible stage, creates an increase in metabolic activity allowing for intermediates accumulated from the chilling stress to be removed, resulting in the restoration of the metabolic balance within the cells. However, if the cell wall or membrane integrity has been compromised and the temperature rises it accelerates the cellular degradation and injury.

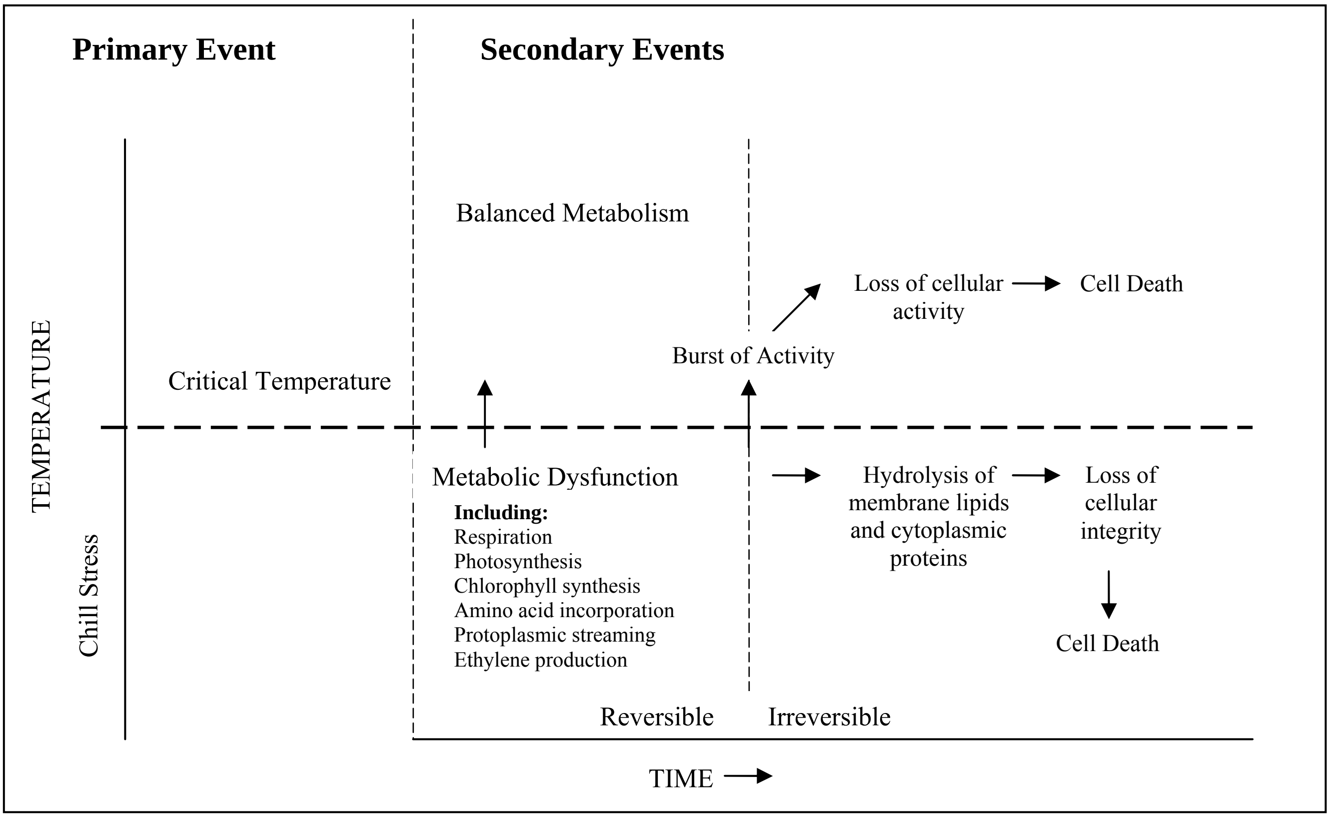

Figure 1.10: Relationship between the primary and secondary events of chilling injury (Raison and Orr, 1990)

5.4. Fruit quality effects

The avocado being of subtropical origin, is considered chilling sensitive and ideally should not be cooled and stored at temperatures close to freezing point due to chilling injury occurring at a temperature much higher than 0°C (Fuchs *et al.*, 1995). The critical/threshold temperature for avocado fruit in cold storage is said to be 8°C (Lyons, 1973). However, for the South African industry to make exporting to the European market possible, a delay in fruit softening is necessary, which is achieved in part by lowering the temperature below the threshold of 8°C (Eaks, 1976).

