EVALUATION OF HOT WATER AND HOT AIR HEAT SHOCK TREATMENTS ON SOUTH AFRICAN AVOCADOS TO MINIMISE THE OCCURRENCE OF CHILLING INJURY

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously, in its entirety or part, been submitted at any university for a degree.

Madeleine Kritzinger

December 2002

ABSTRACT

The South African avocado fruit industry is export driven and the successful storage of fruits for extended periods is, therefore, essential. It was recorded that 7.7 million cartons were exported during the 1995 season. The shipping of the avocados takes approximately 15 days and the fruits are being stored at low temperatures to minimise the possibility of fruits softening. Unfortunately low temperature storage results in chilling injury. A possible method to increase avocado resistance to chilling injury is to administer a heat shock treatment. In this way the fruits are protected from chilling injury by inducing the formation of so-called heat-shock proteins which render the cell membranes more resistant to chilling injury.

The objective of this study was to evaluate different heat shock treatment protocols as a method of preventing or minimising chilling injury and to extend the shelf-life of avocado fruits while exporting at the lowest possible temperature. Examining the effect of different temperatures and exposure times on the quality of the different avocado cultivars pursued this. The exterior chilling injury on each fruit was quantified and the firmness and internal quality parameters evaluated.

A total of 32 Experimental Studies were conducted. The results showed that the Hot Water Heat shock Treatment (HWHST) worked effectively for the South African 'Fuerte' cultivar between 40° and 42°C for exposure times of between 20 and 30 min. The 'Edranol' cultivar also showed promising results between 40° and 42°C for exposure time of between 8 and 22 min. The HWHST was not effective on the South African 'Hass' cultivar. The 'Ryan' cultivar with its thick skin made this cultivar less susceptible to chilling injury and therefore HWHST would be unnecessary. The 'Pinkerton' cultivar had a lot of factors that influenced the results. Therefore, more research needed to be done on the 'Pinkerton' cultivar, before any conclusions could be obtained from this cultivar, although it showed potential. Hot air treatment worked fairly well, but unfortunately the long exposure time needed made this treatment unpractical. Throughout the whole study the importance of maturity surfaced as a major role in all the aspects of post harvest quality.

UITTREKSEL

Die Suid-Afrikaanse avokado vrugtebedryf is hoofsaaklik gerig op die uitvoermark en daarom is dit belangrik dat die vrugte vir 'n bepaalde tyd suksesvol opgeberg kan word. Die sensus opname gedurende die 1995 seisoen het getoon dat 7.7 miljoen bokse avokados uitgevoer is. Die avokados word vir ongeveer 15 dae per boot vervoer, wat kan lei tot vrugte wat sag word. Om dit te verhoed, word die vrugte by lae temperature opgeberg. Ongelukkig veroorsaak lae opbergingstemperature koueskade. 'n Moontlike metode om avokados te beskerm teen lae temperature en koueskade te verminder, is om 'n hitteskok behandeling toe te pas. Op hierdie manier word die vrugte beskerm teen koueskade deur die vorming van sogenaamde hitteskok proteïene wat die selwande meer bestand maak teen koueskade.

Die doel van hierdie studie was om die verskillende hitteskok behandelings protokols te evalueer as 'n metode van beskerming of vermindering van koueskade en om sodoende die rakleeftyd van avocados te verleng as die vrugte by lae temperature uitgevoer word. Eksperimente is uitgevoer om die effek van verskillende temperature en blootstellingstye op die kwaliteit van die verskillende avokado kultivars te bepaal. Die koueskade op die oppervlakte van elke vrug is bepaal en die fermheid en interne kwaliteit parameters is geëvalueer.

In totaal is daar altesaam 32 Eksperimentele Studies gedoen. Die resultate het gewys dat die Warm Water Hitteskok Behandeling (WWHB) effektief was op die Suid-Afrikaanse 'Fuerte' kultivar by temperature tussen 40° en 42°C en by blootstellingstye van tussen 20 en 30 min. Belowende resultate is ook met die 'Edranol' kultivar by temperature tussen 40° en 42°C met blootstellingtye van tussen 8 en 22 min behaal. Die WWHB was oneffektief vir die Suid-Afrikaanse 'Hass' kultivar. Die 'Ryan' kultivar se dik skil het hierdie kultivar minder vatbaar gemaak vir koueskade en daarom was 'n WWHB onnodig gewees. By die 'Pinkerton' kultivar kon daar nog nie 'n gevolgtrekking gemaak word nie, aangesien daar nog baie faktore is wat ondersoek moet word, alhoewel die kultivar baie potentiaal getoon het. Warm lug behandeling het potensiaal gehad, maar die lang blootstellingstye het

hierdie behandeling onprakties gemaak. Gedurende die hele studie is daar klem gelê op die rypheisgraad van die vrugte wat na vore gekom het as 'n belangrike faktor wat 'n hoofrol speel in al die aspekte van die na-oes kwaliteit.

To all my loved ones

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CONTENTS

Language and style used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology.* This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

CHAPTER 1

INTRODUCTION

The South African avocado industry is export driven (Bredell, 1983) and the successful storage of fruits for extended periods are, therefore, essential. The South African avocado industry specifically has to compete internationally. Some of these competitors are near the European market, which gives them the advantage of a much shorter transport distance and they are able to submit their produce early in the season. The European market demands hard avocados without any external or internal disorders. All these characteristics are influenced by horticultural aspects, pre-shipment handling procedures and travel conditions (Ginsberg, 1985).

To compete or even to be in an advantageous position, the South African producers must transport their fruits by airfreight to the European markets, which tends to be extremely expensive. In order to make the South African fruit prices competitive on the international markets airfreight is therefore not a viable option and produce has to be seafreight. However, the sea journey takes approximately 15 days from Cape Town to the nearest European port while other ports may require a further five days of ship storage time. Furthermore, additional time has to be added for harvesting, packing, transport from the subtropical areas to Cape Town, as well as for the distribution of the product in Europe (Bezuidenhout & Eksteen, 1994). During any of these stages, if the storage is not correctly managed deterioration of fruit quality will take place leading to large economic losses (Everett, 1997).

The complicated physiology of the avocado fruit, being a climacteric fruit with a very high climacteric rise (the rise in respiration rate accompanied by the ripening of the avocado) and, therefore, senescent very quickly, makes the avocado a very difficult fruit to preserve by means of post harvest storage (Wills *et al.*, 1989). Avocados are very susceptible to injury during the climacteric rise and especially at the peak of respiration activity (Kosiyachinda & Young, 1976). To slow down the rapid respiration rate, the fruits must

undergo a respiration rate declination and this can only be achieved if the fruits are stored at low temperatures. If not, the fruits will soften, which results in more brown cold development as well as grey pulp development during transport (Eksteen *et al.*, 1997). Temperatures below the critical value can result in an abnormal metabolism which, if prolonged, leads to the development of visible signs of injury (Eaks, 1983).

To submit the fruits to low storage temperatures will not only reduce the ripening rate resulting in physiological and market related benefits, but in addition, the low temperatures may act as a disinfestation treatment for certain insects (Jacobi *et al.*, 1995; Jessup, 1991; Kerbel *et al.*, 1987; Lay-Yee & Rose, 1994; Shellie & Mangan, 1994; Shellie & Mangan, 1996; Shellie *et al.*, 1993). Unfortunately, storage temperatures below 4° to 6°C tend to induce chilling injury (CI) in most avocado cultivars. The CI appears on the surface of the avocado skin as dark sunken, mosaic like patches (Zauberman *et al.*, 1985) and leads to an unattractive appearance and a decrease in the market value. Therefore, lower storage temperatures have a clear preserving potential, but CI remains the limiting factor.

A possible method to increase avocado resistance to CI is to administer a heat shock. In this way the fruits are protected from CI by inducing the formation of so-called 'heat-shock proteins' which render the cell membranes more resistant to CI (Harrington *et al.*, 1994; Nover, 1984). A heat shock may be applied either as hot air, steam or heated water. The treatment has to be administered within certain temperature and time parameters in order to be effective for certain cultivars. These have to be determined through research and a number of trials have already been conducted in other avocado exporting countries (Armstrong, 1994; Nishijima *et al.*, 1995; Paull & McDonald, 1994; Woolf & Laing, 1996).

It has been reported that maturity plays an important role in the sensitivity of the avocado to CI (Swarts, 1982). Early season fruits are more susceptible to CI when stored at low temperatures, because of the high moisture and low oil content. Therefore, if the heat shock treatment is successful and reproducible, this treatment regime will be applied to all the avocado pack houses where fruits are packed for seafreight export. This could

be of great benefit to the farmer in that the cultivars could arrive at the overseas market at a time when the avocado season of the competing producers from other countries, has ended. The heat treatment will not only minimise CI but could also lead to the maintenance of the quality of the fruits during shelf-life for a longer period (Biggs *et al.*, 1988; Eaks, 1978; Maxie *et al.*, 1974; Tsuji *et al.*, 1984). This means that the avocado fruits will arrive in a firm, healthy condition in Europe with minimal external or internal damage, satisfying the importers and distributors.

The objective of this study was initiated and funded by the South African Avocado Growers' Association to evaluate different heat shock treatment protocols as a method of preventing or minimising CI and to extend the shelf-life of avocado fruits while exporting at the lowest possible temperature.

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CHAPTER 2

LITERATURE REVIEW

A. THE SOUTH AFRICAN AVOCADO INDUSTRY

Avocado production in South Africa is centered mainly in the warm subtropical areas of the Northern and Mpumalanga Provinces in the North East of the country, between latitudes 22°S and 25°S. The annual rainfall in most of these areas is high (> 1000 mm p.a.), but there are some orchards in semi-arid regions with a rainfall of ca 400 mm p.a. Approximately 8% of commercial avocado orchards are in the KwaZulu-Natal Province, where the conditions are cooler due to the more southernly latitude (± 30°S) (Donkin, 1999).

The area planted with avocados in South Africa has expanded steadily over the past 30 years, from ca 2 000 ha in 1970 to ca 12 500 ha in 1999. 'Hass' and 'Fuerte' are the major cultivars and account for 33% of area for the 'Hass' cultivar and 42% of area for the 'Fuerte' cultivar under avocados. Due to the European Market's preference for 'Hass', less 'Fuerte' has been planted during the past three years. With 'Hass' accounting for 33% of the new plantings over last three years, whereas 'Fuerte' accounted for only 13% (Donkin, 1999).

The 'Ryan' cultivar accounts for 11% of the total plantings and 12% of new plantings over the past three years. This "late" cultivar reaches good prices on the local market at the end of the season, especially when grown in "late" growth areas. In contrast, the 'Pinkerton' cultivar accounts for only 8.5% of the total plantings but 41% of all new plantings over the last three years. Interest in this cultivar is based on the consistently high yields together with late bearing, although sensitivity of 'Pinkerton' to cold storage is a problem as the majority of South African avocados are exported by sea and thus require a cold storage period of at least 25 days. Intensive research is currently being carried out to address this problem (Donkin, 1999).

The South African avocado season extends usually from mid-March to September, depending on the weather and, therefore, could be earlier. Due

to climatic variability between growing regions, most of the major cultivars are available over an extended period during the season. For example, 'Fuerte' is harvested from mid-March to May in the northern regions, but is harvested in July and August in KwaZulu-Natal (Donkin, 1999).

High summer rainfall (> 1000 mm p.a. in most areas) and warm temperatures contribute to the high incidence of root rot caused by *Phytophthora cinnamomi.* This disease is effectively controlled through phosphorous acid trunk injections integrated with practices that promote root health, such as the addition of organic matter to the soil and mulching. The majority of plantings since the early 1980's, have been on *Phytophthora*-tolerant rootstocks such as 'Duke 7'. A number of locally selected *Phytophthora*-tolerant rootstocks are also currently being tested by the Institute for Tropical and Subtropical Crops (Bijzet *et al.*, 1997).

The South African avocado industry is export orientated, with Europe being the major market. In recent years, small volumes have been exported to the Middle and Far East. The majority of avocados exported by sea in refrigerated containers as airfreight is expensive, and only justified by the abnormally high prices of avocados overseas. Fruits exported by sea are packed and cooled in the production regions and then transported to Cape Town in refrigerated trucks where it is containerised before being loaded onto the ship. Because it takes the fruits about 25 days from packing to reach the European retailer, strict control of all links in the cold chain is vital in order to maintain high standards of fruit quality. In the past three years, there has been a strong move to the use of controlled atmosphere (CA) in integral containers during shipping, due to the beneficial effect on quality and shelf-life of this technology (Ginsberg, 1985).

Since 1973, South African avocado exports have shown a fairly linear growth trend. On an annual basis, however, the effect of alternate bearing cycles is evident. Adverse weather conditions such as drought or excessive rain have also had an impact on the size of the export crop. The industry is aware that a reduction in severity in the alternate bearing phenomenon will be of great benefit. Consequently, more attention is given to orchard practices such as pruning and fertilization so as to address this issue. It is estimated

that approximately 55% of the total avocado crop is exported. In the past, most of the avocados sold in South Africa were sold on the National Fresh Produce Markets in the major cities (Van Zyl & Ferreira, 1995). In recent years, however, sales to the informal sector direct from packhouses have increased steadily, according to the packhouses. Another recent trend in local marketing is the direct supply to supermarket chains from the packhouse (Donkin, 1999). Avocado processing (oil and guacamole), at present, only makes up a very small proportion of the total industry, but with present industry growth, there is great potential for expansion in this area. Statistics on sales to the informal sector are more difficult to monitor as for fruits sold on the National Fresh Produce Markets, making it difficult to estimate total production (Van Zyl & Ferreira, 1995).

Unlike the deciduous and citrus industries in South Africa, marketing of avocados has never been subject to statutory control. Growers export their fruits through exporting companies that operate on a commission basis. Quality standards for export are determined by the South African Avocado Growers' Association (SAAGA) (Toerien, 1994) in association with the National Department of Agriculture. These standards ensure that a good quality product is exported, and include factors such as fruit maturity, size and blemish levels. Quality inspections are carried out by a parastatal organisation, the Perishable Products Export Control Board (PPECB) on a consignment basis prior to shipping. The PPECB also ensures that the standards for refrigerated road transport and refrigerated containers are met (Köhne, 1999, Schroeder, 1994).

SAAGA was formed in the late 1960's to promote the interests of avocado growers in South Africa. SAAGA has a membership of about 500 growers, accounting for 90% of the avocado production in South Africa. The major avocado export companies are also members of the Association and the activities of the association are fully funded by its members. It is the mission of SAAGA to act in the grower's interest to improve the economic viability of production, packing and marketing of avocados. To this end, the Association funds research, market development both locally and in the

European Union, provides extension services, and facilitates co-ordination between exporters (Köhne, 1999).

With the steady increase in production and marketing costs and declining market prices (in real terms) over the past few years, much attention is being paid to improving yields and maintaining orchard viability. Pruning to maintain tree size and prevent inter tree shading is now practised by many growers. Where topography permits, spraying and pruning operations are also being mechanised. Tree nutrition is also receiving attention as an integral part of maintaining a viable productive canopy. Application of fertiliser through irrigation systems (fertigation) is gaining popularity, as it is labour saving and allows for more accurate fertiliser application tailored to the tree's requirements at different stages of its phenological cycle (Tomlinson, 1996; Zekri & Koo, 1992).

European supermarket chains are increasingly placing greater emphasis on the safety of food products on their shelves. This has resulted in a greater awareness of 'Good Agricultural Practice' (GAP) amongst growers and quality assurance systems such as International Standardization Organization for systems (ISO) and Hazardous Analytical Critical Control Point (HACCP) amongst avocado packers. As a part of GAP, there is strong emphasis on Integrated Fruit Production (IFP) to limit the use of harmful agricultural chemicals by employing cultural practices that encourage natural enemies and antagonists of pests and diseases (Donkin, 1999).

The demand for organically grown avocados is also increasing both locally and on the European market. As a result, growers are converting at least some of their orchards to organic farming practices (Donkin, 1999).

B. AVOCADO, THE LIVING ENTITY

Avocado (*Persea americana* Mill.) belongs to the aromatic laurel family (Lauraceae) of which only one other genus, *Cinnamomum*, is appreciably cultivated, yielding cinnamon and campher (Bergh, 1975). The avocado fruit is botanically described as a berry with a thick, fleshy mesocarp surrounding a single seed and ranges in weight from 0.050 kg to 1.0 kg (Whiley & Schaffer,

1994). The cultivars vary in size and are usually pear shaped but can also be round and oval. The flesh has a higher energy value than meat of equal weight and is a good source of fibre, potassium, Vitamins E, C, niacin, thiamine and beta-carotene (Slater *et al.*, 1975; Smith *et al.*, 1983). In addition, the mono-unsaturated fatty acids in avocados effectively reduce blood levels of low-density lipoprotein (cholesterol), which is alleged to contribute to heart disease, while increasing blood levels of heart-protective, high-density lipoprotein (Colquhoun, 1990). The oil from the avocado fruit is widely used in the pharmaceutical industry where it is used as a skin moisturiser and as body lotion preparations, while the seed is reported to contain antibacterial agents (Neeman *et al.*, 1970).

The harvested avocado fruits respirates and utilises oxygen and produces carbon dioxide. The respiration process consists of the metabolism of carbohydrates in the presence of oxygen leading to the production of water, carbon dioxide and heat energy (Combrink, 1996). The avocado has a very high respiration rate (Fig. 1) compared to apples, citrus, grape or kiwifruit (Kader *et al.*, 1985) and, therefore, generates more heat during storage than many other products. It has been reported that the respiration rate differs between cultivars for example, 'Hass' has a higher respiration rate than 'Fuerte' (Kruger, 1996).

The avocado is a climacteric fruit which means its physiology is characterised by an increase in respiration with time, reaching a peak after which the ripening and softening process in the fruit accelerates (Bower & Cutting, 1988)(Fig. 1). The fruits will continue to mature on the tree, but will not soften until picked. The fruit moisture content on a specific time is calculated for the correct maturity and, therefore, the correct harvesting date. The higher the moisture, the lower the oil content and the more immature the fruit. The different cultivars have different moisture contents of 78% (m/m) for 'Fuerte' and 'Ryan', 77% (m/m) for 'Hass', and 75% (m/m) for 'Edranol' and 'Pinkerton' (Kruger, 1996). In South Africa, the harvesting of avocados may start in February and end in August depending on the seasonal rainfall and weather factors (Kruger & Claasens, 1997).

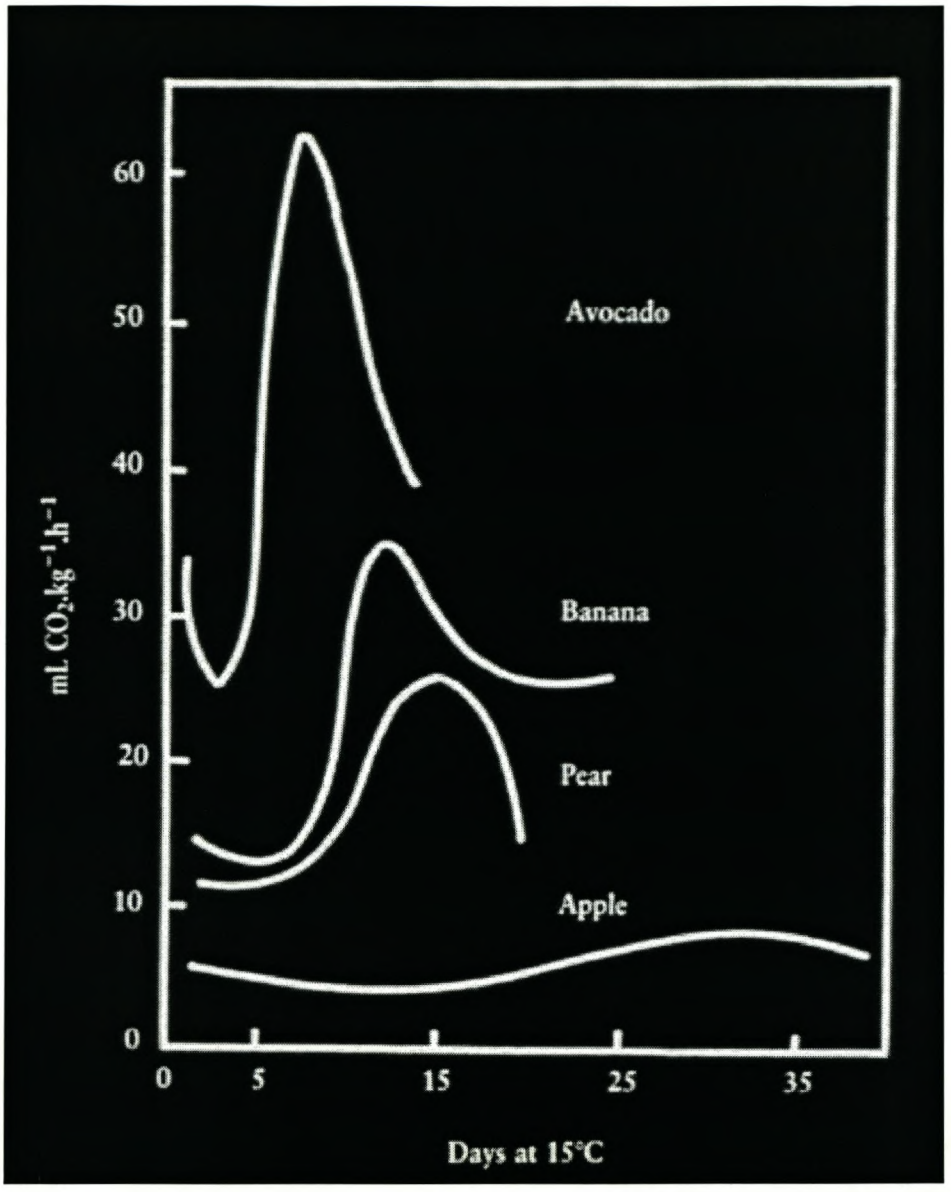

Figure 1. The climacteric peaks of different crops. The very high respiration rate of avocado fruits are shown (Wills *et al.*, 1989).