While it is well established that decreasing the temperature in the storage of avocados can affect the fruit quality if the storage temperature is too low, a compromise is necessary so as to adequately delay ripening yet have minimal chilling injury effects. This has become possible, because chilling injury is dependent on numerous factors, not only temperature. Cultivars differ in tolerance. It was found by Bower and Papli (2006) that 'Hass' for example, can be stored at 1^{o}C for 30 days with fairly commercially insignificant chilling injury symptoms, with no internal damage and only slight external damage. It was also found that the greenskin cultivar 'Pinkerton' can be shipped at 2°C with minimal mesocarp discolouration but with some external injury apparent (Van Rooyen and Bower, 2007). Preliminary results published by Lütge *et al.* (2009) showed that 'Fuerte' stored at 2°C had excellent internal quality but external chilling injury was significantly evident. Early-season fruit have been found to be much more susceptible to chilling injury than fruit harvested later in the season (Swarts, 1980). Therefore early-season fruit will be likely to suffer more damage if stored at low temperatures than later season fruit, and will therefore need to be stored at a slightly higher temperature.

5.5. Prevention of chilling injury

Several experimental treatments have been conducted on a range of avocado cultivars to minimize the occurrence of chilling injury, as the damage may be important to export industries forced by distance from market to ship at low temperature for long periods. Some have been more successful than others, but it must also be realised that pre-harvest conditions are important, and it is not possible to 'cure' bad quality fruit with post harvest treatments (Fuchs *et al*., 1995). Some of the treatments include: low temperature conditioning, which involves keeping the fruit just above the threshold temperature for a period of time before being placed into storage at lower temperature (Woolf *et al.*, 2003; Van Rooyen, 2006). Heat treatments which involve exposing the fruit to temperatures of ±40^{o}C for short time periods (Approximately 90 minutes) before being placed into cold storage were reported to be useful by Woolf (1997), but this treatment makes the fruit even more susceptible to cold chain breaks (Bard and Kaiser, 1996). Waxing and packaging to decrease postharvest water loss have been used to decrease external injury. Bower (2005) found that perforated polypropylene bags were very efficient but the use of waxing was less so, and may even be detrimental. Other experiments include the use of treatments with Ca and other chemicals such as ethylene, abscisic acid, and other natural compounds (Van Rooyen, 2006).

5.6. Influence of anti-oxidants

The definition of an anti-oxidant can be put as “any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate” (Halliwell, 1995). From a plant physiological viewpoint the purpose of anti-oxidants is to scavenge reactive oxygen species (ROS) produced within the plant cell, where the major issue is that of the superoxide radical present due to hydrogen peroxide (Vranová *et al*., 2002). These ROS are produced as by-products of photosynthesis, respiration and energy generating processes during cell metabolism (Vranová *et al*., 2002).
In order for the plant to protect its cells from high levels of ROS when under stressed conditions, such as low temperature storage, the plant tissues contain several anti-oxidant forms, such as non-enzymatic (e.g. L-ascorbic acid, phenols) and enzymatic (e.g. superoxide dismutase (SOD), catalase (CAT), peroxidases (POX)) (Blokhina *et al*., 2003). The presence of anti-oxidant compounds are important due to their protective ability, not only whilst the fruit is developing but also post harvest. Membranes, proteins and DNA can be subject to damage if the reactive oxygen species are not reduced to less reactive compounds (Borg and Schaich, 1988). Low concentrations of anti-oxidants in fruit at the time of harvest could lead to cell component breakdown which would account for increased browning potential at ripeness.

The antioxidant activity 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). Tesfay (2009) reported higher levels of total anti-oxidants in the exocarp and seed than in the mesocarp for the 'Hass' cultivar. 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 due to the exocarp characteristics of 'Hass' being thick and protective, in comparison to thin green skinned cultivars, less C7 sugars need to be transported to the exocarp to provide assistance in reducing damage. Variations in environmental conditions results in plants responding by altering their anti-oxidant metabolism (Litchtentaler, 1996). However, very little is known about the changes in anti-oxidant systems in avocados which occur during low temperature storage.