C. FACTORS THAT INFLUENCE THE RIPENING PROCESS AND QUALITY OF THE FRUIT

Good avocado fruit quality can only be achieved by limiting pre-harvest stress, particularly water stress during the first three months after fruit set. Post harvest environmental conditions must be controlled so that no suffocation of the fruit takes place and the humidity must also be high enough in the cold store rooms (Bower & Cutting, 1987).

A range of factors contribute to the controlling of avocado quality such as respiration, ethylene production, transpiration, the effect of step-down temperatures and storage time. The orchard factors include irrigation, nutrition, mulching, maturity of the fruits, diseases, picking and transport to the pack house. In contrast, the factors in the pack house consist of grading, temperature control, waxing, vapour pressure, packaging and marketing of the cartons. The cold chain during road transport, at the docks and during sea transport is also of the utmost importance, as well as the interrelationship between temperature and time of storage (Donkin, 1999).

To achieve the ideal avocado fruit quality, the correct cultivar choice for the specific climate, as well as the correct geographic position should be considered. External factors like the shape, colour, freedom of blemishes and freedom of disease will depend on good orchard practices and this thus plays an important role. Internally the fruits should be free of physiological disorders, bruising, disease symptoms and also be of excellent and reliable eating quality, in accordance with the SAAGA specifications (Donkin, 1999).

Ripening is a synchronised change involving many biological changes resulting in the synthesis and degradation of pigments, conversion of starch to sugars, changes in firmness and texture, production of volatiles, increased respiration and ethylene senescence (Biale, 1974). Softening during ripening involves structural, as well as chemical modification of cell wall polysaccharides. The mechanism that regulates changes in firmness is still not fully understood, but the cell wall hydrolytic enzymes contribute to softening in climacteric fruit such as apple, avocado, mango, papaya, pear and tomato (Huber, 1983). Cellulase is the main enzyme involved in the

softening process (Pesis *et al.*, 1978). Early in the season cellulase activity is low in freshly picked fruits, however, levels are higher in more mature fruits (Fuchs & Zaubermann, 1987). Fuchs & Zaubermann (1987) concluded that cellulase activity and softening of fruits at 20°C are not triggered by ethylene production, but rather guided by, which means that the concentration of ethylene does not play a role, but only its presence.

Ethylene

Ethylene plays a vital role in the avocado ripening process. It is generally recognised as one of the triggers which induce the enzyme ripening process (Starrett & Laties, 1993). However, Zaubermann *et al.* (1988), showed that the ripening process of avocado fruits requires the continuous presence of ethylene. Gazit & Blumenfeld (1970) and Köhne (1985) showed that immediately after harvest, fruits will not readily respond to ethylene induced ripening. They explained this response as being due to an endogenous ripening inhibitor present in the fruits while the fruits were still hanging on the tree and they also postulated that this inhibitor remained functional for a limited time after harvest.

The unravelling of the biochemical pathway of ethylene biosynthesis in plants (Fig. 2) has been one of the most interesting biochemical stories of recent years (Reid, 1987). Further research showed that the application of the amino acid methionine greatly stimulated ethylene production in apples and this compound was then considered to be the starting point of ethylene biosynthesis. The S-adenosyl-methionine (SAM) synthase enzyme forms another key component, (Fig. 2), which is converted to an unusual cyclic amino acid, aminocyclopropane-1-carboxylic acid (ACC) (Fig. 3), the precursor of ethylene.

ACC synthase (Fig. 2) is the enzyme that controls the rate at which the pathway operates and is activated by a common co-factor, pyridoxal phosphate. Inhibitors of enzymes that require pyridoxal phosphate such as amino-ethoxyvinyl glycine (AVG) and amino-oxyacetic acid (AOA) can also be

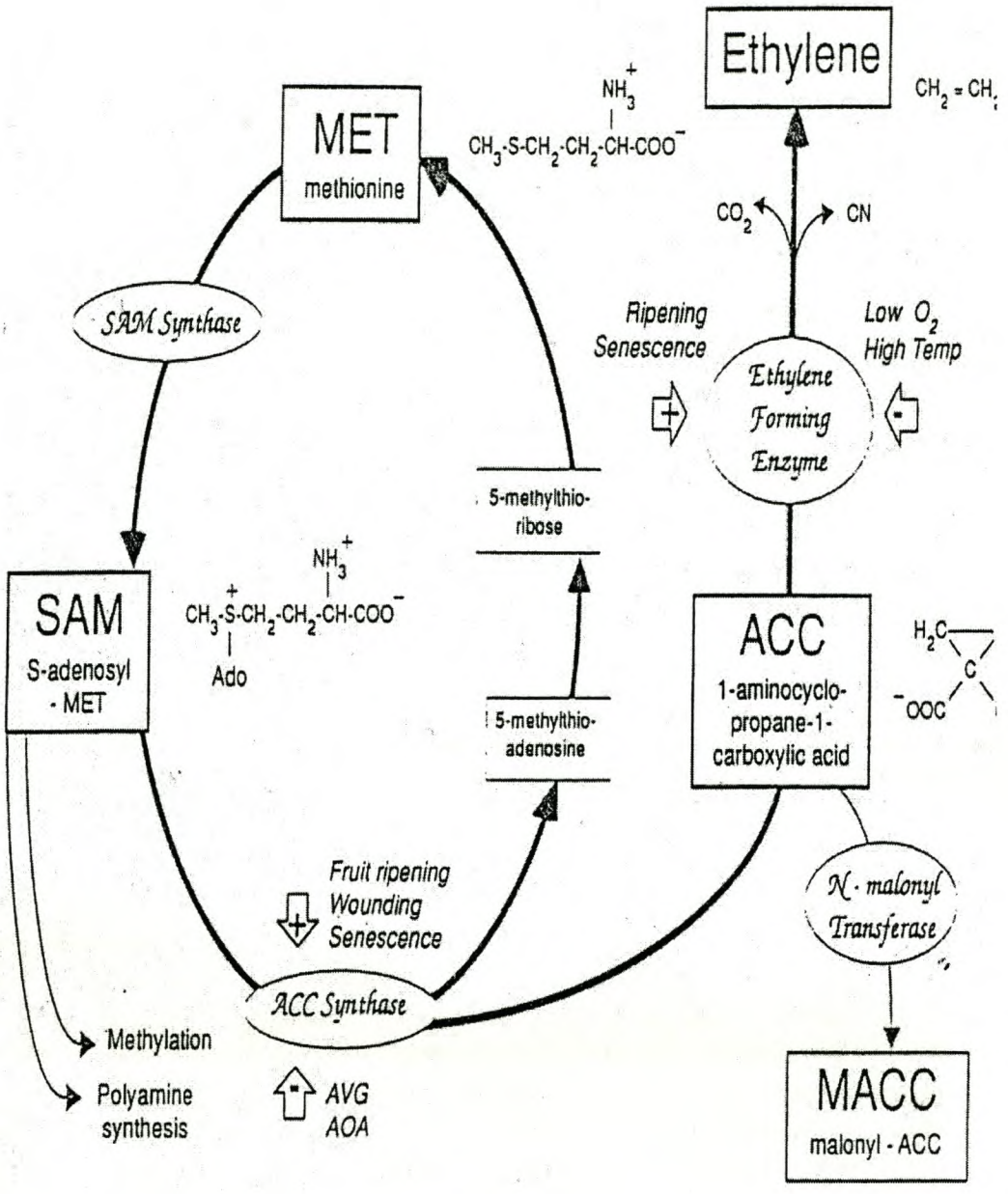

Figure 2. Pathway of ethylene biosynthesis (Redrawn from Yang, 1987).

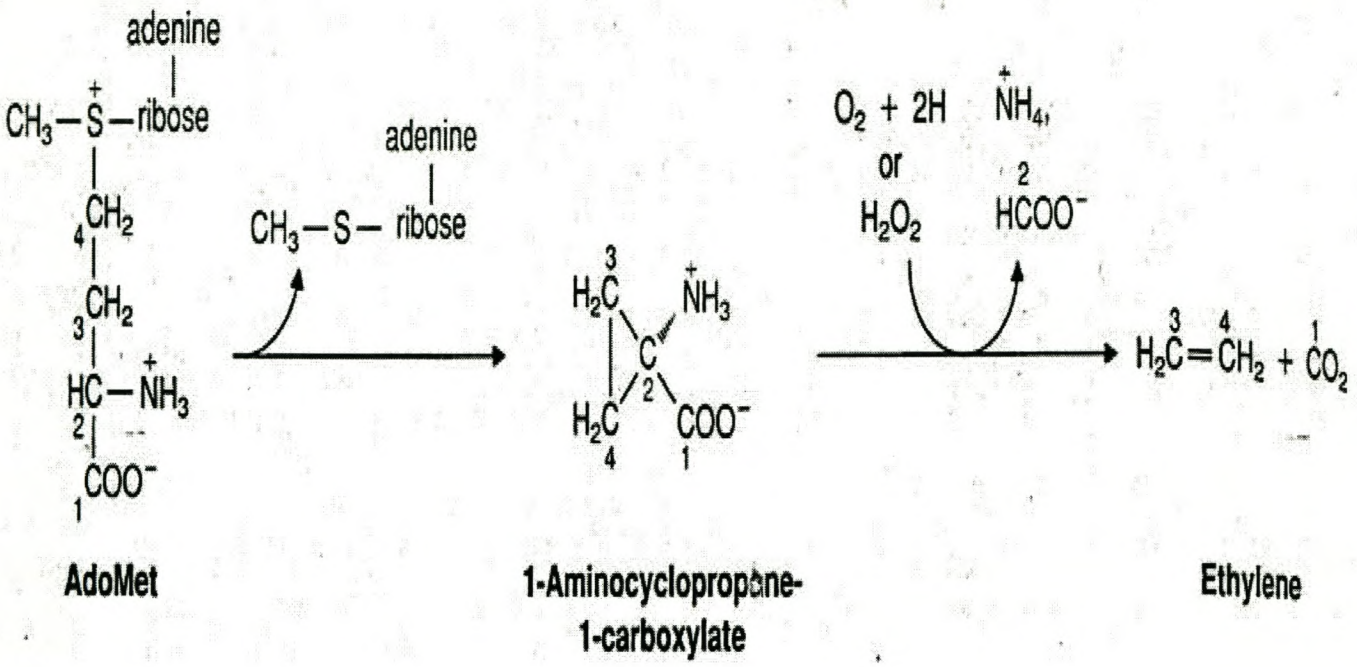

Figure 3. 1-Aminocyclopropane-1-carboxylate (ACC). Precursor of ethylene (Mathews & Van Holde,1990).

used to inhibit ethylene production (Yang, 1987). The final step in the pathway, the ethylene forming enzyme (EFE) (Fig. 2), can be influenced by high temperature and low oxygen (O_2) levels, which can reduce ethylene production. The biochemical pathway in which ethylene induces the ripening effects, is by means of binding to a protein called ethylene (C_2H_4)-binding site (Fig. 4). By binding to this protein it stimulates the release of the so-called second messenger molecule to instruct the deoxyribonucleic acid (DNA) (Fig. 4) in the nucleus to form messenger ribonucleic acid (mRNA), which carry the specific characteristics of the ethylene function (Reid, 1987). These molecules are then "translated" into proteins by polyribosomes and the proteins that are formed are the enzymes that cause the actual ethylene response (Yang, 1987).

Ethylene induces an enhancement of tissue permeability, respiration and ripening (Bangerth, 1979). Ethylene production by fruit can be increased as a result of mechanical injuries, disease incidence and increased temperatures (Kader *et al.*, 1985). However, a heat shock will change the structure of the fruit to such an extent that it will inhibit ripening. This can be explained by the fact that ethylene synthesis is inhibited within hours in both apples and tomatoes after a heat shock (Biggs *et al.*, 1988; Klein, 1989).

Tingwa & Young (1974) showed that infiltration of avocado fruit with calcium in the form of calcium sulfate ($CaSO_4$) or calcium chloride ($CaCl_2$) depressed and delayed the peak of ethylene production if applied prior to the climacteric peak. Hofman *et al.* (1995) showed that the respiration rate of 'Fuerte' fruits could be increased by the presence of levels as low as 0.01 μl^{-1} of ethylene during storage at temperatures of 10° - 14°C. The reduction of ethylene levels during long-term storage is, therefore, of vital importance and sufficient air exchanges are essential in container vessels while under CA conditions. It is, therefore, very important to control and inhibit ethylene production in avocado fruits otherwise the fruits will arrive soft on the European market. From the literature (Bangerth, 1979; Biggs *et al.*, 1988; Klein, 1989; Zaubermann *et al.*, 1988) it was reported that by controlling ethylene production with a heat shock, the fruits stayed harder for longer and

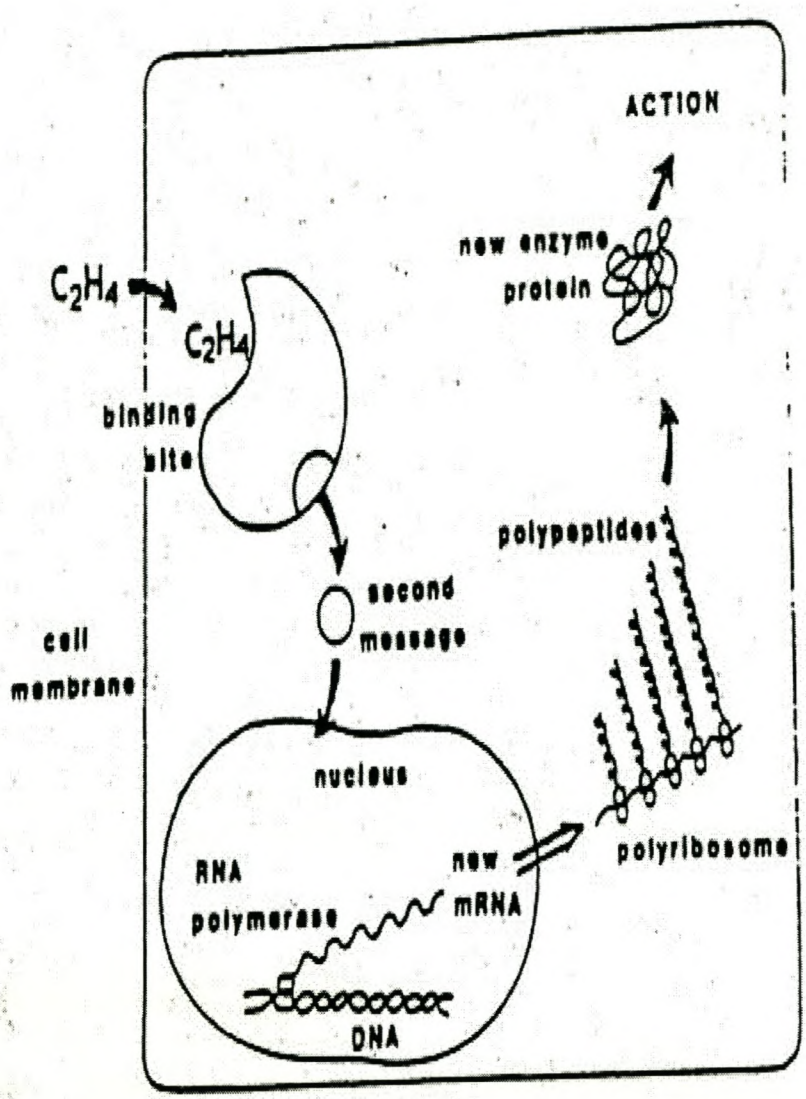

Figure 4. Mechanism of ethylene action (Reid, 1987).

thus an investigation into the impact of heat shock treatments on the ripening process is of great importance.

Transpiration

Transpiration is the process of water loss by the fruit and is an important factor in determining fruit quality. The fruit is covered with a waxy film on the surface of the exocarp, which is interrupted by stomates in the young fruit and in time, can become plugged and less active (Blanke & Bower, 1990; Cummings & Schroeder, 1942). Diurnal fluctuation in fruit diameter due to water loss has been reported with a maximum loss at about 8 h and minimum at 15 h (Schroeder & Wieland, 1956). After misting the canopy with water, tree transpiration can be reduced and the fruit shrinkage prevented, which will result in the diameter increasing after a short time (Schroeder & Wieland, 1956).

Water stress during critical stages of fruit ontogeny has also been shown to lead to defects in internal fruit quality. Bower *et al.* (1989) related different irrigation schedules to the activity of the browning enzyme, polyphenol oxidase in the mesocarp of mature fruit after harvest. When the soil matric potential measured at 300 mm was allowed to fall to –80 kPa before re-wetting (Bower *et al.*, 1978), there was increased activity of polyphenol oxidase in fruit compared to those grown where the soil matricpotential did not exceed –55 kPa. Fruit from the latter treatment had higher calcium levels in the mesocarp during the first 16 weeks of growth. This is known to improve membrane structure and reduce physiological fruit disorders (Bangerth, 1979; Chaplin & Scott, 1980; Ferguson, 1984; Millaway & Wiersholme, 1979).

Moisture evaporation is strongly affected by temperature, relative humidity, air velocity, surface coatings such as waxes or wrappers and packaging. Rind moisture loss has been shown to have a significant effect on fruit quality and the degree of cold injury in 'Fuerte' fruits stored at 5.5°C (Bower & Cutting, 1987; Donkin & Cutting, 1994).

Water loss is certainly one of the important factors leading to fruit deterioration because it acts as a stimulus for ripening (Adato & Gazit, 1974)

and an associated change in abscisic acid (ABA) concentration in the mesocarp, which leads to the browning of the flesh (Cutting & Bower, 1987). Increased moisture loss resulting in stress during storage, not only enhances polyphenol oxidase (PPO) activity and visual symptoms of physiological disorders, but also increases the prevalence of pathological disorders (Bower & Cutting, 1987). Cutting *et al.* (1992) showed that more mature fruits in storage are less subjected to moisture loss than relatively immature fruits. The relative humidity in the storage atmosphere, therefore, plays a vital role.

A basic rule in heat dynamics is that the greater the temperature and less the volume of air in the system, the higher the moisture loss will be from the fruit. By decreasing the volume of air (i.e. by using a cooling system with a bigger capacity) and restricted temperature, moisture loss can be limited. The design of a cooling system in a pack house, therefore, plays a major role in preventing water loss from fruits and in final fruit quality (Vorster *et al.*, 1990). Thus, if the fruits are given a heat shock, it will render a slow ripening fruit with a lower respiration and transpiration rate.

Temperature

Temperature is an extremely important factor when planning the long term storage of avocados. However, it is not just temperature *per se*, but the maintenance of the total cold chain through to the consumer that is of importance. The impact of a break in the cold chain was clearly shown by Swarts (1982) who demonstrated when such break in cooling occurred, a subsequent increase in softening of fruits occurred. It is, therefore, clear that a good management strategy, where the time and temperature both are controlled, be implemented if good fruit quality is to be achieved.

After experiencing problems with South African fruits that were soft on arrival in Europe, a detailed analysis of seasonal data was made by Bezuidenhout (1992). He showed that a deviation in holding temperature of at least 1°C higher than recommended for a 22-day transit time, increased the softness of fruits from 25 to 35 firmometer units. Furthermore, a similar increase of 1°C over a total transit period of 28 days resulted in an increase in softness from 32 to 46 firmometer units.

In 1983, Eaks showed the relationship between ethylene production and the climacteric and ripening of 'Hass' avocados at various temperatures. Bezuidenhout (1983) constructed a climacteric model of 'Fuerte' fruits and was able to establish that excessive cold prior to the climacteric is favourable for chilling injury (CI) and pulp-spot to develop but once the climacteric period had passed, temperatures can be lowered without CI development. He also found that large 'Fuerte' fruits are more susceptible to physiological disorders than smaller fruits. His climacteric model also showed that pulp-spot susceptibility decreases later in the season, whereas grey-pulp increases steadily, especially if high temperatures occur in the post-climacteric phase (Bezuidenhout, 1983).

Vorster *et al.* (1987) carried out trials on various cultivars and found that early season 'Fuerte' fruits were very sensitive to external cold injury. The use of 7.5°C as storage temperature for the first week, followed by 5.5°C for two weeks and 3.5°C for one week, reduced the incidence of early CI during the first half season, when compared with the standard of 5.5°C for four weeks. The step-down temperature also resulted in a significant reduction in pulp-spot symptoms. Vorster *et al.* (1987) and Eksteen & Bester, (1987) also proposed a step-down temperature regime, but not only during the storage period, but throughout the season.

By 1990, a more sophisticated schedule of shipping temperatures, based on the moisture content of the fruit, had been developed (Vorster *et al.*, 1990). Interestingly, the step-down schedule of cooling was found to be unnecessary for 'Fuerte' fruits grown in Kwazulu-Natal, where continuous storage at 5.5°C was found to be satisfactory in terms of external quality and internal physiological disorders (Donkin *et al.*, 1995). Various researchers (Jessup, 1991; Zauberman & Jobin-Décor, 1995) have shown the potential of storing 'Hass' fruits at temperatures as low as 2°C. However at 1°C, severe CI was found to occur (Vuthapanich & Hofman, 1997). The pre-conditioning of 'Hass' fruits to induce tolerance to low temperatures (Woolf *et al.*, 1995; Woolf *et al.*, 1996) showed great potential. Thus, it was concluded that storage temperature greatly influenced the final quality of the fruits, but that maturity will determine the temperature at which the fruits have to be stored

and must be complimented by a step-down conditioning regime. To be able to use lower storage temperatures, a heat shock of the fruits are probably necessary to protect the fruits from CI.

Storage period

Swarts (1979) reported that there is a clear relationship between time and storage temperature and that CI increased with reduced temperatures and longer storage times. Vorster *et al.* (1988) also showed that dramatic increases in external cold injury in 'Fuerte' occurred if the storage time is extended from 21 to 28 days. Similarly, grey-pulp defects were found to increase in both 'Pinkerton' and 'Hass' as the storage period was extended. Bower (1988) also reported that total post-harvest disorders increased from 14% after 21 days to 30% after 30 days and to 58% after 44 days.

Brown-cold damage is more common on old fruits and excessive moisture loss during transit and cold storage is thought to play a major role in the development of this symptom. This type of damage definitely appears to be correlated to the age of the fruits (after picking) and this, coupled with low temperature storage for a long period aggravates the problem (Champ *et al.*, 1993). For example, fruits placed at ambient temperature on arrival in Europe (22 days after packing) showed no symptoms, however, after a further 10 days of cold temperature storage, showed clear symptoms of brown-cold damage. Once again, this disorder appears to be time versus temperature related. Desiccation may, however, also play a role (Milne, 1994). These observations emphasise the importance of reducing respiration by storing the fruits at low temperature in case the sea journey is extended for unplanned reasons. Therefore, the preservation of fruits at low temperatures without the occurrence of CI, needed to be investigated. It is possible that all these ripening factors could be manipulated with the application of a heat shock treatment.

D. HEAT SHOCK REACTIONS

Heat shock

Heat shock reaction is considered the adaptive response a living entity is experiencing, when it is suddenly exposed to a rise in temperature (Vierling, 1991). To understand the concept more clearly, the organism *Escherichia coli,* was studied (Cowing *et al.*, 1985). This organism grows normally at 30°C but when it suddenly experiences a temperature shock of 40°C, an increase in certain proteins appear based on this reaction more than 19 genes react to this impulse. These genes code for heat shock proteins (HSP) that form part of the High Temperature Protection (HTP) regulator gene and are present in the *E.coli* genome which are found in two operator genes (Cowing *et al.*, 1985).

The synthesis of HSP is part of the response of all organisms to heat stress (Linquist, 1986). The formation of HSP gives the organism or plant temporary protection against the harmful effect of any sudden temperature increase or variation. All these proteins are transcripted under normal conditions but only at very low levels. Although all the specific functions of the HSP are not known, they do include the ability to protect the cell against a drastic temperature shift. It can only be speculated that at high temperatures the proteins in the cell start to denaturate and they loose their 3-D structure and, therefore, loose some of their functionality. These HSPs can then assist in the demolishing of these denaturated proteins or they help as "chaperones" by assisting cellular proteins to fold in their functional 3-D structure or to help move them to the cellular position where they are needed (Cowing *et al.*, 1985).

A correlation between the development of thermotolerance and the synthesis of HSP has been found (Li *et al.*, 1982; Vierling, 1991), as well as a correlation between the loss of thermotolerance and the disappearrance of HSP. The development of thermotolerance is dependent on the incubation temperature, but it must be high enough and sudden enough to initiate the synthesis of HSP. The best temperature range, where the HSPs were activated, were between 35° and 49°C, but this was depended on the type of

fruit, as well as the specific cultivar (Champ *et al.*, 1993; Chan, 1986a; Paull & Chen, 1990; McDonald *et al.*, 1998; Woolf *et al.*, 1996).

Elevated temperatures can cause ACC (Fig. 4) to accumulate in apple and tomato tissue and simultaneously can decrease the ethylene production (Yu *et al.*, 1980; Atta Aly, 1992). However, raising the temperature or holding the fruits longer at higher temperatures will cause the dissappearance of ACC (Klein, 1989; Atta Aly, 1992). The accumulation of ACC is caused by the rapid loss of ACC oxidase enzyme activity, which occurs in many fruits after a short period of high temperature exposure (Chan, 1986a and 1986b; Dunlap *et al.*, 1990; Paull & Chen, 1990). This is primarily due to a decrease in ACC oxidase mRNA and cessation of enzyme synthesis (Lurie *et al.*, 1996). Therefore, the inhibition of ripening of fruits after a heat treatment could be related to the influence heat has on the ripening hormone, ethylene. This could probably explain the occurrence of "heated" fruits being more advanced in some ripening characteristics than "non-heated" fruits while maintaining their quality longer during shelf-life (Lurie, 1997).

Only recently it has been found that heat stress can condition plants to low temperatures. This resistance to low temperature injury or CI was found to correlate with the presence of HSP (Lafuente *et al.*, 1991; Sabehat *et al.*, 1996) and it can be concluded that the use of a heat shock treatment on avocados so as to prevent cold-damage, is worth investigating.

Chilling Injury

The brown mosiac pattern type indentations on avocado fruit skin after cold storage are called CI (Fig. 5). These brown areas on the fruits are possibly caused by the accumulation of total-phenols under low temperatures, the rupturing of the cell walls and thereafter, the polyphenol oxidation, which leads to the formation of the brown pigmentation. There appears to be a correlation between the total-phenol content of fruits stored at low temperatures (-1° to 2°C), the electrolyte leakage rate and the occurrence of CI. The cell membrane degeneration and the CI may be related to the rapid increase of the total-phenol content (Oogaki *et al.*, 1990).

Figure 5. Brown indentations found on the skin of the avocado as a result of chilling injury.

The presence of PPO (polyphenol oxidase) activity in the plastids of normal mesocarp tissue, and the absence in the plastids of the abnormal brown mesocarp tissue, indicate a possible involvement of PPO in the development of mesocarp browning in avocados. The movement of the PPO through the thylakoid membranes causes no visible structural changes in the membranes. This movement is probably triggered by the presence of the substrate in the surrounding cytoplasm (Engelbrecht, 1987).

The reduction of sensitivity to CI in fruits may not be solely due to the presence of HSP. CI has long been thought to begin with membrane damage (Lyon, 1973), and the heat treatment may cause membrane alterations as well. The examination of the lipid composition of apple plasma membranes and the total tissue lipids of tomato showed that after heat treatment and subsequent cold storage, more phospholipids and greater fatty acid unsaturation appeared than in non-heated fruits (Lurie *et al.*, 1995; Lurie *et al.*, 1997). This would suggest more fluid membranes in heated fruits and the corresponding reduction of indiscriminate leakage from the tissue of heated fruits and vegetables.

Tissue damage complicates the technique for achieving an effective time-temperature regime that will produce the desired effect (disinfestation, fungal control and reduction in CI) without damaging the fruit (Lurie *et al.*, 1995; Lurie, 1997; Lurie *et al.*, 1997). Damage can be internal, as well as external. External damage generally appears as brown peeling (Kerbel *et al.*, 1987; Klein & Lurie, 1992; Lay-Yee & Rose, 1994; Woolf & Laing, 1996) while internal tissue damage will result in an increased decay development (Jacobi & Wong, 1992; Jacobi *et al.*, 1993; Lay-Yee & Rose, 1994). It can also include flesh browning of fruits such as avocados, litchis, citrus and nectarines (Jacobi *et al.*, 1993; Shellie *et al.*, 1993; Lay-Yee & Rose, 1994; Shellie & Mangan, 1994 and Shellie & Mangan, 1996). If the produce is stored at a low temperature after the heat treatment, the heat damage can be misidentified as CI, which has similar symptoms.

E. CONCLUSION

For the South African avocado industry to have a competitive advantage over the rest of the world and present the best possible quality avocado fruits to the European market, the avocado has to be hard without CI indentations and no internal browning. Although airfreight would result in better quality fruit, the problem lies with quantity and expenses. To justify airfreight cost with the price receiving for avocados, bulk supply would be necessary, making it impossible with airfreight. With seafreight, black cold injury develops due to the low temperature storage. It is, therefore, a CI that becomes more severe when the storage temperature is reduced to counteract fruit softening (Eksteen *et al.*, 1998). If the application of a heat shock treatment can help facilitating the above mentioned criteria, the avocado producers will subsequently gain credibility and will be guaranteed a stable distribution area on the European market.

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CHAPTER 3

EVALUATION OF HOT WATER HEAT SHOCK TREATMENTS ON SOUTH AFRICAN AVOCADOS IN ORDER TO MINIMISE THE OCCURRENCE OF CHILLING INJURY

Abstract

Avocado fruits were subjected to different heat shock treatments. The initial study was undertaken with a broad spectrum of hot water temperatures and exposure times according to different literature studies. A large number of trials were done with South African 'Fuerte' and 'Hass' as these were the most important cultivars in South Africa. Only preliminary work was done with 'Pinkerton', 'Ryan' and 'Edranol' cultivars. From the results it was clear that 'Hass' was less susceptible to chilling injury (CI) than 'Fuerte', except at the beginning of the season. It further appeared that fruit maturity played an important role in the susceptibility of CI, as well as the most suitable treatment temperature and exposure time for the different cultivars. The results also indicated that it was beneficial to wax the fruits before the treatment. The most effective treatment period for the 'Fuerte' cultivar was 20 - 30 min in an optimal temperature range of approximately 42°C. 'Hass' showed no specific treatment benefit at that stage.

This heat shock treatment technique requires absolute precision and a number of external factors can impact the treatment. However, more research needs to be done before a final decision on a possible commercial application can be reached.

Introduction

The South African avocado industry is export driven and the successful storage of fruits for extended periods is, therefore, essential (Bredell, 1983). The sea journey to the European markets takes approximately 15 days. In

addition there should also be five days for distribution of the fruit. This long process of delivery leads to the possibility of the fruits arriving soft on the European market, resulting in severe financial losses for the producers (Eksteen *et al.*, 1997; Kruger *et al.*, 1997). Subjecting the fruits to low storage temperatures will not only reduce the ripening rate resulting in physiological and market related benefits, but may also act as a disinfestation treatment for certain insects (Jacobi *et al.*, 1995; Jessup, 1991; Kerbel *et al.*, 1987; Lay-Yee & Rose, 1994; Shellie *et al.*, 1993; Shellie & Mangan, 1994; Shellie & Mangan, 1996). Unfortunately, storage temperatures below 4° to 6°C tend to induce CI to most avocado cultivars. The CI appears on the surface of the avocado skin as dark sunken, mosaic like patches (Zauberman *et al.*, 1985) and leads to an unattractive appearance and a decrease in the market value. Therefore, lower storage temperatures have a clear preserving potential, but CI remains the limiting factor. A possible method to increase avocado resistance to CI is by administrating a heat shock.

Heat shock treatment is a technique where the fruits are subjected to high temperatures for specific time intervals. This treatment depends on the type of fruit, as well as the avocado cultivars (Armstrong *et al.*, 1995; Donkin & Wolstenholme, 1995; Jacobi *et al.*, 1995; Jessup, 1991; Kerbel *et al.*, 1987; Lay-Yee & Rose, 1994; Shellie *et al.*, 1993; Shellie & Mangan, 1994; Shellie & Mangan, 1996; Woolf & Laing, 1996; Woolf *et al.*, 1995). The heat shock process renders the fruit more tolerable to colder temperature storage and, therefore, minimises the occurrence of CI (Harrington *et al.*, 1994; Nover, 1984).

Heat shock treatments have been conducted on an experimental basis with variable success in other avocado exporting countries. Examples of successful studies include 'Sharwil' avocados with a pre-treatment of 38°C for 8 - 12 h in Hawai (Nishijima *et al.*, 1995); 'Hass' avocados treated in hot air at 38°C for 3, 6 and 10 h and at 40°C for 0.5 h in New Zealand (Woolf *et al.*, 1995) and 'Hass' avocado pre-treated at 38°C in hot water for 1 h and then treated for 1 - 10 min at 50°C in New Zealand (Woolf & Laing, 1996).

The aim of this study was to determine the best temperature and exposure time combination of hot water heat shock treatments on South African avocados in order to minimise the occurrence of CI.

Material and methods

Feed back from SAAGA indicated that certain producers had trouble with their crops arriving overseas (Donkin, 1996). These avocado orchards were selected for sampling. The two main export cultivars namely 'Hass' and 'Fuerte' needed to be studied. Both these two cultivars are early season cultivars and consisted of the bulk volume overseas (Donkin, 1996). For interest sake, preliminary studies were also conducted on the 'Ryan' (late season cultivar), 'Pinkerton' and 'Edranol' cultivars (both mid season cultivars). Weather also played a role in the maturity of the fruits as Kruger & Claassens (1997) explained and, therefore, no specific harvesting date or maturity levels of the fruits were needed. The main idea of the experiments were not only to minimise CI, but also to simulate the actual reality of what will happen when the pack houses receive avocados from producers of different localities, as well as maturity.

A total of 14 Experimental Studies were conducted during the 1996 avocado season, five with the 'Fuerte', six with the 'Hass' and one each with the 'Pinkerton', 'Ryan' and the 'Edranol' cultivars. The fruits were obtained from four locations, namely Burgershall (Weirich Brothers and Andy McQueen), Nelspruit (Institute for Tropical and Subtropical Crops (ITSC) and HL Hall & Sons), KwaZulu-Natal Midlands (Everdon Estates) and Tzaneen (Westfalia Estates).

The fruit were harvested randomly in harvesting boxes and transported to the laboratory. A standard cleaning method was used which included the following: washing of the fruits with 0.5% (m/m) hypochlorite solution to remove sooty blodge (black fungus that causes infection after ripening), after which the fruits were rinsed with tap water to protect the fruits against lenticel damage. The different hot water heat shock treatments, as applied in the different Experimental Studies, were administered with a average of 10 – 12 replicas

included and after the fruits had cooled, a wax (TAG 1:1, Dormas chemicals, Johannesburg) was applied by means of dipping the fruits into the wax. After the fruits had air dried by means of fans, they were packed into carton boxes and cold stored according to the different Experimental Studies layouts.

After the storage period of each study, the fruits were evaluated according to the CI percentage on the whole surface of each fruit. The average of each treatment together with all the replicas represented the CI (% (v/v)) are given in the result tables. Other parameters such as firmness that was measured by a firmometer (Swarts, 1981) and internal disorders by means of a scale of 0 – 3 were also evaluated. These parameters are not discussed in this thesis because the problem that was under investigation was external appearance of CI of the exported fruits. The statistical differences between the different treatments were measured with Duncan's multiple range test (P = 0.05). The experimental designs (summary in Table 1) of the different Experimental Studies are given in chronological order. Because of the multitude of data of each treatment and replicas only the results of the best treatment of each study, as well as the controls were discussed in this thesis.

Experimental Study I: Influence of hot water treatments at 36°, 38°, 40° and 42°C and exposure times of 30, 60, 180, 630 and 660 min on 'Fuerte' fruit quality.

Four hundred and ten 'Fuerte' fruits from the Burgershall area were cleaned using the standard cleaning method and the moisture content of a random sample was determined. The method used to determine moisture content consisted of the calculation of the difference between wet and dry mass. This was done by cutting the fruit in half and removing the skin so that the flesh could be grated by a grater into fine shreds to make the drying process easier and faster. The drying bowls were weighed before the 10 g of grated flesh was placed in it. A representative sample consisted of 10 fruits. The drying took place overnight in a drying oven at a temperature of approximately 35°C.

Table 1. Summary of the different experimental designs.

Exp. Study	Hot water temperatures	Exposure Time (Minutes)	Cultivar	Sample size	Area	Other treatment
I	36°,38°, 40°, 42°C	30, 60 , 180, 630, 660	Fuerte	410	Burgershall	
II	33°, 36°, 39°, 42°C	5, 10, 15, 20, 30, 45, 70, 90, 105, 120	Fuerte	410	Burgershall	
III	33°, 36°, 39°, 42°C	5, 10, 15, 20, 30, 45, 70, 90, 105, 120	Hass	410	Burgershall	
IV	33°, 36°, 39°, 42°C	10, 20, 30, 40	Hass	340	ITSC Nelspruit	Step-down 6° - 4° - 2°C
V	33°, 36°, 39°, 42°C	10, 20, 30, 40	Fuerte	340	Halls & Sons Nelspruit	Step-down 6° - 4° - 2°C
VI	39°, 41°, 43°, 45°C	5, 10, 15, 20, 25	Fuerte	160	Halls & Sons	Pre-waxing
VII	39°, 41°, 43°, 45°C	5, 10, 15, 20, 25	Hass	500	ITSC Nelspruit	0.5 % chlorite treatment + no treatment
VIII	39°, 41°, 43°, 45°C	5, 10, 15, 20, 25	Pinkerton	240	Burgershalll	
IX	33°, 36°, 39°, 42°C	5, 10, 20, 30	Edranol	112	Burgershalll	
X	33°, 36°, 39°, 42°C	5, 10, 20, 30	Ryan	112	Burgershall	
XI	39°C 41°C 43°C 45°C	25 + 30 25 + 30 10 + 20 5 + 10	Hass	180	Halls & Sons	Pre-waxing
XII	41°C 43°C	20 + 25 10	Hass late season	80	Everdon Estates, Natal	
XIII	39°C 41°C 42°C	30 25 20	Hass late season mature	60	Everdon Estates, Natal	Pre-waxing
XIV	39°C 41°C 42°C	30 25 20	Fuerte out of season mature	60	Tzaneen	Pre-waxing

The following day the mass of the dried fruits were determined by weighing and further drying by microwave oven on medium high until no change in weight was detected (to ensure total dryness) before the moisture content was calculated. This moisture content was used to determine the maturity of the avocado fruit (Kruger & Claassens, 1996).

The heat shock temperatures evaluated were 36°, 38°, 40° and 42°C and the time intervals are 30, 60, 180, 630 and 660 min. The samples consisted of 20 fruits for each treatment and a control of 10 fruits.

A data-logger (Cotton System data-logger) probe was connected to each waterbath (stainless steel baths with Carel thermostats) and a probe was inserted into each fruit. The thermocouples (Cotton System thermocouples) were used to measure the water, the fruit pulp temperatures in the waterbaths, the ambient fruit pulp temperatures, as well as the fruit pulp temperatures of the fruits as it cooled down in the cold room at 2°C. The fruits were then submitted to the hot waterbaths set at 36°, 38°, 40° and 42°C to give individual specific exposure times of 30, 60, 180, 630 and 660 min. After the heat shock the treated fruits and the controls were waxed with a polyethylene type wax (TAG – Dormas Chemicals, Johannesburg) at a concentration of one part wax and one part water and then stored for three weeks at 2°C in order to induce CI. After storage, the fruits were moved to room temperature for a week to ripen. A final evaluation was then conducted.

The most important evaluation parameters used were external and internal heat and CI (CI), as well as fruit firmness and physiological disorders. External temperature related injury was scored on a scale from 0 to 100% (v/v), where 0 indicated no injury and 100% (v/v) represented complete surface injury. Internal browning was expressed on a scale from 0 to 3, where 0 represented no browning, 1 slight browning at the base of the seed, 2 indicated injury under the skin and around the seed, and 3 represented complete discolouration of the fruit flesh. All following experimental studies were similarly conducted.

Experimental Study II: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min exposure times on 'Fuerte' fruit quality.

Four hundred and ten 'Fuerte' fruits from the Burgershall area were subjected to heat treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min time intervals and storage at 2°C for three weeks in order to induce CI. The changes in the time:temperature combination used in this study were based on the data obtained in Experimental Study I where it was found that a lower temperature range was required than in ES I. The time:temperature combinations were also chosen to establish a database of the different reactions of the fruits at different time and temperature ranges.

Experimental Study III: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min exposure times on 'Hass' fruit quality.

Four hundred and ten 'Hass' fruits from the Burgershall area were subjected to heat treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min time intervals and storage at 2°C for three weeks in order to induce CI. This study was done on the same day as Experimental Study II, but with the 'Hass' cultivar.

Experimental Study IV: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 10, 20, 30 and 40 min exposure times on 'Hass' fruit quality.

Three hundred and forty 'Hass' fruits from the ITSC at Nelspruit were used for this trial. In this study no cleaning of the fruits were done. The treatment temperatures were 33°, 36°, 39° and 42°C at the time intervals of 10, 20, 30 and 40 min. These time intervals were selected based on the results of Experimental Studies II and III where it was found that a shorter exposure time was required to prevent CI. Half the fruits were stored at 2°C for three weeks and half were conditioned for a day at 6°C and then a second day at 4°C before storage at 2°C. This is called a step-down, which is the recommended storage method of the PPECB (Perishable Product Export Control Board) (Eksteen, 1996).

Experimental Study V: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 10, 20, 30 and 40 min exposure times on 'Fuerte' fruit quality.

Three hundred and forty 'Fuerte' fruits from Halls & Sons were treated at temperature settings of 33°, 36°, 39° and 42°C for 10, 20, 30 and 40 min. These settings are the same as in Experimental Study IV. Half of the fruits were placed in a cool room at 6°C and were conditioned by means of a 2°C step for a period of one day until 2°C were reached. This was the step-down regime that was recommended by the PPECB for the export fruits at that stage (Eksteen, 1996). The other half of the fruits were stored at 2°C for three weeks.

Experimental Study VI: Influence of hot water treatments at 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Fuerte' fruit quality.

One hundred and sixty 'Fuerte' fruits from Halls & Sons pack house were used for this trial. Half of the fruits were washed and waxed in the pack house while the other half were not. The fruits were treated at temperatures of 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min and stored at 2°C for three weeks.

Experimental Study VII: Influence of hot water treatments at 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Hass' fruit quality.

Before the hot water heat shock treatment, half of the fruits from a sample of 500 'Hass' fruits from the ITSC were washed with a 0.5% (m/v) sodium hypochlorite solution while the other half was not washed. This was done to establish if the chlorite solution could possibly be the cause of more lenticel and chilling damage. The temperature settings evaluated were 39°, 41°, 43° and 45°C and the time intervals were 5, 10, 15, 20, 25 and 30 min and the fruits were then stored at 2°C for three weeks. These heat shock settings were the same as in Experimental Study VI, because the two studies were done simultaneously.

Experimental Study VIII: Influence of hot water treatments at 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Pinkerton' fruit quality.

Two hundred and forty 'Pinkerton' fruits from the Kiepersol area were exposed to hot water at 39°, 41°, 43° and 45°C for 10, 15, 20 and 25 min exposure time and stored at 2°C for three weeks. These heat shock settings were used because this study was also done concurrently to Experimental Studies VI and VII.

Experimental Study IX: Influence of hot water treatments at 33°, 36°, 39° and 42°C for *5, 10, 20 and 30 min exposure times on 'Edranol' fruit quality.*

One hundred and twelve 'Edranol' fruits from the Kiepersol area were treated at 33°, 36°, 39° and 42°C for 5, 10, 20 and 30 min exposure time and stored at 2°C for three weeks.

Experimental Study X: Influence of hot water treatments at 33°, 36°, 39° and 42°C for *5, 10, 20 and 30 min exposure times on 'Ryan' fruit quality.*

One hundred and twelve 'Ryan' fruits from the Kiepersol were treated at temperature settings of 33°, 36°, 39° and 42°C for intervals of 5, 10, 20 and 30 min exposure time and stored at 2°C for three weeks. The same settings used in Experimental Study IX were used on this cultivar.

Experimental Study XI: Influence of hot water treatments at 39°C for 25 and 30 min, 41°C for 25 and 30 min, 43° for 10 and 20 min and 45°C for 5 and 10 min exposure times on 'Hass' fruit quality.