5.6.1 Ascorbic acid

Ascorbic acid (AsA) is one of the important anti-oxidant compounds present within the fruit. The main presence of this compound is within the exocarp and seed whereas the concentration within the mesocarp is significantly lower (Tesfay *et al*., 2010). It has also been noted that the concentration levels decline during the ripening process (Wills and Tirmazi, 1982). AsA plays a significant role in the resistance to environmental stresses, including photo-oxidation (Smirnoff, 2000; Conklin *et al*., 1996; Noctor and Foyer, 1998; Foyer, 1993) where it acts as a free radical reductant in plant tissues, reducing and limiting the amount of oxidative damage (Torres del Campo, 2005). AsA is an effective detoxifying compound of reactive oxygen species due to its ability to donate electrons in a wide range of enzymatic and non-enzymatic reactions. This compound can be used in the regeneration of tocopherol (which is also present in avocado fruit), which is an anti-oxidant that plays an important role in limiting membrane damage (Thomas *et al*., 1992). AsA is the main ROS-detoxifying compound in the aqueous phase due to its ability to donate electrons in many enzymatic and non-enzymatic reactions (Blokhina *et al*., 2003).

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 total anti-oxidant compounds (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°C and then increased to a level similar to the level at harvest, indicating the fruits ability to increase levels of antioxidants in response to stress. However, knowledge on the effect of storage temperature on AsA concentrations in avocados is limited.

5.7. Influence of sugars on chilling injury

Sugars within the avocado fruit play many significant roles with respect to ripening, but may also be important with respect to cold storage. Sugars have an influence on chilling injury in that they are able to reduce cell water potential within the cell, which allows a decrease in water loss as well as lowering the freezing point of the water within the cell (Popp and Smirnoff, 1995). Sugars can also stabilize enzymes and cell membranes when acting as osmoprotectants by binding to constitutive molecules. Sugars also act as a critical energy source for the anabolic processes whilst the fruit is within storage (Purvis, 1990). The antioxidant ability of certain C7 sugars, particularly mannoheptulose, could also reduce the severity and occurrence of chilling injury. Avocado fruit contain large amounts of C7 sugars, most notably *D*-mannoheptulose and perseitol, whose function were largely unknown. Liu *et al*. (1999) reported that these C7 sugars were the major non-structural carbohydrates present in avocado fruit tissues. Further research by Tesfay (2009) indicated that *D*-mannoheptulose is of paramount importance as a transport sugar. Perseitol on the other hand acts as the storage product of *D*-mannoheptulose, which can be easily converted into *D*-mannoheptulose. The identification of the exact roles of sugars found within avocado fruit is still an area of research which needs more investigation, however the general belief is that the collection of C7 sugars in the mesocarp tissue of avocados plays an important role in postharvest quality. The importance of this is that it has been found that altering of storage temperature and duration may lead to changes in carbohydrate storage and usage and ultimately affect final fruit quality of the avocado fruit (Eaks, 1990; Spalding, 1976). The common sugar glucose also plays a role in being a precursor to ascorbic acid which is a major antioxidant found within the avocado exocarp tissue (Tesfay *et al*., 2010).

6. Cold Chain Breaks

Although many studies have been conducted on the effect of temperature alterations on the quality of avocado fruit, little information is available on the effects of a break in the cold storage temperature. A study conducted by Dodd *et al.* (2007) showed concern that cold chain breaks do occur as does temperature abuse, such as significant temperature fluctuations above and below the set point, in many critical links within the cold chain logistics while exporting perishable products from South Africa. There are numerous points within the logistics chain that exchange the fruit from one form of transport to another, and therefore potential periods where the temperature of the fruit may rise. Recommendations and guidelines have been made available by the Perishable Produce Export Control Board (PPECB) for the export of avocados that give an outline for the correct handling of fruit in terms of refrigerated storage to minimize increases in pulp temperature (PPECB, 2011).

6.1. Cold chain

To understand how the fruit can be subject to breaks in the cold chain, the actual process of the logistics should be known. The first link in the cold chain starts at the pack-house after the fruit have been harvested and packed. Fruit are stored and cooled in the pack-house for a certain period, however it should not take longer than 24 hours for fruit to be cooled from "field temperature" to "shipping temperature" (SAAGA, 2007). The fruit then need to be transported either by a refrigerated road motor trailer (RRMT) or via road or rail in an integral container to the harbour. Fruit are then inspected by PPECB inspectors to ensure the correct pulp temperature is attained, and only then are unloaded and packed into a container which will be placed onto the ship. When the fruit eventually reach their destination, they are unloaded again onto a refrigerated road truck and taken to a market (Dodd *et al*., 2007; Kremer-Köhne, 1998). As explained, there are several opportunities for a break or a delay in the cold chain to occur in the logistics system if not managed correctly.