Half of the 180 'Hass' fruits from HL Halls & Sons were waxed before, as well as after hot water heat treatment while the other half were waxed only after the heat treatment. The following temperature time permutations were evaluated: 39°C for 25 and 30 min; 41°C for 25 and 30 min; 43°C for 10 and 20 min; and 45°C for 5 and 10 min. The fruits were then stored at 2°C for three weeks. These settings were selected as they resulted in the most promising results for all the 'Hass' experimental studies.

Experimental Study XII: Influence of hot water treatments at 41°C for 20 and 25 min and 43°C for 10 min exposure times on late season 'Hass' fruit quality.

Eighty late season 'Hass' fruits from Everdon Estates in KwaZulu-Natal were used for this trial. All the fruits were washed and waxed in the pack house before being dispatched to Nelspruit via Tzaneen. The settings evaluated were 41°C for 20 and 25 min, as well as 43°C for 10 min exposure time and stored at 2°C for three weeks. These settings were selected to determine if the promising results from the previous Experimental Study (XI) could be further refined.

Experimental Study XIII: Influence of hot water treatments at 39°C for 30 min, 41°C for 25 min, 42°C for 20 min exposure times on late season 'Hass' fruit quality.

Sixty late season, but more mature, 'Hass' fruits from Everdon Estates in Natal were used in this trial. The fruits were waxed an hour before the hot water treatment was applied and again after the treatment. The settings evaluated were 39°C for 30 min, 41°C for 25 min and 42°C for 20 min exposure time and the fruits were then stored at 2°C for three weeks. These settings were selected because the data from the previous Experimental Study (XII) showed poorer results than was expected and the settings were changed to accommodate the differences in maturity.

Experimental Study XIV: Influence of hot water treatments at 39°C for 30 min, 41°C for 25 min, 42°C for 20 min exposure times on late season 'Fuerte' fruit quality.

Sixty out of season, but more mature, 'Fuerte' fruits from Tzaneen were waxed an hour before the hot water treatment and again after the treatment. The settings evaluated were 39°C for 30 min, 41°C for 25 min and 42°C for 20 min and the fruits were then stored at 2°C for three weeks. Because of the maturity of these fruits the same scenario was used as in Experimental Study XIII.

Results and discussion

To facilitate the discussion of the results obtained, the Experimental Studies were grouped into cultivar groupings.

'Fuerte'

Experimental Study I: Influence of hot water treatments at 36°, 38°, 40° and 42°C for 30, 60, 180, 630 and 660 min exposure times on 'Fuerte' fruit quality.

The results obtained with the 'Fuerte' cultivar in Experimental Study I are given in Table 2. In Fig. 1, the statistical differences based on Duncan's multiple range test (P = 0.05) are illustrated. The data showed that the best treatment under these study conditions was 40°C for 30 min. Although the second best treatment was 38°C for 60 min and statistically did not significantly differ from the best treatment, the third treatment differed statistically from the best and not from the second, therefore, 40°C for 30 min was selected as the best treatment with 15.7% (v/v) CI. The CI was determined by the percentage (v/v) CI scars on the whole surface of each avocado fruit. The average of all the replicas gave the total score of 15.7% (v/v) CI. In comparison with the control with 70.5% (v/v) CI, the best treatment showed significantly less CI on the fruits.

Experimental Study II: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min exposure times on 'Fuerte' fruit quality.

The best treatment obtained in this study was the 42°C for 70 min combination with 55% (v/v) CI compared to the control of 85% (v/v) CI (Table 2). The same method used in ES I was used to determine the results. Although the cold room dipped to near zero °C, the data still showed that the heat shock treatment had performed better than the control and showed potential in extreme temperatures.

Table 2. Chilling injury induced after the hot water heat treatments of 'Fuerte' fruits and storage at 2°C for 21 d.

Exp. Study (ES)	Moisture content (%)(g.100g^{-1})	Experimental Design	Best temperature and time combinations		Chilling Injury (CI) (% (v/v) surface area with CI)	
			Temp (°C)	Time (min)	Control % (v/v) CI	Best % (v/v) CI
ES I	72.18	Standard	40	30	70.5	15.7
ES II	71.62	Standard	42	70	85.0	55.0*
ES V	69.05	Standard	42	20	14.5	5
		Standard (Step-down)	42	30	5.5	2
ES VI	63.90	Standard	43	5	30 Fig.4+5	3
		Pre – waxed	41	20	30 Fig.6+7	0
ES XIV#	68.93	Pre – waxed	42	20	36.7	14

*The cold rooms in which the fruits were stored had a deviation of less than ± 0.5°C, but in this specific case the temperature dipped below zero for a day and a half, which resulted in higher CI (v/v) scores.

#Out of season fruits from Tzaneen.

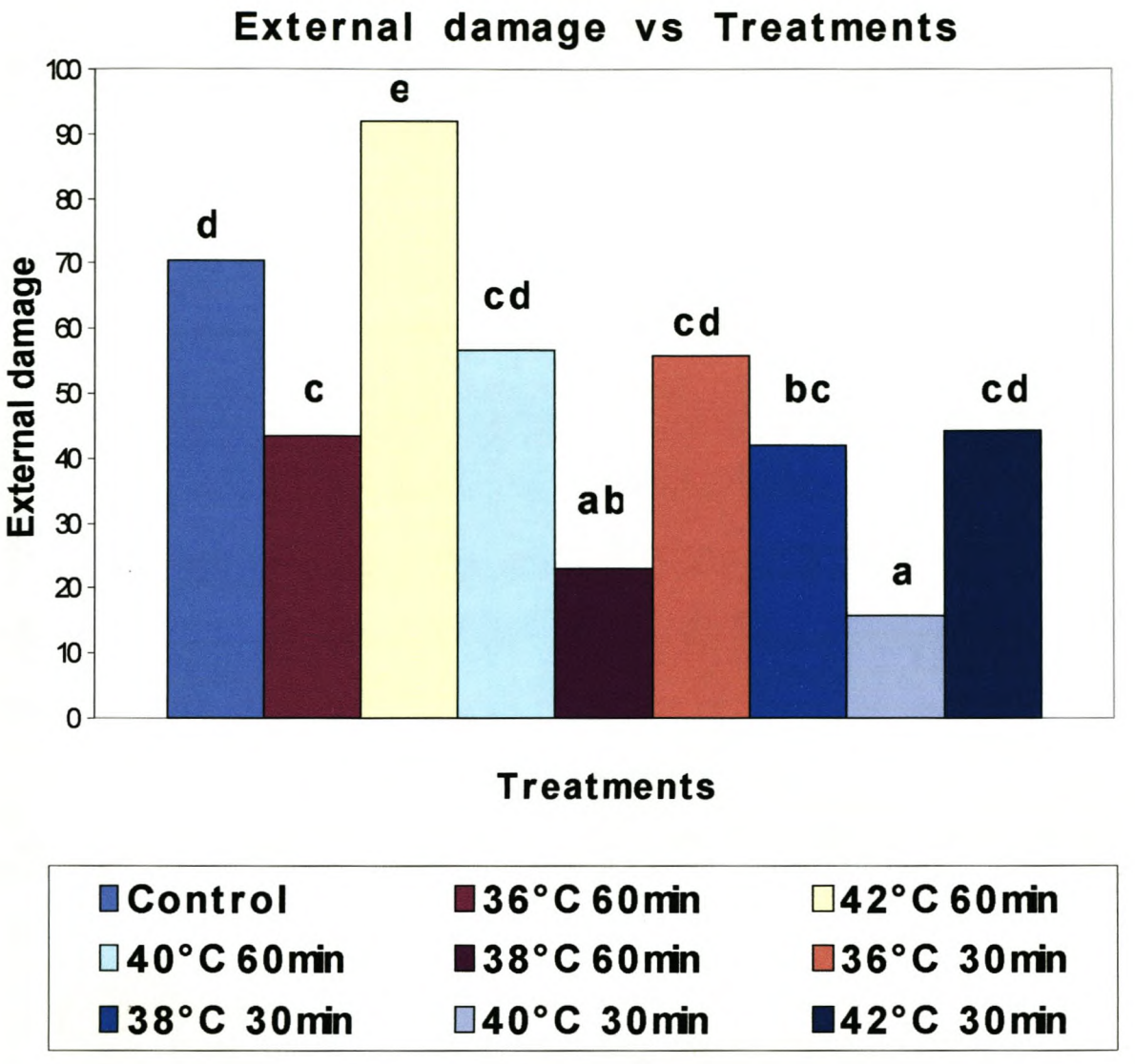

Figure 1. External damage resulting from the different treatments on the 'Fuerte' cultivar (Experimental Study I but all the other ES results were done the same way). The statistical difference was measured with Duncan's multiple range test (P = 0.05).

This information is valuable in the sense that it showed that during shipping, temperature drops could occur, leaving disastrous consequences for the exporters if they don't know what to expect the quality and condition of the fruits would be arriving overseas. This batch of 'Fuerte' fruits were from the same origin as those used in Experimental Study I, but these fruits were more mature with a 71.62% ($g.100g^{-1}$) moisture content compared to a 72.18% ($g.100g^{-1}$) moisture content of the Experimental Study I. In this case the difference in maturity, but mainly the extreme cold exposure played an important role in the effective temperature and exposure time combination, explaining the difference in results obtained. It is known that early on season fruits are more sensitive and susceptible to CI than late season fruits (Eksteen, 1998; Kruger & Claassens, 1997). Based on the lower moisture and higher oil content, which determines the maturity of the fruits (Fig. 2), lower temperatures were chosen to treat these fruits. The assumption was made that with higher oil, better heat transfer was expected and, therefore, lower heat temperature regimes seemed necessary. In Experimental Study I, temperatures of 36°, 38°, 40° and 42° were used, but in Study II, to compensate for the more mature fruits, lower temperatures such as 33°, 36°, 39° and 42°C were evaluated. Shorter exposure times of 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min instead of 30, 60, 180, 630 and 660 min were also used since the results obtained in Experimental Study I showed that the exposure times were too long and heat damage occurred. Although in this case with the unusual extreme conditions, the best treatment of 42°C for 70 min showed a definite improvement over the control values and it can still be concluded that the hot water treatment did help to minimise the CI even under such extreme low storage temperatures. In Fig. 3 the time the fruit pulp takes to reach the specific temperature, as well as the fluctuations of the different temperatures in the waterbaths are indicated. This gave an idea of how the heat conductivity inside the avocado fruit worked. For example, the fruit took approximately 30 – 40 min to reach a temperature of 33°C.

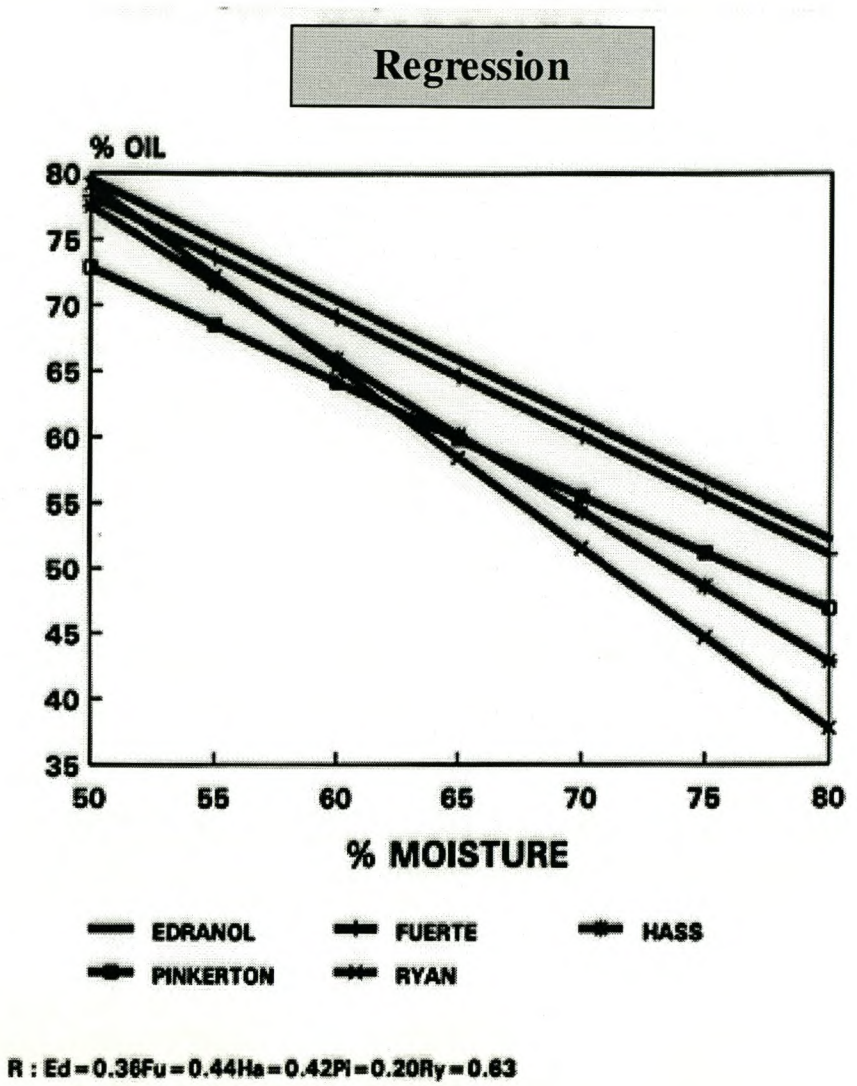

Figure 2. The linear regression between moisture and oil content of the different cultivars (Kruger & Claassens, 1996).

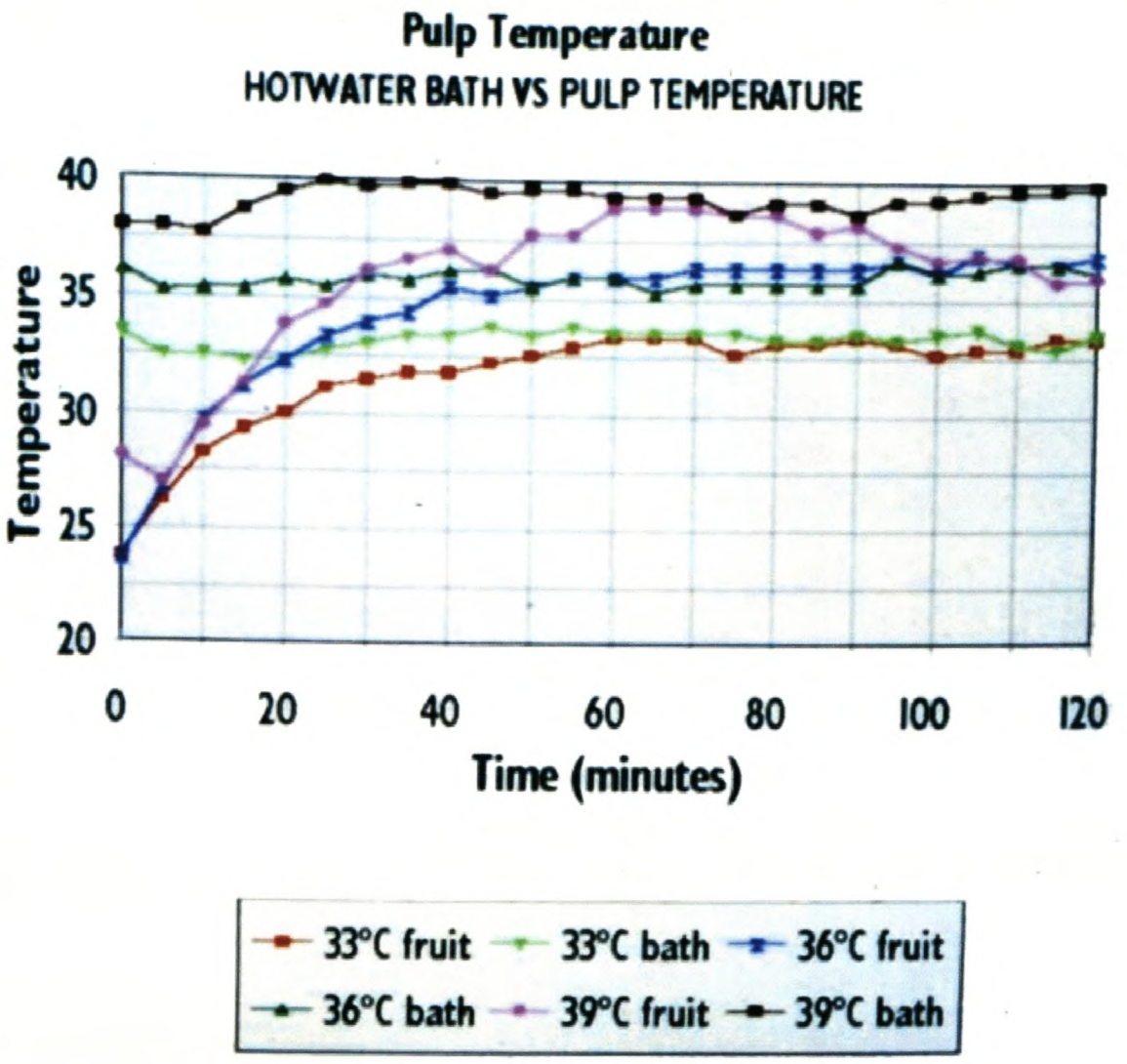

Figure 3. The fluctuation in temperatures and the time it takes for the pulp of the fruit to reach different temperatures using probes in the hot waterbaths and inside the fruit.

The probes in the hot waterbaths gave an indication of how accurate the temperature of the waterbaths were kept. This exercise was very important for the reason that no inaccurate predictions could be made. From the results of the probe readings, the waterbaths worked effectively and from the results of the fruit pulp temperatures the next Experimental Study could be planned.

Experimental Study V: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 10, 20, 30 and 40 min exposure times on 'Fuerte' fruit quality.

The best temperature and time combination obtained by means of the standard method was 42°C with a 20 min exposure time resulting in a CI of 5% (v/v) compared to the control of 14.5% (v/v) CI (Table 2). This result was obtained with the same method used in ES I. By means of a step-down temperature regime (6° - 4° - 2°C), the best combination was 42°C for 30 min exposure time with 2% (v/v) CI compared to the control of 5.5% (v/v) CI (Table 2). The step-down method showed potential when the two controls of the different methods were compared. With the step-down regime the control showed only 5.5% CI compared to 14.5% CI of the control at 2°C. Which indicated that not only, heat shock treatments could minimise CI, but also the use of a step-down regime. It must also be taken into consideration that the fruits in this study were at a moisture level of 69.05% ($g.100g^{-1}$) and were very mature, which makes the fruits less susceptible to CI, making a heat shock treatments more effective. Each study, therefore, on its own showed valuable information in the sense that progressing of the seasons and maturity, influenced heat transfer in the avocado fruit.

Experimental Study VI: Influence of hot water treatments of 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Fuerte' fruit quality.

The best temperature and time combination obtained in this study for the standard method (fruits waxed after the heat shock treatment), was 43°C for 5 min (Fig. 4 and 5) with CI of 3% (v/v) compared to the control of 30% (v/v) (Table 2). The fruits that were pre-treated with TAG-wax before the heat shock was administered, showed even better results than the standard method with the best time:temperature combination of 41°C for 20 min (Fig. 6 and 7) with no

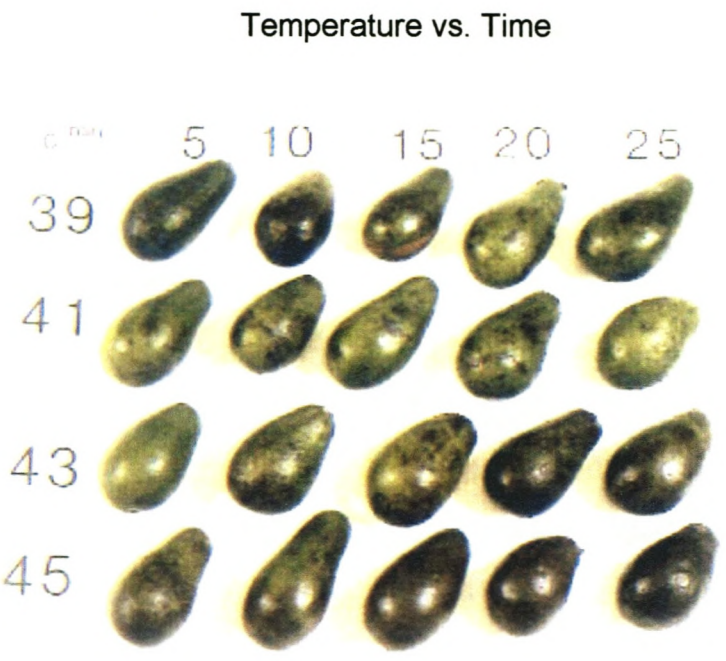

Figure 4. Grid layout of a representative sample of each heat treatment of the 'Fuerte' fruits after storage that were not pre-waxed before treatment. The best treatment was 43°C for 5 min.

Temperature vs Time

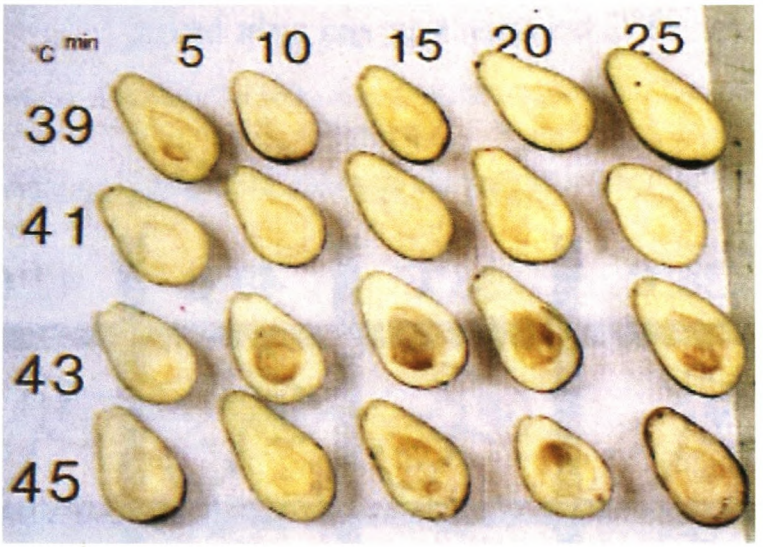

Figure 5. Grid layout of sectioned 'Fuerte' fruits that were not pre-waxed before treatment.

Temperature vs Time

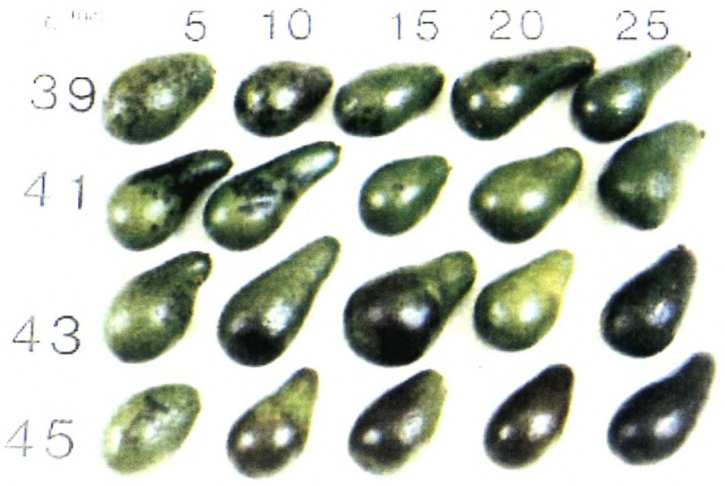

Figure 6. Grid layout of a representative sample of each heat treatment of the 'Fuerte' fruits after storage that were pre-waxed before the heat treatment. The best treatment was 41°C for 20 min.

Temperature vs Time

Figure 7. Grid layout of sectioned, pre-waxed 'Fuerte' fruits.

CI compared to the control that had 30% (v/v) CI (Table 2). The same batch of fruits were used for the standard, as well as for the pre-waxed treatment and two different results were obtained. This indicated that the wax protected the fruits and made the heat shock treatment more effective. From the two grid layouts, where a representative sample of each heat treatment versus each exposure time was chosen (Fig. 4 and 6), showed that a very precise time:temperature combination is required for the heat shock treatment to be effective. It was also found that the fruits treated at the lower temperatures showed more CI than those subjected at middle temperatures, while the higher temperatures showed heat damage, which could easily be mistaken for CI (Fig. 4 and 6). The sectional fruits, as shown in Fig. 5 and 7, showed that the higher temperature of 45°C lead to more darkening of the flesh around the inside of the skin, due to heat damage. From the results obtained in Experimental Studies I – V, a definite pattern was found especially with the 'Fuerte' fruits. Higher temperature settings were required to be effective because of the more mature fruits.

Experimental Study XIV: Influence of hot water treatments at 39°C for 30 min, 41°C for 25 min, 42°C for 20 min exposure times on late season 'Fuerte' fruit quality.

The best temperature and time combination obtained in this study was 42°C for 20 min exposure time, which resulted in 14% (v/v) CI for the best combination and 36.7% (v/v) CI for the control (Table 2). The same method was used in determining the results as in ES I. The same temperature and time intervals were used as in Experimental Study V. Interesting facts were obtained through these two ES. Although, this study was not as successful as Study V, probably because of late season 'Fuerte' fruits from Natal, which has a totally different climate than that is found in Mpumalanga and were grown differently, the fruits had more or less the same maturity level and, therefore, the same best time:temperature combination of 42°C for 20 min. This indicated that maturity does definitely determine the choice of heat shock treatment. Making this technique too complicated for pack houses, where different producers and different maturity fruits arrive.

'Hass'

Experimental Study III: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 5, 10, 15, 20, 30, 45, 70, 90, 105 and 120 min exposure times on 'Hass' fruit quality.

The 'Hass' results of this study are summarised in Table 3, while the evaluation design of the different treatments and repetitions are shown in Fig. 8. The best time:temperature combination for this study was 30°C for 90 min, with a 40% (v/v) CI compared to the 80% (v/v) CI of the control (Table 3). This study was done on the same day as Experimental Study II and the same problem that occurred with the cold room that was below zero for a day and a half was experienced here. However, this incident was considered an excellent opportunity to see if the heat shock treatment was really effective. The moisture content was 75.57% ($g.100g^{-1}$), which indicated that these fruits were 'beginning of the season' fruits. Fruits from the early stage of the season are more susceptible to CI than fruits from later in the season (Eksteen, 1998; Kruger & Claassens, 1997). Under these extreme condition of near zero °C storage the heat treatment of 30°C for 90 min showed potential.

Experimental Study IV: Influence of hot water treatments at 33°, 36°, 39° and 42°C for 10, 20, 30 and 40 min exposure times on 'Hass' fruit quality.

In this study the best time:temperature combination for the standard method was 36°C for 20 min exposure time with 25% (v/v) CI compared to the control with 82% (v/v) CI (Table 3). The step-down method, where the fruits were subjected to a temperature of 6°C for one day, then at 4°C for another day before being stored at 2°C for the rest of the storage period showed better results. This method had the best combination of 42°C for 30 min exposure time and the CI was 25% (v/v) compared to the control of 49% (v/v) CI. In this study the step-down method gave better control fruits, 49% CI, than the standard method of 80% CI.

Table 3. Chilling injury induced after different heat treatments of 'Hass' fruits and storage at 2°C for 21 d.

Exp. Study (ES)	Moisture content (%) ($g.100g^{-1}$)	Experimental Design	Best temperature and time combination		Chilling Injury (CI) (% (v/v) surface area with CI)	
			Temp (°C)	Time (min)	Control	Best
ES III	75.57	Standard	30	90	80	40
ES IV	65.20	Standard	36	20	82	25
		Step – down	42	30	49	25
ES VII	67.40	Standard	None	None	80	84
		No hypo-chlorite	None	None	70	86
ES XI	66.00	Standard	41	25	5	2.8
		Pre - waxed	41	25	5	0
ES XII	66.80	Pre - waxed	41	20	2	2
ES XIII	64.30	Pre - waxed	42	20	34	22

Figure 8. Experimental set-up showing the different treatments, as well as the repetitions of 'Hass' from Experimental Study III.

This indicated that by gradually bringing the temperature in the cold room down, could condition the fruits and protected them from cold inducing temperatures. These findings correlated with data found in Experimental Study V and showed that a step-down temperature regime that conditions the fruits to cold storage temperatures was more efficient than a constant cold inducing temperature storage regime at 2°C. Although the two heat treatments of the standard and the step-down gave both 25% CI, the option of a step-down regime is more practical for a pack house than to waste 20 - 30 min on a waterbath.

Experimental Study VII: Influence of hot water treatments at 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Hass' fruit quality.

In this study the standard use of hypochlorite was compared against no hypochlorite treatment and the data (Table 3) showed clearly that there was no best treatment for either the standard option or the treatment without hypochlorite. In both cases (Table 3) the control showed a lower CI than were found for the treated fruits. This indicated that the heat shock treatments used in this study were not efficient and that further investigation is needed.

Experimental Study XI: Influence of hot water treatments at 39°C for 25 min and 30 min, 41°C for 25 and 30 min, 43°C for 10 and 20 min and 45°C for 5 and 10 min exposure times on 'Hass' fruit quality.

With a moisture content (maturity) of 66% ($g.100g^{-1}$), the fruits used in this study were very mature, and the best combination temperature and exposure time for the standard method was 41°C for 25 min with 2.8% (v/v) CI and for the control 5% (v/v) CI (Table 3). The results obtained in this ES were determined by the same method that was used in ES I. The very low CI of 2.8 – 5% will be acceptable on the overseas market. With the pre-waxed fruits, the best combination was also 41°C for 25 min with no CI and 5% (v/v) CI for the control. From the data obtained in this study it showed that pre-waxing gave extra heat protection for the fruits. It appeared that the heat shock treatments (the best standard and pre-waxed treatment) did work an extend than when compared to the control, which showed less CI (due to the difference in percentages). Physically the fruits looked the same. Therefore, the data

obtained indicated that at very high maturity levels (low moisture content), a heat shock treatment is not necessary (Table 3).

Experimental Study XII: Influence of hot water treatments at 41°C for 20 and 25 min and 43° for 10 min exposure times on late season 'Hass' fruit quality.

The fruits evaluated in this study were from Natal and underwent a long journey before arriving in Nelspruit. The fruits were very mature and from the results, the best treatment combination and the control had the same level 2% (v/v) CI (Table 3), while the rest of the heat treated fruits had much higher CI damage. These higher CI scores indicated that the wrong temperature and time exposure to hot water treatment could do more damage than good. It is, therefore, of the utmost importance that the precise temperature and time exposure be determined before any decision on a heat shock treatment for any kind of fruit, is made. The reason for including these data and findings was because it showed that long journeys need to be cold chained, for fruits arriving on the different export ports, could already have softened.

Experimental Study XIII: Influence of hot water treatments at 39°C for 30 min, 41°C for 25 min, 42°C for 20 min exposure times on late season 'Hass' fruit quality.

These 'late season' fruits had a maturity of 64.3% ($g.100g^{-1}$), which was lower in moisture content (%) than those used in the previous Experimental Study (XII). The best temperature and exposure time combination was 42°C for 20 min with 22% (v/v) CI and 34% (v/v) CI for the control (Table 3). The data showed that even the best combination treatment had a high CI score. This could possibly be as a result of the long transportion time and excessive moisture loss, which could have resulted in more skin damage and CI. Thus, it is recommended that the fruits must be transported directly to the pack house after harvesting as quickly as possible, where it will be treated, packed and cold stored before much moisture is lost (Table 3). Even though no usable results were obtained from this study, every study on its own gave enough other information that could be useful in other research.

The following cultivars were investigated as preliminary experiments.

'Pinkerton'

Experimental Study VIII: Influence of hot water treatments at 39°, 41°, 43° and 45°C for 5, 10, 15, 20 and 25 min exposure times on 'Pinkerton' fruit quality.

This study was undertaken to investigate 'middle season' fruits with a maturity of 69.19% ($g.100g^{-1}$). The best combination for this study was 43°C for 20 min (Table 4) with no CI and the control also had no CI. The rest of the 'Pinkerton' treatments from this study (not shown in table) had high CI levels. This phenomena, where all the rest of the heat treatments showed CI (not shown in table) except for 43°C for 20 min and the control, showed again the importance of the right temperature and exposure time. The conclusion that could be made from this ES was that where the control had no CI, the fruits were mature enough to withstand the chilling inducing low storage temperatures.

'Edranol'

Experimental Study IX: Influence of hot water treatments at 33°, 36°, 39° and 42°C for *5, 10, 20 and 30 min exposure times on 'Edranol' fruit quality.*

This study was undertaken to investigate the behaviour of 'early season' fruits of a late season cultivar with a maturity of 74.77% ($g.100g^{-1}$). The temperature settings used with this cultivar were as in Experimental Studies II – IV because the other cultivars, 'Hass' and 'Fuerte', showed promising results with these temperature settings at the beginning of the season. However, different time settings were used because the results obtained from Experimental Studies II – IV indicated that a lower exposure time could be used. In this study it was found that two treatments (39 °C for 20 min and 42 °C for 10 min) resulted in no CI compared to the control which had 20% (v/v) CI (Table 4).

Table 4. Chilling injury induced after hot water heat treatments and storage at 2°C for 21 d of the 'Pinkerton', 'Edranol' and 'Ryan' cultivars.

Cultivars and Exp. Study (ES)	Moisture content (%) ($g.100g^{-1}$)	Experimental design	Best temperature and time combinations		Chilling Injury (CI) (% (v/v) surface area with CI)	
			Temp (°C)	Time (min)	Control	Best
Pinkerton ES VIII	69.19	Standard	43	20	0	0
Edranol ES IX	74.77	Standard	39 42	20 10	20 20	0 0
Ryan ES X	68.74	Standard	42	20	0	0

'Edranol' is a cultivar that has a very thin and smooth skin that can easily be damaged. The heat shock treatment technique for this cultivar, therefore, showed excellent potential and worked very well with the best combinations which had no CI compared to the control.

'Ryan'

Experimental Study X: Influence of hot water treatments at 33°, 36°, 39° and 42°C for *5, 10, 20 and 30 min exposure times on 'Ryan' fruit quality.*

This study was undertaken to investigate the behaviour of 'late season' fruits with a late season cultivar that had a maturity of 68.74% ($g.100g^{-1}$). The best treatment for this study (42°C for 20 min), as well as the control showed no CI (Table 4). The rest of the treatments in this study had CI, which showed again that it's important not to use the wrong temperature and time regime as the data showed that the non-treated fruits (control) appeared better with no CI than the treated fruits. The data also indicated that the 'Ryan' fruits did not benefit from the heat shock treatments. This could be because of their thick skin and natural toughness.

Conclusion

It can be concluded that each Experimental Study must be seen on is own, as each study had its own unique situation and influencing factors. From all the studies, valuable information was gained, for example, how the different cultivars, as well as the different maturity levels reacted to different heat shock treatments. This was an excellent exercise for what might happen when pack houses receive different producer's avocados with different maturities. The mixture of different maturities are inevitable when the producers harvest their crop, because the whole tree is harvested at once resulting in different maturity stages on the same tree (Fig. 9).

From the data obtained in the Experimental Studies it was found that the results of the 'Hass' cultivar were not as promising as those reported for the New Zealand 'Hass' by Woolf & Laing (1996).

Figure 9. The difference in fruit maturity on the same tree (Kruger & Claassens, 1996).

The same conclusion was confirmed by the results obtained by another South African researcher (Kremer-Köhne, 1998). In this study it was found that 'Hass' fruits were less susceptible to CI than 'Fuerte' fruits, except that at the start of the season all the avocados had high moisture contents and low oil contents. This high moisture content could be the cause of the avocados being more susceptible to CI in the beginning of the season. With 'Fuerte', a pattern was detected where the most promising results were obtained between 40° and 42°C and the exposure time between 20 and 30 min. Although a definite pattern was not clear for 'Hass', but from the available results it would appear that the permutations for this cultivar might also fall within the above ranges.

Of the other cultivars the thicker skin type ('Ryan' and 'Pinkerton') were found to be less susceptible to CI than the thin skin 'Edranol'. The range of temperatures and exposure time combinations that procured protection was also wider for 'Edranol' than for the 'Fuerte' or 'Hass' cultivars.

The effectiveness of the heat treatment technique was markedly influenced by the maturity of the fruits. This was expected, but data confirming and proving the difficulty of the technique was necessary for future reference and research. There is no non-destructive method currently available to predict the maturity of the avocados online in the pack house or even in the orchard at harvesting. This makes the technique even more complicated. Therefore, there is no way what so ever to work with definite maturity norms (Chapter 6).

At the beginning of the season, hot water heat treatments reduced CI, but it was not possible to eliminate it completely (ES I, III and IX), whereas towards the end of the season, CI was preventable, but very little CI occurred on the control fruits (ES V, VI, VIII, X, XI and XII).

Pre-treatment waxing of the fruits appeared to improve the effectiveness of the treatment by giving the fruits extra protection against CI. It was also found that the use of pre-conditioning method where the fruits were treated by means of a step-down storage regime also lead to lower CI values.

From all the results obtained, the use of very precise temperature and exposure time was needed for the heat shock treatment to be totally effective. It is, therefore, of the utmost importance that further research be done before a pack house attempt to use heat shock treatments on the fruits. The reason is

because the incorrect use of the heat shock treatment technique can do more damage than no treatment at all.

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CHAPTER 4

RE-EVALUATION AND OPTIMIZATION OF COMBINED HOT WATER AND HOT AIR HEAT SHOCK TREAMENTS ON SOUTH AFRICAN AVOCADOS IN ORDER TO MINIMISE THE OCCURRENCE OF CHILLING INJURY

Abstract

Refined methodology of heat shock treatments was used. Chapter 3 showed only results of hot water heat shock treatments. In this study hot air heat shock treatments were also used. The temperature and time intervals were narrowed to fall within the most effective ranges identified in Chapter 3. Chilling inducing regimes, which were 2° to 4°C lower than the commercial regime but followed the same stepping pattern were also implemented. Preliminary results in the previous Chapter indicated specific trends, but were not consistent with published reports from New Zealand on this matter. The results obtained in this Chapter were consistent with results in Chapter 3. From the results obtained, 'Hass' proved to be unresponsive to the treatment while a measure amount of success was obtained with 'Fuerte' and 'Edranol'. Although the technique minimised the occurrence of CI, the practicality over rules the effectiveness of this technique. The technique further proved to be unnecessary with 'Ryan' while certain encouraging trends with step-down together with conditioning were observed in 'Pinkerton', which could help this cultivar with its poor storage potential. Maturity also manifested as a fluctuating influence, which made the heat conductivity from the heat shock technique variable and, therefore, the effectiveness of this technique unreliable to use. Unless a non-destructive maturity measurement apparatus could be installed to sort fruits according to different maturity grades before ripening, the incorporation of a heat shock treatment in a pack house should not be considered.

Introduction

In the previous Chapter of this thesis (Chapter 3) it was shown that hot water and hot air treatments render the fruits more tolerable to lower temperature storage and this is generally known as heat shock treatment. The fruits are protected from chilling injury (CI) by inducing the formation of heat-shock proteins which render the cell membranes more resistant to CI (Harrington *et al.*, 1994; Nover, 1984). Not only must the treatments be administered within certain temperature and time parameters in order to be effective, but it was also found that these temperatures and exposure times had to be determined per cultivar and a number of trials have already been conducted in other avocado exporting countries (Nishijima *et al.*, 1995; Woolf *et al.*, 1995; Woolf & Laing, 1996).

It was also found that maturity played an important role in the sensitivity of the avocado to CI, which confers the work of Eksteen (1998). This Chapter was funded by SAAGA for more refined work, the experiments were conducted even though the knowledge existed that the maturity of the fruits will determine the heat conductivity and, therefore, the effectiveness of the technique. But valuable information regarding the different producers and geographic position of their orchards, as well as maturity information was gained. This information lead to further research projects. Previous data also showed that early season fruits are more susceptible to CI when stored at low temperatures, as predicted by Kruger & Claassens (1997). Therefore, if the heat shock treatment is successful and repeatable, this treatment regime will be applied at all the avocado pack houses where fruits are packed for sea freight export. This will be of benefit to the South African producers in that the cultivars will arrive at the overseas market at a time when the avocado season of competing producers from other countries, has ended. The heat treatment will not only minimise CI but it will also maintain the quality of the fruits during shelf-life for a longer period (Biggs *et al.*, 1988; Eaks, 1978; Maxie *et al.*, 1974; Tsuji *et al.*, 1984).

After careful evaluation and consideration of the data obtained in Chapter 3 of this thesis, the Experimental Studies of Chapter 4 were planned.

A pattern with 'Fuerte' fruits were obtained and the most effective temperatures were between 40° and 42°C for an exposure time of between 20 and 30 min. The 'Hass' cultivar had no specific pattern and showed little sensitivity towards low temperature storage. The 'Ryan' and 'Pinkerton' cultivar showed no response to the heat shock, while the 'Edranol' cultivar showed promising results. A more refined approach and the use of more sensitive settings are required to define the exact temperature and exposure time schedules.

The aim of this study was, therefore, to evaluate the best temperature and time regime refined from the results obtained in Chapter 3 of this thesis.

Materials and methods

A total of twenty trials were conducted during the 1997 season. Eight trials were done using 'Fuerte', five with 'Hass', two each with 'Pinkerton' and 'Edranol' and three with the 'Ryan' cultivar. The fruits originated from four locations, namely, Burgershall (Research Station), Kiepersol (Koeltehof & Koos van Heerden), Levubu (Research Station) and Barberton (Michael van Schalkwyk).

The fruits were harvested in lug boxes and transported to the laboratory. A standard cleaning method was used which included the following: washing of the fruits with 0.5% (m/v) hypochlorite solution to remove sooty blodge (black fungus, causing infection after ripening), after which the fruits were rinsed with tap water to protect the fruits against lenticel damage and then a natural Carnauba coating, Stafresh at a concentration of 1:1, was applied by dipping the fruits in the wax. The fruits were stored for 21 days in the cold rooms at the different temperatures stated in each ES and were then placed at ambient temperature for a week to ripen. The fruits were evaluated according to the CI percentage on the whole surface of each fruit. The average of each treatment together with all the replicas represented the CI (%) as given in the result tables. Other parameters such as firmness and internal disorders were also evaluated, but because the problem that was under investigation was external appearance of CI of the exported fruits, these parameters were not mentioned,

but was necessary to explain the results obtained from each ES. The various storage regimes employed are depicted in Fig. 1.

The experimental designs as summarised in Table 1 of the different experimental studies, are given in chronological order.

Experimental Study I: Influence of hot water bath treatments at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min exposure times on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to hot water heat shock treatments (HWHST) at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min. Storage Temperature (ST) step-down from 5.5°C for seven days and further step-down with a half of a degree every day until 4°C for the rest of the storage period.

Experimental Study II: Influence of hot water bath treatments at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min exposure times on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to HWHST at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min. ST step-down from 5.5° to 4°C.

Experimental Study III: Influence of hot water bath treatments at 39°, 40°, 41° and 42°C for 20, 22, 24 and 26 min exposure times on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to HWHST at 39°, 40°, 41° and 42°C for 20, 22, 24 and 26 min. ST step-down 6° - 5°C, 4° - 3°C and 2.5° - 1°C were used to evaluate the different temperature regimes.

Experimental Study IV: Influence of a pre-treatment of hot air of 30°C for 30 min together with hot water treatments at 40° for 26 min and 42° for 24 min exposure times on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to PR (pre-treatment) hot air at 30°C for 30 min and HWHST at 40°C for 26 min and 42°C for 24 min. ST step-down 5° - 4°C. No 2°C cold storage.