The PPECB has the responsibility bestowed upon them by the Department of Agriculture, Forestry and Fisheries in South Africa to monitor, manage and record all activities occurring within the cold chain of the products involved. The main stages which are scrutinized are at each point of loading or trans-shipping from pack-house to final loading on the ship (Figure

1.11 and 1.12). Pulp temperatures are recorded to ensure no changes have occurred. (Dodd *et al*., 2007; SAAGA, 2007)

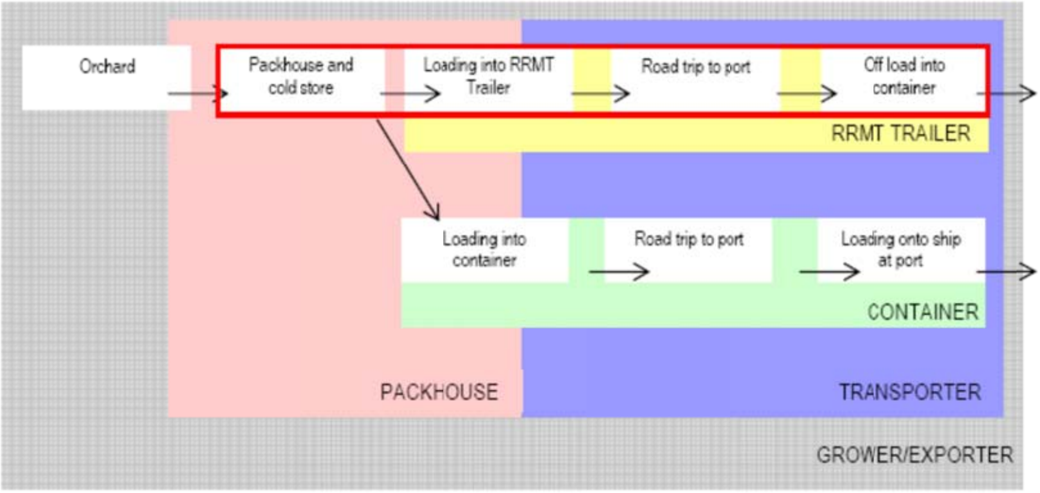

Figure 1.11: Processes involved in the logistics of avocado fruit from orchard to loading of ship. (Dodd *et al*., 2007)

The pack-house has been found to be a major area of concern. There have been indications that fruit from 33% of the pack-houses analysed had off-loading temperatures 2^{o}C warmer than the loading temperatures (Dodd *et al*., 2007). This could be due to the pack-houses not having adequate cooling systems in place to allow for correct and constant cooling of all fruit in the pallets. The other problematic area found was related to the RRMT as 60% of the RRMT loads had temperatures 2^{o}C warmer than the pack-house, as well as 62% of the loads >2^{o}C (warmer) than the original set point temperature. RRMT vehicles are only able to maintain the set temperatures if all fruit has been correctly cooled before loading, due to the small cooling units.