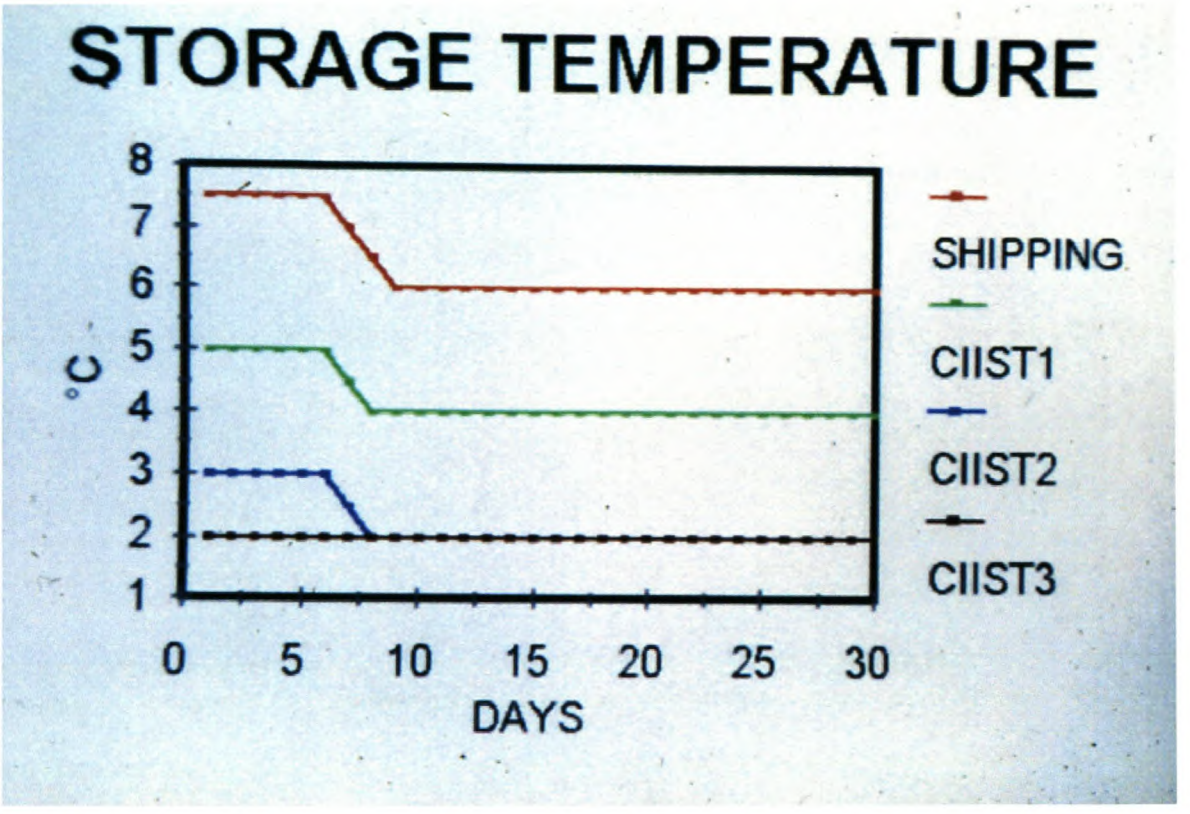

Figure 1. The various storage regimes include the standard shipping regime of 7.5°C for 7 days and then step-down to 7°C for one day followed by 6.5°C for one day and then step-down to 6°C for the rest of the storage period of ± 21 days. The chilling inducing injury step-down temperatures (CIIST) include 5° (seven days) – 4.5° (one day) - 4°C (rest of storage), 3° (seven days) – 2.5° (one day) - 2°C (rest of storage) and a constant storage temperature of 2°C.

Table 1. Summary of the different experimental designs.

Exp Study	Hot water temperatures	Exposure Time (Minutes)	Cultivar	Sample size	Area	Other storage temp. (21 day)
I	HWHST* 40°, 41°, 42°, 43°C	22, 24 , 26, 28	Fuerte	119	Burgershall	Step-down, 5.5° - 4°C
II	HWHST 40°, 41°, 42°, 43°C	22, 24 , 26, 28	Fuerte	119	Burgershall	Step-down, 5.5° - 4°C
III	HWHST 39°, 40°, 41°, 42°C	20, 22, 24, 26	Fuerte	350	Kiepersol	6° - 5°C, 4° - 3°C, 2.5° - 1°C
IV	HAHST* 30°C HWHST 40°C 42°C	60 26 24	Fuerte	70	Levubu	Step-down 5° - 4° , 2°C
V	HAHST 38°C 40°C	180, 360, 600 30	Fuerte	100	Kiepersol	2°C
VI	HAHST 38°C 40°C	180, 360, 600 30	Fuerte	100	Kiepersol	2°C
VII	HAHST 36°, 38°, 40°C	30, 180, 360, 600, 720	Fuerte	320	Levubu	Step-down 5° - 4°C, 2°C
VIII	HWHST 40°, 41°, 42°, 43°C	22, 24, 26, 28	Hass	360	Burgershall	Step-down 5.5° - 4°C 7.5° - 5.5°C
IX	HWHST 40°, 41°, 42°, 43°C	22, 24, 26, 28	Hass	360	Burgershall	Step-down 5.5° - 4°C 7.5° - 5.5°C
X	HWHST 40°C 41°C 42°C 43°C	24, 26, 28 24, 26, 28 22, 24 20, 22, 33	Hass	130	Burgershall	Step-down 3.5° - 2°C
XI	HAHST 38°C 40°C	360, 540, 1440 30	Hass	250	Burgershall	2°C
XII	HAHST		Hass	250	Burgershall	2°C

	38°C 40°C	360, 540, 1440 30				
XIII	HWHST 39°C 40°C 42°C 43°C	 12, 15, 18 15, 18, 20 18, 20, 22 12, 15, 18	Pinkerton	130	Kiepersol	Step-down 5° - 3.5°C
XIV	HAHST 30°C 20°C HWHST 39°C 40°C 42°C 43°C	 600 240 15 15 15 15	Pinkerton	150	Kiepersol	 2°C
XV	HWHST 40°C 41°C 42°C 43°C	 18, 20, 22, 25 18, 22, 25 15, 18, 22 15, 18, 22	Ryan	140	Kiepersol	Step-down 5.5° - 2.5°C, 2°C
XVI	HAHST 36°, 38°, 40°C	 540, 600, 660	Ryan	210	Kiepersol	Step-down 5° - 4°C, 3.5° - 2°C, 2°C
XVII	HAHST 36°, 38°, 40°C	 30, 180, 360, 600, 720	Edranol	240	Barberton	Step-down 5° - 4°C, 3° - 2°C, 2°C
XVIII	HWHST 36°C 40°C 41°C 42°C	 22, 28, 30 20, 22, 25 15, 18, 20 8, 10, 15	Edranol	390	Kiepersol	Step-down 5° - 4°C, 3° - 2°C, 2°C

* HWHST = hot water heat shock treatment

HAHST = hot air heat shock treatment

Experimental Study V: Influence of hot air treatments at 38°C for 180, 360, and 600 min, as well as hot water treatment at 40°C for 30 min exposure time on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to Hot Air Heat shock Treatments (HAHST) at 38°C for 180, 360 and 600 min and then 40°C for 30 min. ST was kept at 2°C for the whole storage period.

Experimental Study VI: Influence of hot air treatments at 38°C for 180, 360 and 600 min, as well as hot water treatment at 40°C for 30 min exposure time on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to Hot Air Heat shock Treatment (HAHST) at 38°C for 180, 360 and 600 min, 40°C for 30 min. ST was kept at 2°C for the whole storage period.

Experimental Study VII: Influence of hot air treatments of 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min exposure times on 'Fuerte' fruit quality.

'Fuerte' fruits were submitted to HAHST at 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min, respectively. ST step-down 5° - 4°C and 2°C.

Experimental Study VIII: Influence of hot water treatments of 40°, 41°, 42° and 43°C at 22, 24, 26 and 28 min exposure times on 'Hass' fruit quality.

'Hass' fruits were submitted to HWHST at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min. ST steps 5.5° - 4°C and 7.5° - 5.5°C.

Experimental Study IX: Influence of hot water treatments of 40°, 41°, 42° and 43°C at 22, 24, 26 and 28 min exposure times on 'Hass' fruit quality.

'Hass' fruits were submitted to HWHST at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min. ST steps 5.5° - 4°C and 7.5° - 5.5°C.

Experimental Study X: Influence of hot water treatments of 40°C for 24, 26 and 28 min; 41°C for 24, 26 and 28 min; 42°C for 22 and 24 min and 43°C for 20, 22 and 33 min exposure times on 'Hass' fruit quality.

'Hass' fruits were submitted to HWHST at 40°C for 24, 26 and 28 min; 41°C for 24, 26 and 28 min; 42°C for 22 and 24 min; 43°C for 20, 22 and 33 min. ST step-down 3.5° - 2°C.

Experimental Study XI: Influence of hot air treatments of 38°C for 6, 9 and 24 h, as well as 40°C for 30 min exposure times on 'Hass' fruit quality.

'Hass' fruits were submitted to HAHST at 38°C for 6, 9 and 24 h and 40°C for 30 min. ST at 2°C.

Experimental Study XII: Influence of hot air treatments of 38°C for 180, 360 and 600 min, as well as 40°C for 30 min exposure times on 'Hass' fruit quality.

'Hass' fruits were submitted to HAHST at 38°C for 180, 360 and 600 min and 40°C for 30 min. ST at 2°C.

Experimental Study XIII: Influence of hot water treatments of 39°C for 12, 15 and 18 min; 40°C for 15, 18 and 20 min; 42°C for 18, 20 and 22 min and 43°C for 12, 15 and 18 min exposure times on 'Pinkerton' fruit quality.

'Pinkerton' fruits were submitted to HWHST at 39°C for 12, 15 and 18 min; 40°C for 15, 18 and 20 min; 42°C for 18, 20 and 22 min and 43°C for 12, 15 and 18 min. ST step-down 5° - 3.5°C.

Experimental Study XIV: Influence of a pre-treatment of hot air at 38°C for 600 min together with a second hot air treatment of 20°C for 240 min together with hot water treatments of 39°, 40°, 42° and 43°C for 15 min exposure times on 'Pinkerton' fruit quality.

'Pinkerton' fruits were submitted to PR1 (HAHST), 38°C for 600 min; PR2 (HAHST) 20°C for 240 min + HWHST at 39°, 40°, 42°C, and 43°C for 15 min. ST at 2°C.

Experimental Study XV: Influence of hot water treatments of 40°C for 18, 20, 22 and 25 min; 41°C for 15, 18 and 22 min; 42°C for 15, 18 and 22 min and 43°C for 15, 18 and 22 min exposure times on 'Ryan' fruit quality.

'Ryan' fruits were submitted to HWHST at 40°C for 18, 20, 22 and 25 min; 41° for 18, 22 and 25 min; 42°C for 15, 18 and 22 min; 43°C for 15, 18 and 22 min. ST step-down 5.5° - 2.5°C and 2°C.

Experimental Study XVI: Influence of hot air treatments of 36°, 38° and 40°C for 540, 600 and 660 min exposure times on 'Ryan' fruit quality.

'Ryan' fruits were submitted to HAHST at 36°, 38° and 40°C for 540, 600 and 660 min. ST at 3.5° - 2°C, 5° - 4°C and 2°C.

Experimental Study XVII: Influence of hot water treatments of 39°C for 22, 28 and 30 min; 40°C for 20, 22 and 25 min; 41°C for 15, 18 and 20 min and 42°C for 8, 10 and 15 min exposure times on 'Edranol' fruit quality.

'Edranol' fruits were submitted to HWHST at 39°C for 22, 28 and 30 min; 40°C for 20, 22 and 25 min; 41°C for 15, 18 and 20 min; 42°C for 8, 10 and 15 min. ST at 5° - 4°C, 3° - 2°C and 2°C.

Experimental Study XVII: Influence of hot air treatments of 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min exposure times on 'Edranol' fruit quality.

'Edranol' fruits were submitted to HAHST at 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min. ST step-down 3° - 2°C, 5° - 4°C and 2°C.

Results and discussion

'Fuerte' results

Experimental Study I: Influence of hot water bath treatments at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min exposure times on 'Fuerte' fruit quality.

The results obtained with 'Fuerte' in Experimental Study I are given in Table 2.

Table 2. Hot water heat shock treatments (HWHST) results of 'Fuerte' in ES I - IV. Standard deviation is given in brackets.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
40°,41°,42°,43°C for 22,24, 26, 28min (ES I)	Step 5.5° - 4°C	72.84	42°C for 24 min	10.3 (6.1)	33.3 (7.2)
40°,41°,42°,43°C for 22,24,26,28min (ES II)	Step 5.5° - 4°C	73.55	40°C for 26 min	18.4 (9.5)	26.8 (13.5)
39°,40°,41°, 42°C for 20,22,24,26 min (ES III)	Step 6° - 5°C	71.5	N/a*	N/a*	4.3 (2.3)
	4° - 3°C		40°C for 26 min	2.5 (2.6)	7 (11.3)
	2.5° - 1°C		40°C for 24 min	3.3 (3.4)	29.3 (19.6)
PR: 30°C for 30 min, 40°C for 26 min, 42°C for 24min. No PR (ES IV)	Step 5° - 4°C	54.80	PR + 42°C for 24 min	0 (0)	8 (8.4)
	2°C		40°C for 26 min	20 (7)	27 (9.7)

N/a* = No treatment at this temperature, only the control as reference of the currently used shipping regime.

The fruits that were submitted to 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min and stored at 5.5°C and stepped-down to 4°C, showed that the best treatment was 42°C for 24 min with 10.3% surface area CI and the control with 33.3%. In Figures 2 to 4 the linear trends are given for this cultivar and Experimental Study. All these results were statistically proven by the statistical differences based on Duncan's multiple range test (P = 0.05) as illustrated in Fig. 1 of Chapter 3.

Experimental Study II: Influence of hot water bath treatments at 40°, 41°, 42° and 43°C for 22, 24, 26 and 28 min exposure times on 'Fuerte' fruit quality.

The same set of treatments were used in this study as were used in Experimental Study I, but the moisture content differed, although not much, it indicated an influence. This influenced the outcome of the treatment in such a way that the best treatment was 40°C for 26 min (Fig. 5). The reason could possibly be that the higher moisture content and, therefore, a more CI susceptible state, needed a temperature range lower than in Study I and for a longer period to activate the HSP (heat shock proteins) (Table 2).

Experimental Study III: Influence of hot water bath treatments at 39°, 40°, 41° and 42°C for 20, 22, 24 and 26 min exposure times on 'Fuerte' fruit quality.

The best treatment in this study was 40°C for 26 min at the storage range of 4° - 3°C with 2.5% surface area CI and the control with 7% CI. Whereas for the lower storage temperature range of 2.5° - 1.0°C, the best treatment was 40°C for 24 min with 3.3% CI and the control with 29.3% (Table 2). The current shipping storage range was 6° - 5°C and was used as a control with 4.3% CI occurring. The results showed that with different storage temperature ranges, different heat shock treatments resulted in the best time:temperature combinations. The 2.5°C storage range with 3.3% CI showed an improvement when compared to the control with 29.3% CI, but overall the step-down range of 4° - 3°C worked the best (Table 2).

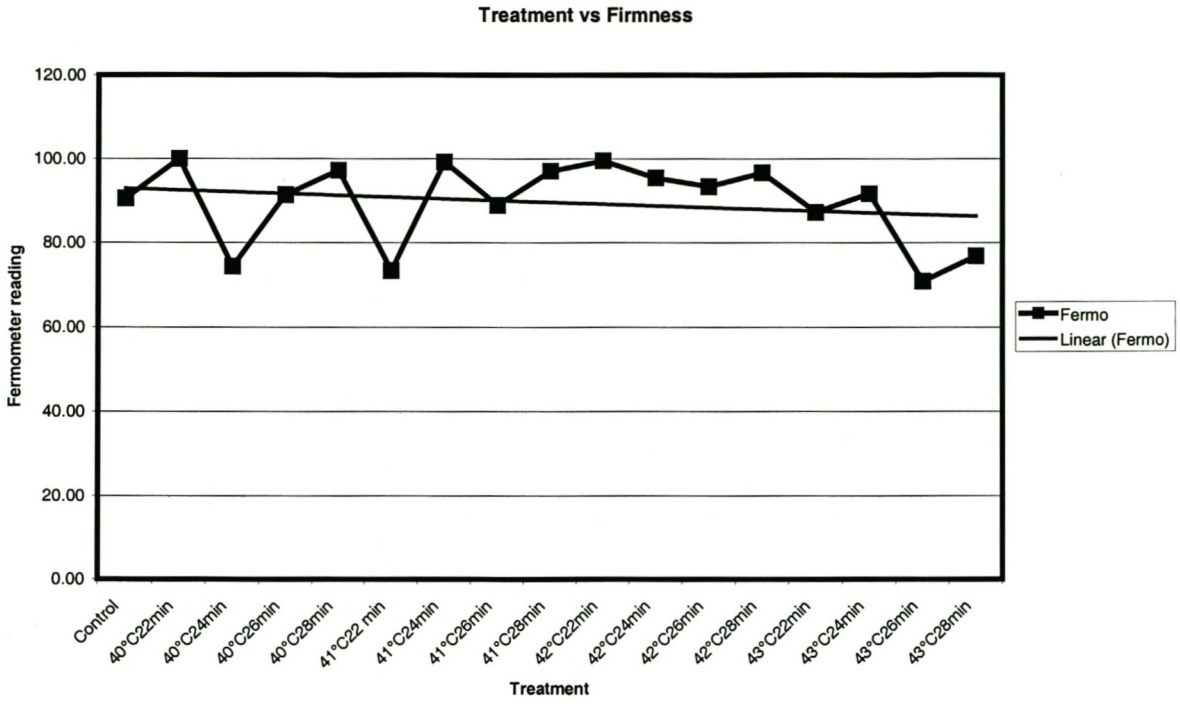

Figure 2. The Firmometer readings versus the different heat shock treatments.

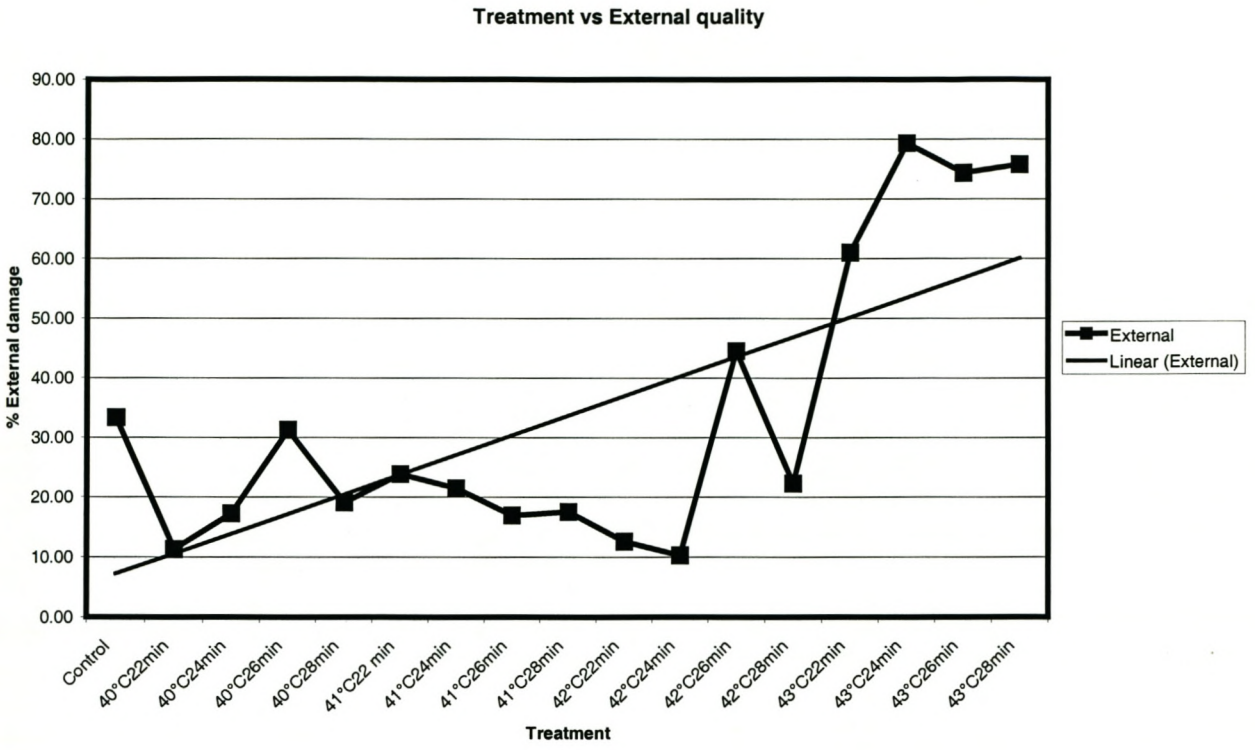

Figure 3. The percentage external damage versus the different heat shock treatments.

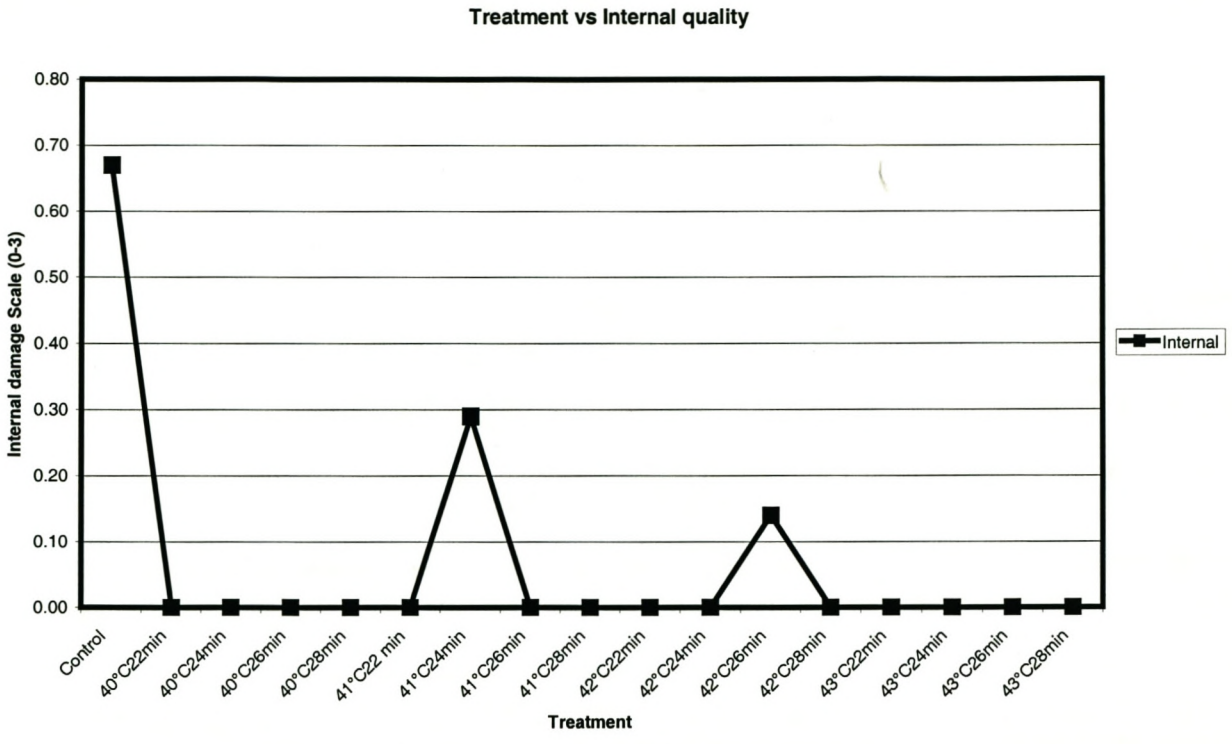

Figure 4. The internal damage versus the different heat shock treatments.