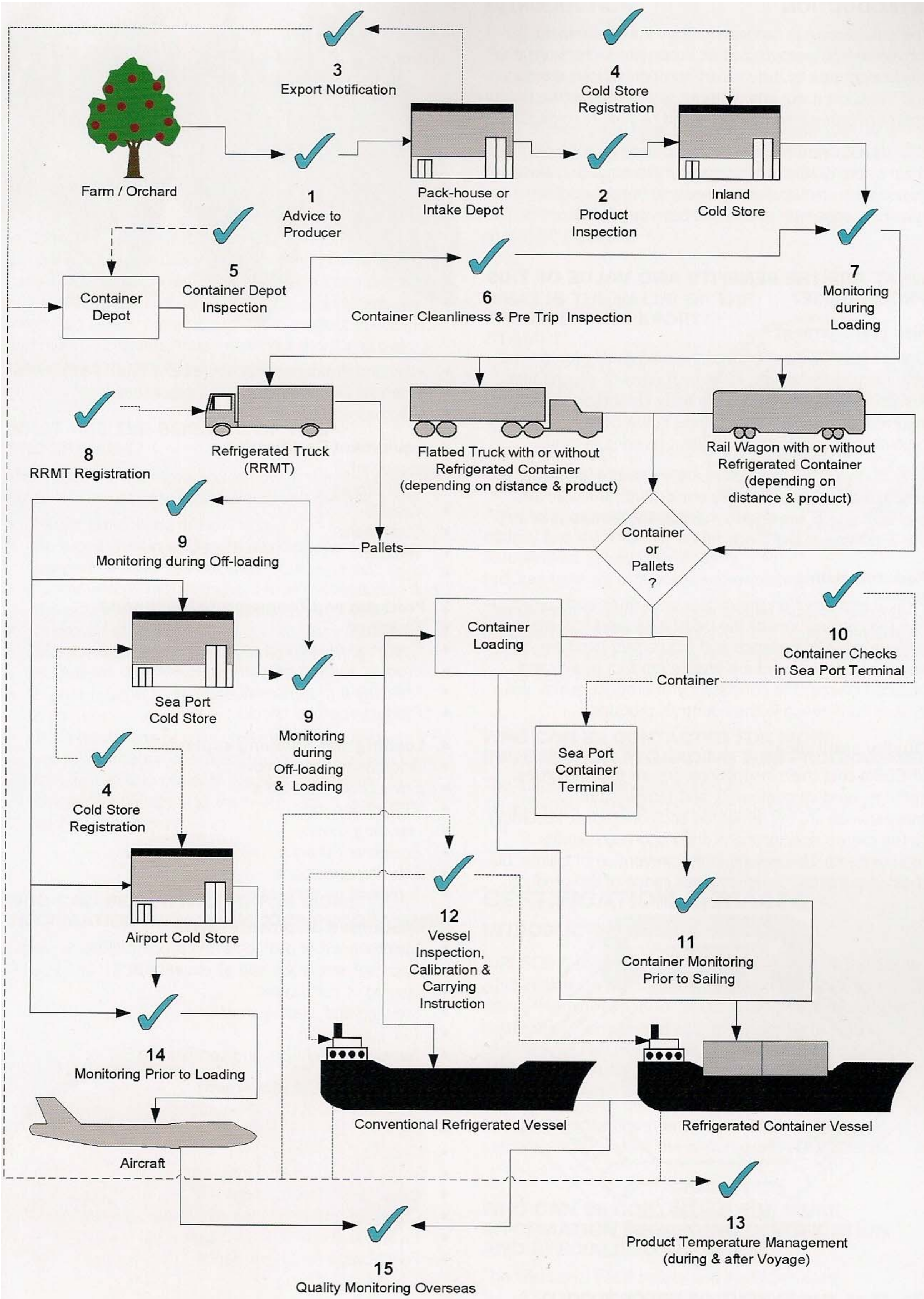

Figure 1.12: PPECB processes and checks throughout the cold chain for the export of any perishable product. (PPECB, 2011)

6.2. Effects of cold chain breaks

With minimal work having been conducted on the effects of cold chain breaks, the information on the physiological changes and possible consequences for fruit quality are not clear. While increasing temperature of storage will enhance respiration and result in softening (Biale, 1941) little is known of quality effects. Studies conducted with 'Hass' avocado fruit showed there were definite signs of mass loss, flesh softening and increase in enzyme activity (Dodd *et al*., 2007; Undurraga *et al*., 2007; Burdon *et al*., 2008). Negative effects due to a delay before controlled atmosphere(CA) establishment, such as skin colour development and delayed ripening after storage have also been reported (Burdon *et al*., 2008). However not all the results were negative because it was found that the taste of the fruit can be improved with a 25°C break for 2 days after 15 days of storage (Undurraga *et al*., 2007).
Other fresh fruit commodities such as tomatoes also severely decreased in quality after cold chain breaks, with the most striking influences being found in time taken to reach maturation as well as susceptibility to disease development (de Castro *et al*., 2005).

6.3. Solutions

In order to decrease the incidence of cold chain breaks, enhanced logistics and management systems will be needed, including adequate cooling and storage facilities, road transporters and containers, as well as efficiency in loading and unloading. While there may be a theoretical need for enhanced logistics, the practical need cannot be determined until more is understood of the physiological changes that occur, as not all parts of the chain may be equally deleterious if out of protocol, since there is even evidence of enhanced quality.