Figure 5. Grid layout of a representative 'Fuerte' fruits of the different heat shock treatments. The most effective temperature and time combination was 40°C for 26 min.

Experimental Study IV: Influence of a pre-treatment of hot air of 30°C for 30 min together with hot water bath treatments at 40°C for 26 min and 42°C for 24 min exposure times on 'Fuerte' fruit quality.

The pre-treatment definitely pre-conditioned the fruits and helped to minimise CI, but a higher HW treatment of 42°C was necessary to be effective at this temperature range of 5° - 4°C than at the 2°C range with 40°C. This showed that the pre-treatment gave the fruits protection and extra coverage and, therefore, a higher temperature range of 42°C was necessary for heat conductivity through the skin of the fruits. The best treatment was 30°C for 60 min (pre-treatment hot air) and then 42°C for 24 min (hot water bath) resulting in fruits with no CI and the control with 8% CI at 5°- 4°C storage range. At the 2°C storage range, the best treatment was 40°C for 26 min with 20% CI and the control with 27% CI, which did not showed a difference (Table 2).

Experimental Study V: Influence of hot air treatments at 38°C for 180, 360, and 600 min, as well as hot water bath treatment at 40°C for 30 min exposure times on 'Fuerte' fruit quality.

These hot air treatments showed no promising results (Table 3). The treatments were unpractical and the best treatment of 38°C for 600 min and the control had almost the same CI of 59.4 and 62.4%, respectively. Therefore, the data showed that the technique was not effective.

Experimental Study VI: Influence of hot air treatments at 38°C for 180, 360 and 600 min, as well as hot water bath treatment at 40°C for 30 min exposure times on 'Fuerte' fruit quality.

The same parameters as used in Experimental Study V were repeated in this study, but the fruits were from a different producer with different moisture content. The results were more promising than with ES V, but still the control and the best treatment had the same CI of 31 and 29%, respectively (Table 3). The results showed that the technique was again not effective for hot air treatments.

Table 3. Hot air heat shock treatments (HAHST) of 'Fuerte' in ES V - VII.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best Combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
38°C for 180, 360, 600 min and 40°C for 30 min (ES V)	2°C	63.66	38°C for 600 min	59.4 (0.7)	62.4 (0.6)
38°C for 180, 360, 600 min and 40°C for 30 min (ES VI)	2°C	68.85	38°C for 600 min	29 (0.8)	31 (0.6)
36°,38°,40°C for 30, 180, 360, 600, 720 min (ES VII)	Step 5° - 4°C 2°C	54.80	40°C for 30 min 40°C for 360 min	0 (0) 3.4 (4.2)	0 (0) 31 (16.7)

Experimental Study VII: Influence of hot air treatments of 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min exposure times on 'Fuerte' fruit quality.

These were very late season fruits with no CI on the control, as well as the best treatment of 40°C for 30 min in 5° - 4°C storage range. The 2°C storage range showed promising results with the best treatment of 40°C for 360 min with 3.4% CI and the control with 31.0% CI. But the 360 min exposure time is a too long heat treatment and impractical for a pack house to treat fruits for 360 min. In contrast, the step-down regime gave better results (Table 3).

'Fuerte' discussion and conclusions

The best combination heat shock results obtained with 'Fuerte' cultivar for each ES are listed in Table 2 and 3. Although the choice of temperature and time combinations was based on the most promising combinations identified in Chapter 3 of this thesis, the same pattern were attained during the previous season.

In 3 of the 7 experiments, significant differences were noticed between the control and the best treatments. Two of these (Experiments I and III) were HWHST and in both, the exposure time was 24 min. The effective temperature was found to be between 40° and 42°C. The third was a HAHST (Experimental Study VII) where the best temperature was again found to be 40°C, but the application was for 360 min and due to the time needed at 40°C the treatment was found to be impractical.

The impact of fruit maturity on the resistance of 'Fuerte' to CI and ultimately on the effectiveness of HWHST was highlighted in the previous Chapter. It was found that the technique is ineffective at the beginning of the season, but it is found to be more effective during the middle of the season. Towards the end of the season it was found to be difficult to induce CI. The continuously lowering step-down temperature regimes applied in the present study allowed for more consistency with regard to the induction of CI, but the effectiveness of the heat shock techniques remained erratic. In conclusion it would, therefore, appear that although a measure of success is attainable with 'Fuerte', the repeatability of the results are such that the technique was

found to be inappropriate for continuous use in a pack house. Therefore, the heat shock treatment will not be used for the 'Fuerte' cultivar.

'Hass' results

Experimental Study VIII: Influence of hot water bath treatments of 40°, 41°, 42° and 43°C at 22, 24, 26 and 28 min exposure times on 'Hass' fruit quality.

The same treatments that were used with 'Fuerte' (Table 2) in Experimental Study I was used with 'Hass' (Table 4). The fruits developed more CI (12.4%) on the control fruits at the temperature (7.5° – 5.5°C), that was recommended at that stage by the Perishable Product Export Control Board (PPECB), than with the control fruits of the step-down temperature range of 5.5° - 4°C (8.6% CI). This explained the reason why the fruits arrived overseas with CI. The temperature range for the fruit was not correct for this maturity stage. The best combination of 40°C for 24 min, at the 5.5°C step temperature range, gave only 2.7% CI, making the HST (heat shock treatment) effective and profitable at the right storage temperature.

Experimental Study IX: Influence of hot water bath treatments of 40°, 41°, 42° and 43°C at 22, 24, 26 and 28 min exposure times on 'Hass' fruit quality.

This was a repeat of Experimental Study VIII, but the fruits in this case had a 76.85% moisture content, which was less than the previous study of 77.75% (Table 4). A different best combination temperature and exposure time was identified namely 42°C for 24 min with 4.1% CI and the control with 7.8% CI at the 5.5°C temperature range. This showed that the moisture content played an important role in the determination of the best temperature and time combination. The control at the 7.5°C temperature range also had less CI (3.4%) than the previous Experimental Study showing that this temperature range is effective for this maturity stage.

Table 4. Hot water heat shock treatments (HWHST) results of 'Hass' in ES VIII – X.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
40°, 41°, 42°, 43°C for 22, 24, 26, 28 min (ES VIII)	5.5° - 4°C 7.5° - 5.5°C	77.75	40°C for 24 min N/a*	2.7 (2.1) N/a*	8.6 (7.4) 12.4 (9.4)
40°, 41°, 42°, 43°C for 22, 24, 26, 28 min (ES IX)	Step 5.5° - 4°C 7.5° - 5.5°C	76.85	42°C for 24 min N/a*	4.1 (3.7) N/a*	7.8 (4.2) 3.4 (2.3)
40°C for 24, 26, 28 min, 41° C for 24, 26, 28 min, 42°C for 22, 24 min 43°C for 20, 22, 33min (ES X)	Step 3.5° - 2°C	70.83	43°C for 22 min	1.5 (3.0)	1 (1.7)

N/a* = No treatment at this temperature, only the control as reference of the currently used shipping regime.

Experimental Study X: Influence of hot water bath treatments of 40°C for 24, 26 and 28 min; 41°C for 24, 26 and 28 min; 42°C for 22 and 24 min and 43°C for 20, 22 and 33 min exposure times on 'Hass' fruit quality.

The best combination for this study was 43°C for 22 min with 1.5% CI and the control with 1% CI (Table 4). The fruits in this study were already very mature and had a lower moisture content, which showed that even at a very low storage temperature range of 3.5 – 2°C, the control had almost no CI and the best treatment only a few spots of indentations. These findings emphasised the fact that more mature fruit (lower moisture content) tend to be less susceptible to CI and would be able to withstand lower storage temperatures.

Experimental Study XI: Influence of hot air treatments of 38°C for 360, 540 and 1440 min, as well as 40°C for 30 min exposure times on 'Hass' fruit quality.

The same fruits were used in this study than in the previous study. In this Experimental Study (Table 5), hot air was used and the control had 6.6% CI, which was much lower than the best combination of 38°C for 1440 min with 39.6%, while in the previous study (Table 4) the control had 1% CI and the best combination had 1.5%. This incident showed that the step-down regime (HWHST 3.5° - 2°C) of the previous Experimental Study X reduced the occurrence of CI when the controls of ES X and ES XI were compared. The best treatment had a very high % of CI and also a long exposure period, making hot air treatment not effective and very impractical to implement.

Experimental Study XII: Influence of hot air treatments of 38°C for 180, 360 and 600 min, as well as 40°C for 30 min exposure times on 'Hass' fruit quality.

With this study fruits with even lower moisture content were used. The best combination of 38°C for 360 min differed from the previous study, where it was 38°C for 1440 min. This can probably be ascribed to the higher maturity of the 'Hass' fruits and, therefore, a shorter exposure time was more effective (Table 5). But still the treatment was ineffective and impractical. The heat shock treatment resulted in more damage than good.

Table 5. Hot air heat shock treatments (HAHST) results of 'Hass' in ES XI - XII.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
38°C for 360, 540, 1140 min 40°C for 30 min (ES XI)	2°C	70.83	38°C for 1140 min	39.6 (0.4)	6.6 (0.4)
38°C for 180, 360, 600 min 40°C for 30 min (ES XII)	2°C	68.80	38°C for 360 min	36.3 (0.7)	30.7 (0.6)

'Hass' discussion and conclusions

The best results obtained with 'Hass' are summarised in Tables 4 and 5. The results were consistent with previous results in Chapter 3, that the technique was found to be totally ineffective. Great care was taken to accurately control the treatment temperatures and chilling inducing regimes. The favourable results reported by Woolf *et al.* (1996) with New Zealand 'Hass' was not attained in this study. This could probably be the result of different orchard practices and climate differences of different countries. It again showed that for South African avocados, different post harvest methods needed to be investigated before implementation of any kind of method adopted from overseas is used.

'Pinkerton' results

Experimental Study XIII: Influence of hot water bath treatments of 39°C for 12, 15 and 18 min; 40°C for 15, 18 and 20 min; 42°C for 18,20 and 22 min and 43°C for 12, 15 and 18 min exposure times on 'Pinkerton' fruit quality.

The best combination for 'Pinkerton' fruits with a HWHST for this study was 39°C for 15 min at the temperature range of 5.5° - 3.5°C. There were no CI found with any of the treatments or with the controls, making the step-down regime on its own a very effective method to reduce CI (Table 6).

Experimental Study XIV: Influence of a pre-treatment of hot air of 38°C for 600 min together with a second hot air treatment of 20°C for 240 min together with hot water bath treatments of 39°, 40°, 42° and 43°C for 15 min exposure times on 'Pinkerton' fruit quality.

The best combination for this study was the PR1 (HAHST) of 38°C for 600 min together with the PR2 (HAHST) of 20°C for 240 min and the HWHST of 43°C for 15 min with no CI, whereas the control had 30.4% CI at a storage temperature of 2°C. The same fruits as were used in the previous study was used and showed that the step-down regime of 5° - 3.5°C worked better than the 2°C constant storage temperature. This study also showed that the pre-conditioning of the fruits with hot air made the fruits more tolerable to higher temperature shocks of up to 43°C that resulted in no CI (Table 6).

Table 6. Heat shock treatments (HWHST and HAHST) results of 'Pinkerton' in ES XIII - XIV.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
Hot Water 39°C for 12, 15, 18 min 40°C for 15, 18, 20 min 42°C for 18, 20, 22 min 43°C for 12, 15, 18 min (ES XIII)	Step 5° - 3.5°C	74.41	39°C for 15 min	0 (0)	0 (0)
HA PR1= 30°C 600 min + PR2 = 20°C for 240 min, HW = 39°, 40°, 42°, 43°C for 15 min, PR1; PR1 + PR2, PR1+ HW, PR1+ PR2 + HW (ES XIV)	2°C	74.41	38°C for 600 min + 20°C for 240 min + 43°C for 15 min	0 (0)	30.4 (15.6)

'Pinkerton' discussion and conclusions

In the case of the 'Pinkerton' cultivar (Table 6), the HWHST, which was preceded by a HAHST pre-treatment, conferred a measure of protection to the fruits. In view of the physiological problems currently encountered with 'Pinkerton' after cold storage, it was necessary to investigate this cultivar as to gather data on how this cultivar would react to this technique. This cultivar became a popular cultivar, because of its high and constant yields. It was, therefore, important to seek any possibility to improve the cold storage potential of this cultivar. Detailed observations were made during the present study on the incidence of physiological disorders, as well as fungal infections causing pathological problems in these fruits when the final evaluations were done of each treatment (not showing in this thesis). In general, the prevalence of physiological disorders were not influenced by the treatments, except in extreme permutations of the highest temperatures combined with the longest exposure times. In the latter case, severe internal browning of the fruit flesh set in.

The 'Pinkerton' fruits used in the trials were from the same origin and the trials were all conducted within a 24 h period. From the results in Chapter 3 and 4 it showed that the HWHST and HAHST did not affect the incidence of internal browning. However, the fruits stored at 2°C had a significantly higher incidence of internal browning and epidermal discolouration than those stored at the 5° - 3°C regime. It would, therefore, appear as if temperatures, which are not sufficiently low to induce CI, might lead to browning of the fruits. It is, therefore, recommended that more experimentation on appropriate temperature regimes (Schutte, 1994) still needed to be conducted combined with controlled atmosphere experiments at higher temperature regimes. From all these ES on this cultivar, data was generated to support other and further research other than heat shock treatments. Therefore, although the studies showed that the heat shock technique was not practical other valuable information was gathered.

'Ryan' results

Experimental Study XV: Influence of hot water bath treatments of 40°C for 18, 20, 22 and 25 min; 41°C for 15, 18 and 22 min; 42°C for 15, 18 and 22 min and 43°C for 15, 18 and 22 min exposure times on 'Ryan' fruit quality.

The best combination treatment for this study was 40°C for 22 min with no CI, the same as for the control at the step-down storage regime of 5.5° - 2.5°C. These results again showed that the use of a step-down regime reduced the occurrence of CI. The control of the constant 2°C storage temperature showed 13.3% CI and the best combination was 43°C for 22 min with only 2% CI. At the 2°C storage the heat shock treatment helped to reduce the occurrence of CI considerably (Table 7).

Experimental Study XVI: Influence of hot air treatments of 36°, 38° and 40°C for 540, 600 and 660 min exposure times on 'Ryan' fruit quality.

No CI was obtained with the controls at 2°C and 5° - 4°C storage regimes. Although the best combination for this study was 38°C for 540 min at 2°C had no CI, all the other treatments had CI (data not given in thesis). This showed that the wrong treatment could cause more damage than improve the CI incidence. A low CI of 1% appeared on the fruits at the best combination of 38°C for 660 min at 3.5° - 2°C and only 2.5% CI on the control fruits. Although the hot air heat shock method helped to prevent CI, the practicality of the treatments of such long exposure times complicated the application of this method and made it impractical to implement (Table 7).

'Ryan' discussion and conclusions

The best results obtained with 'Ryan' cultivar are shown in Table 7. As can be deduced from the data, relatively little CI was observed in the controls. In the one case where 13.5% CI was induced, the hot water treatment at 43°C for 22 min apparently resulted in a measure of protection, as the mean percentage CI recorded was 2%.

Table 7. Heat shock treatments (HWHST and HAHST) results of 'Ryan' in ES XV - XVI.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
Hot water 40°C for 18, 20, 22, 25 min 41°C for 18, 22, 25 min 42°C 15, 18, 22 min 43°C for 15, 18, 22 min (ES XV)	Step 5.5° - 2.5°C 2°C	66.29	40°C for 22 min 43°C for 22 min	0 (0) 2 (2.7)	0 (0) 13.3 (32.6)
Hot air 36°, 38°, 40°C for 540, 600, 660 min (ES XVI)	Step 3.5° - 2°C 5° - 4°C 2°C	64.50	38°C for 660 min N/a* 38°C for 540 min	1.0 (1.9) N/a* 0 (0)	2.5 (4.6) 0 (0) 0 (0)

N/a* = No treatment at this temperature, only the control as reference of the currently used shipping regime.

The HWHST seemed to have worked well with 'Ryan' cultivar, but unfortunately too many factors influences the outcome of the treatment and, therefore, makes this treatment difficult to repeat and impractical.

'Edranol' results

Experimental Study XVII: Influence of hot water bath treatments of 39°C for 22, 28 and 30 min; 40°C for 20, 22 and 25 min; 41°C for 15, 18 and 20 min and 42°C for 8, 10 and 15 min exposure times on 'Edranol' fruit quality.

The best combinations for the different storage regimes showed the following results: the 5° - 4°C regime with 38°C for 180 min with no CI and the control with 5%; the 3° - 2°C with 36°C for 30 min with no CI and the control with 4.5%; and the 2°C storage regime with 36°C for 600 min with no CI and the control with 10% (Table 8). The only practical application was the 3° - 2°C storage regime for 30 min at 36°C. The different regimes had potential combinations that worked effectively, but the exposure times of these treatments were 600, 180 and even 30 min, making the application impractical for a pack house.

Experimental Study XVIII: Influence of hot air treatments of 36°, 38° and 40°C for 30, 180, 360, 600 and 720 min exposure times on 'Edranol' fruit quality.

The best combination at the 5° - 4°C regime was 42°C for 8 min with no CI and the control had 23.1%. At the 3° - 2°C regime 40°C for 22 min had no CI and the control had 15%, and with the 2°C the best combination was 42°C for 15 min, which gave 17.5% with the control 30% (Table 8). The hot water treatment showed better application possibilities that the hot air applications that had been used in Experimental Study XVII.

'Edranol' discussion and conclusions

The best results obtained in the two 'Edranol' trials are shown in Table 8. A number of interesting observations can be made from the results. The fruits used in the two trials were of similar maturity (approximately 70% moisture content).

Table 8. Heat shock treatments (HWHST and HAHST) results of 'Edranol' in ES XVII – XVIII.

Treatment	Storage Temperature	Moisture (%) ($g.100g^{-1}$)	Best temperature and time combination	Best treatment (% surface area with CI)	Control (% surface area with CI)
Hot air 36°,38°,40°C for 30, 180, 360, 600, 720 min (ES XVII)	Step 5° - 4°C 3° - 2°C 2°C	70.66	38°C for 180 min 36°C for 30 min 36°C for 600 min	0 (0) 0 (0) 0 (0)	5 (8.5) 4.5 (9.6) 10 (12.2)
Hot water 36°C for 22, 28, 30 min 40°C for 20, 22, 25 min 41°C for 15, 18, 20 min 42°C for 8, 10, 15 min (ES XVIII)	Step 5° - 4°C 3° - 2°C 2°C	69.85	42°C for 8 min 40°C for 22 min 42°C for 15 min	0 (0) 0 (0) 17.5 (15.5)	23.1 (26.2) 15 (19.4) 30 (17.8)

The experiments were further done on two consecutive days and the fruits were kept in the same cold rooms. Yet, the CI induced in the controls was higher in the HWHST trial than in the HAHST. The fruits used in the former trial originated from the Kiepersol area where the temperatures fluctuates less than in the area where the fruit for the second trial, Kaapsehoop, were obtained. The importance of lower orchard temperatures in conditioning the fruits against CI, as shown by Swarts (1982), has again come to the fore in these experiments. Although the percentage CI induced differed between the two trials, the order was identical in that the more severe storage regimes induced the highest percentage of CI. Both the water and air treatments further appeared to confer a measure of protection against CI. As indicated in the results the different producers, as well as different localities also influences the out come and effectiveness of the heat shock treatments.

With all these information and conclusions at hand, it became apparent that in a pack house with different cultivars and different producers, too many variances and factors appeared, which influenced the technique and its effectiveness making implementation impossible and impractical.

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CHAPTER 5

SCANNING ELECTRON MICROSCOPY OF THE SKIN OF AVOCADO FRUIT

Abstract

To understand what chilling injury (CI) or cold damage is, chemical analysis was done on the cold damaged areas, as well as the healthy green areas of a avocado fruit. The data showed that a higher calcium content was present in cold damaged areas of avocado skin than in the healthy areas. An investigation was, therefore, conducted to understand the reason why the calcium was higher and also why the cold damaged areas appeared as brown/black indentations. The skin of an avocado was studied under a scanning electron microscope in order to compare the difference between the cells of black cold damaged areas and healthy green areas. The microscopy of the cells of the healthy green areas was taken as the control and used as reference to compare the microscopy of the cold damage areas. The photos of the healthy cells showed that many tiny oil cells developed in the cells and in some cases even gathered together to form large oil balls. The photo of the cold damaged cells showed that the cell walls were all ruptured and compacted with no trace of oil. This indicated that the cold damaged cells were all dead, whereas the green healthy cells were all well structured. Through this study, the reason why the calcium concentration was higher in the damaged cell sample compared to the healthy cell sample was answered. It was ascribed to the dilution effect of the area per concentration, resulting in a larger amount of cell walls clustered together in the case of the compressed cell walls and, therefore, leading to more calcium being present. From this study it became apparent that to prevent the cell walls from rupturing and causing polyphenol oxidation (the brown colouration) and compactions leading to the indentations, the cell walls must be strengthened with calcium during the pre- and post-harvest periods.

Introduction

After avocados have been stored at a low temperature for a few days, CI sets in. In some cases only one or two fruits showed signs of CI whereas sometimes the whole fruit is covered with brown/black indentations (Fig. 1). The reason that only certain areas were affected led to the investigation of their chemical compositions, as well as structural construction on cellular level. The avocado fruits have a high oil content and to understand the consistency of the avocado, more information on the oil cells was essential. Oil cells are idioblastic, secretory structures that differentiate in the ground parenchyma of many taxonomically diverse angiosperm species but occur most commonly in species of the woody Ranalean complex (West, 1969). In the parenchyma cells, the lipids occur as many individual cytoplasmic bodies (Platt-Aloia & Thomson, 1981) (Fig. 2). Polyphenol oxidase positive reaction products are present in the thylakoids of plastids from normal mesocarp tissue and absent in the thylakoids from brown abnormal mesocarp tissue (Engelbrecht, 1987). This suggests that the brown tissue had already lost the polyphenol oxidase enzyme because of the rupturing of the cell walls that had occurred and an oxidation process took place, which lead to the browning effect. This left the cells broken, dead and compacted to form the brown/black CI indentations on the surface of the avocado skin. It is well known that calcium plays an important role in the strengthening of cell walls (Bangerth, 1979). The calcium bonds to chains of galacturonic acid making a cross linkage (Noggle & Fritz, 1983). By cross-linking, the chains are being reinforced making the structure stronger and secure.

Therefore, the aim of the study was to determine why CI appeared as brown/black indentations and why a higher calcium content was present in the damaged skin than in the healthy skin.

Figure 1. An example of the difference in maturity of the fruits found in a box. Some fruits have a few chilling injured brown/black indentations and others are almost totally covered.

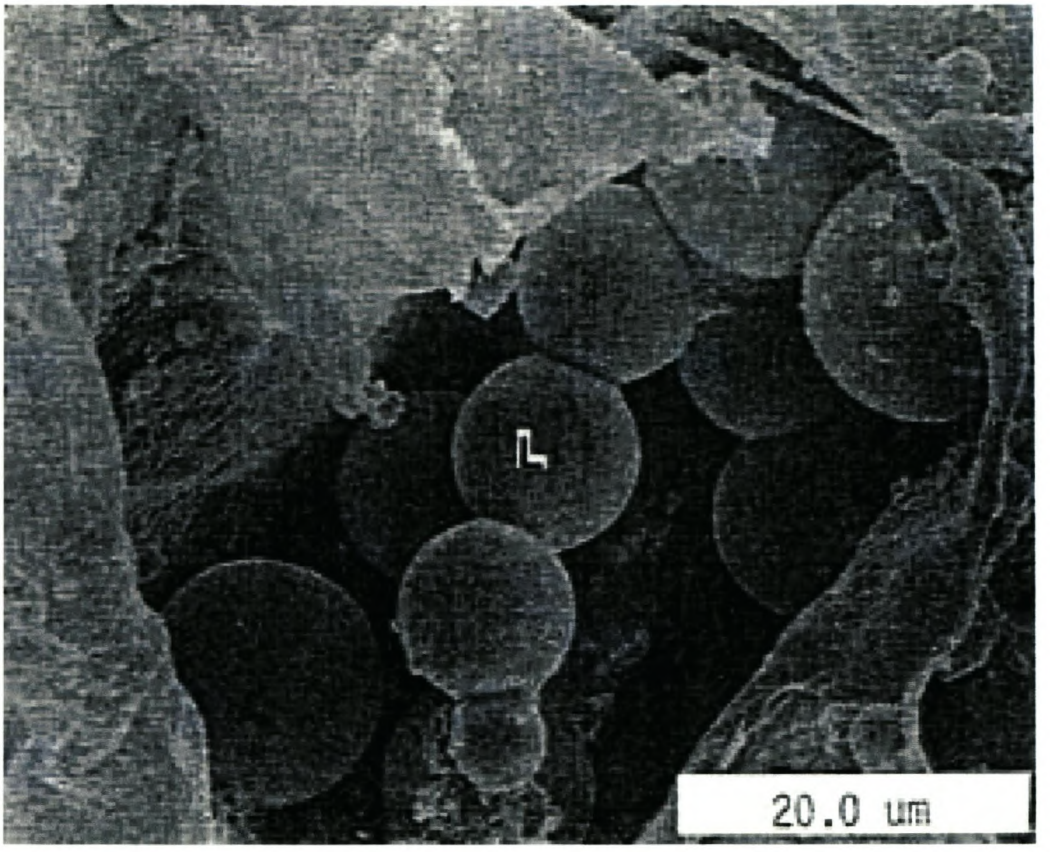

Figure 2. The lipid bodies/oil cells in a parenchiem cell (L = Lipid body) (Kaiser *et al.*, 1992).

Material and methods

A. Chemical analysis

Two avocado fruits, which had both cold damaged areas, as well as healthy green areas were used after cold storage. The cold damaged areas on the avocado skin, as well as the healthy areas were cut out by means of a scalpel and divided into two sections and then analysed by means of their chemical compositions at the Institute for Tropical and Subtropical Crops (ITSC) chemical laboratory. The data were given as mg of calcium present in a kg of sample (ppm). The place where CI occurred the most, on the ball of the fruit, was studied by means of four healthy fruits without CI scars. The skin of the four fruits were taken and divided into a neck section and into a ball section and then chemically analysed.

B. Scanning electron microscopy

A closer investigation on cellular level had to be done to investigate cells by means of a scanning electron microscopy conducted on different parts of the avocado's skin. A scanning electron microscope is a device where any material could be investigated under million times of enlargement. These devices are situated only at universities and research foundations and for this particular study, the scanning electron microscope at the University of Pretoria was used under guidance of Mr. Chris van der Merwe.

Fruit preparation

The skin was used from cold stored chilling injured avocado fruits. The fruits, which had both cold damaged and healthy cells was dissected with a new sharp razor to assure smooth and clean cut 1 x 2 mm dimension cubes. The cold damaged areas and the skin with the healthy cells were carefully separated from each other. The fresh avocado skin samples that were under investigation had to be fixed immediately to capture the freshest possible sample.

Sample fixation

The chemicals needed to perform this task were as follows: 2.5% (m/v) glutaraldehyde (Dormas Chemicals - Johannesburg); 0.075 M sodium phosphate buffer (Dormas Chemicals - Johannesburg); 0.5% aqueous OsO_4 (Dormas Chemicals - Johannesburg); and 30, 50, 70, 90 and 3 x 100% ethanol solution (Dormas Chemicals - Johannesburg).

The primary fixation consisted of 2.5% (m/v) glutaraldehyde in 0.075 M sodium phosphate buffer at a pH of 7.4 (Malick & Wilson, 1975; Platt-Aloia & Thomson, 1980). The samples were left in this solution for 2 - 4 h and were then removed to be washed with 0.075 M sodium phosphate buffer and left to stand for 10 - 20 min. This washing step was repeated three times while changing the buffer each time.

The post-fixation of the samples was done in a 0.5% (v/v) aqueous OsO_4 for 2 h. Thereafter, the samples were thrice rinsed every 10 min with distilled water. The samples were then dehydrated with different concentrations of ethanol ranging from 30, 50, 70, 90 and 3 x 100% (v/v) for 15 min standing time each.

The samples were dried using the critical drying point method with CO_2 as described by Anderson (1951) using a Tuisimas CO_2 critical point dryer. After drying, the samples were ready to be mounted on aluminum stubs with conductive carbon cement as describe by Anderson (1951).

Electron microscopic parameters

The samples were cemented onto an aluminum platform to keep them in place before they were sputter coated with gold in a Polaron E5800 sputter coater (with the help of Mr Chris van der Merwe at the University of Pretoria).

The sample was viewed with a JEOL 840 SEM scanning electron microscope. The images were enlarged 500 - 1900 times and were 10 μm in diameter, the image was captured with a microcomputer and printed out (Platt-Aloia & Thomson, 1981). Different settings were investigated until the setting, which highlighted the problem the best was chosen.

Results and discussion

It became apparent from the chemical analysis that the cold damaged skin had more calcium than the healthy skin (Table 1). This did not make sense, otherwise these damaged cells would have been strong (Bangerth, 1979). From the results obtained from the chemical analysis of the neck and ball sections (Table 2), the reason why more CI appeared on the ball of the fruits than on the neck (Fig. 1) was explained by the higher calcium content in the neck. The higher calcium in the neck helped with the strengthening of the cell walls. To explain the results of Table 1, the second phase of investigation was necessary.

In the first part of this study the healthy cells of the avocado skin samples were studied to obtain a set standard of the appearance of healthy cells under an electron microscope enlargement (Fig. 3). Then the damaged sections (Fig. 4) were studied and it was found that the epidermis differed from that of the healthy skin (Fig. 3). Both the healthy and the damaged samples showed the cuticle and epidermis cells very clearly. The epidermis cells of the healthy tissue illustrated in Figures 3 and 5, differed from the chilling injured tissue (Fig. 4). The healthy skin was found to be strong and filled with oil cells, while the damaged cells had no oil cells, only a compaction of cell walls.

The damage appearance (Fig. 4) together with the data in Table 1 facilitates the explanation of the occurrence of higher calcium presence in damaged cells than in healthy green cells. The cell walls consist of many components (Bangerth, 1979) and calcium plays an important role in securing a strong structure. The calcium in the dead cells was higher because of the amount of cell walls pressed together and because there were more compacted cell walls per area than found in healthy areas. This probably contributed to the higher calcium content in the injured cells.

Table 1. The calcium content of the chilling injured avocado skin areas and the clean healthy avocado skin areas.

Avocado skin	Calcium ppm (mg calcium per kg sample)	
	Fruit no. 1	Fruit no. 2
Black cold areas	900	651
Healthy green areas	138	61

Table 2. The calcium content (on dry mass base - DMB) of the avocado skin from the top part of the fruit (neck) and the bottom part (ball/round area).

Skin	Calcium ppm (mg calcium per kg of sample)			
	Fruit no. 1	Fruit no. 2	Fruit no. 3	Fruit no. 4
Ball	10	46	100	139
Neck	51	72	457	655

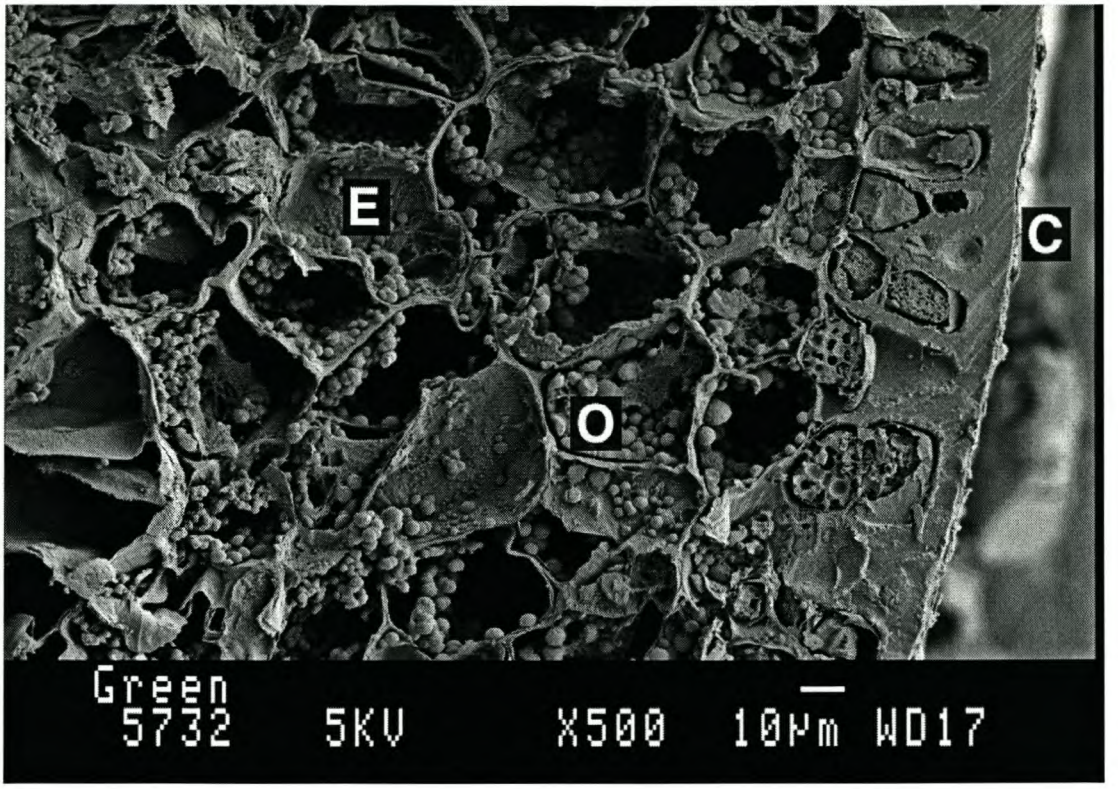

Figure 3. Crosscut of the healthy skin sample filled with the oil balls/cells (C = cuticle, E = Epidermis, O = Oil cells).

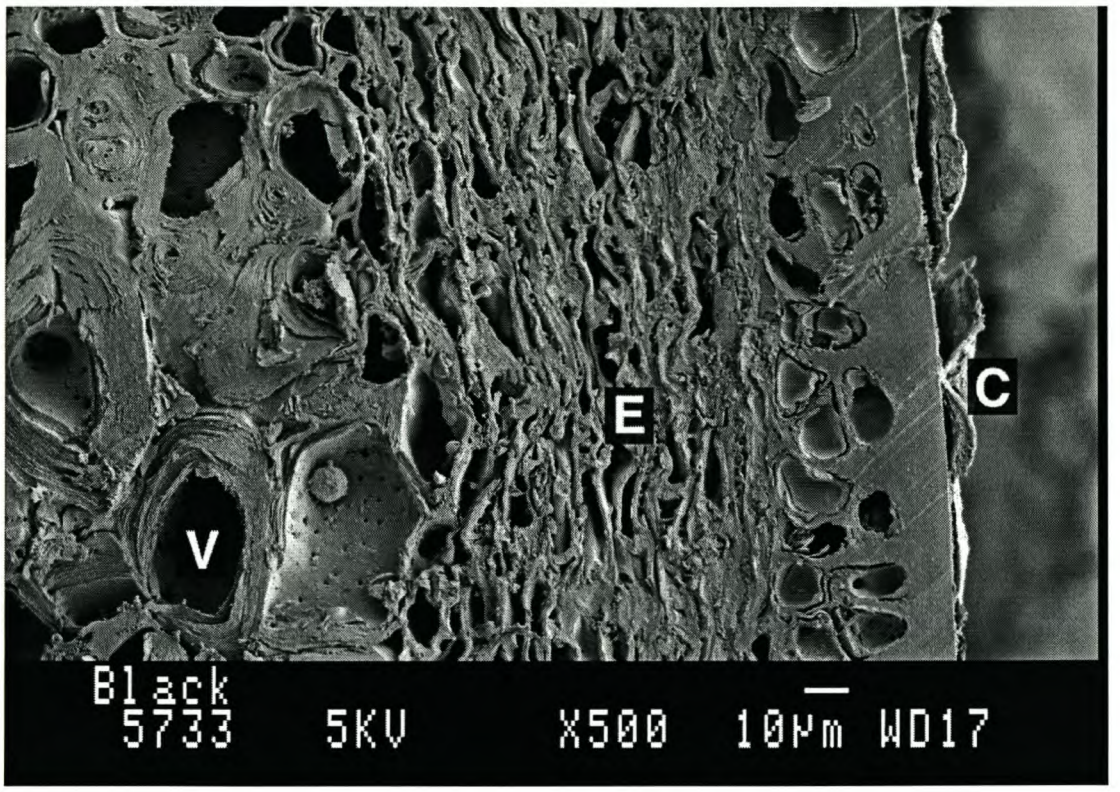

Figure 4. Crosscut of a chilling injured area of avocado skin, which shows the compaction of cell walls with no oil cells/balls (C = cuticle, E = Epidermis, V = Vascular tissue).

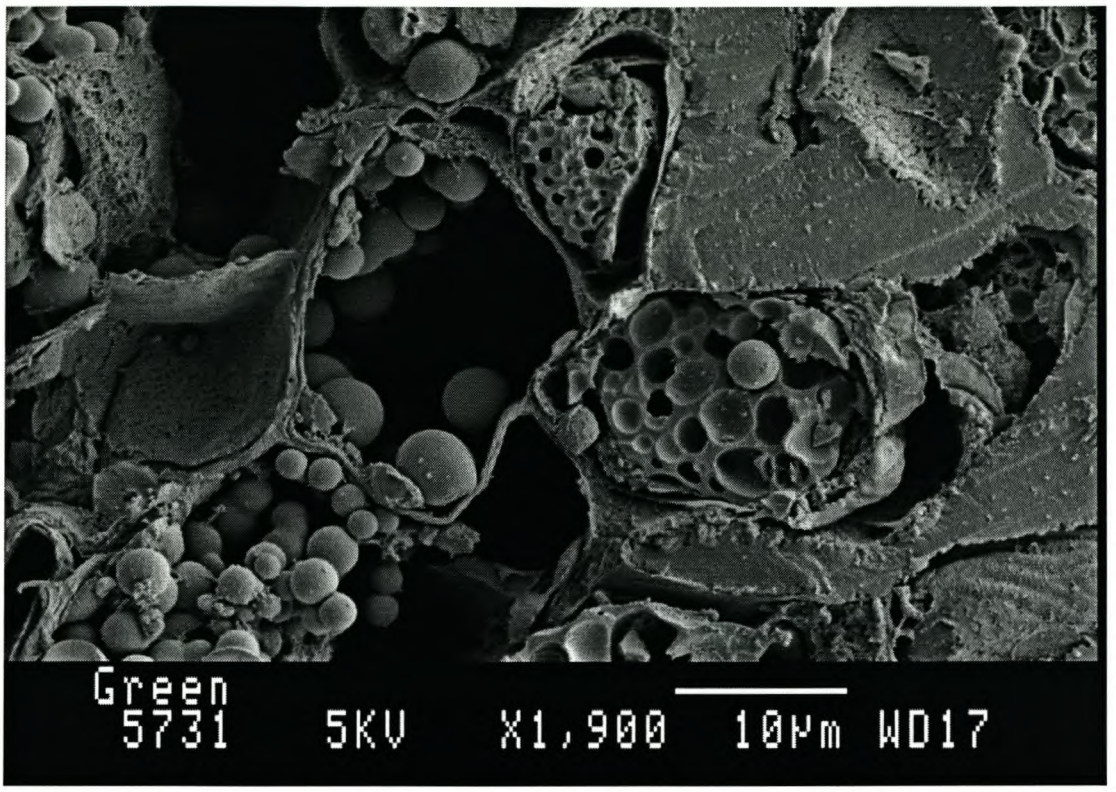

Figure 5. The gathering of oil cells to from larger oil balls.

From this study the assumption can be made that the cells in the damaged samples must have been dead cells with the cell walls ruptured and the cell contents being discarded. If that is the case, then the ruptured cell walls leaked out the cell content, leading to the polyphenol oxidase enzymes reacting with the substrate causing brown colouring (Engelbrecht, 1987) on the avocado skin, which was exactly what seemed to have happened in this study.

Conclusion

The aim of the study was to determine why CI appeared as black/brown indentations, as well as the reason why a higher calcium content was present in damaged areas than in healthy areas. This study explained a possible reason for all these questions as cell wall collapsing. From the results it seemed that weak cell walls could not withstand the cold stress of low temperature storage, which resulted in the leakage of polyphenol oxidase enzymes and resulted in brown colouring of the avocado skin. The weak cell walls then collapsed and compacted, forming the indentations.

All these findings lead to one conclusion and that is that the cell walls were not strong enough to withstand cold stress and, therefore, a method of strengthening the cell walls with calcium, if possible applied after harvest, must be investigated (Penter & Stassen, 2000). If this type of application is successful, less CI will occur on the fruits resulting in more profitable produce on the export market.

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CHAPTER 6

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

The South African subtropical fruit industry and, therefore, the avocado industry are export driven and the successful storage of fruit for extended periods is essential (Bredell, 1983). To slow down the rapid respiration rate, the fruit must undergo a respiration rate declination and this can only be achieved if the fruit is stored at low temperatures. Temperatures below the critical value can result in an abnormal metabolism which, if prolonged, leads to the development of visible signs of injury (Eaks, 1983).

It is known that storage temperatures below 4° to 6°C tend to induce chilling injury (CI) in most avocado cultivars. The CI appears on the surface of the avocado skin as dark sunken, mosaic like patches (Zauberman *et al.*, 1985) and leads to an unattractive appearance and a decrease in the market value. From research done it has been shown that lower storage temperatures add valuable preserving potential, but CI remains the limiting factor. A possible method to increase the resistance of the avocado to CI is to administer a heat shock. In this way the fruit is protected from CI by inducing the formation of so-called heat shock proteins which render the cell membranes more resistant to CI (Harrington *et al.*, 1994; Nover, 1984).

In this thesis a method to minimise the prevalence of CI caused by low storage temperatures was investigated by means of heat shock treatments. From the results obtained in Chapters 3 and 4 of this thesis, a general conclusion was reached that heat shock treatments reduced the susceptibility of CI on the South African 'Fuerte', 'Edranol' and 'Pinkerton' cultivars. However, the technique was found to be ineffective with the South African 'Hass' cultivar, while the 'Ryan' cultivar was found to be relatively resistant to CI whether treated or not.

Based on the data obtained it is recommend that pack houses should not attempt to apply this technique at this stage as the various cultivars reacted differently to the technique within different parameters. This is probably due to variations in terms to maturity as illustrated in Figures 1 and 2

Figure 1. The difference in maturity of the fruits in one box.

Figure 2. The difference in fruit maturity on the same tree (Kruger & Claassens, 1996a).

of the different fruits, as well as physiological differences between cultivars. Kruger & Claassens (1996a) explained the reason for such a big difference in maturity that can be found in one box and this could be ascribed to the difference in maturity of fruit on the same tree. They also found that this depends on the position of the fruit on the tree, as well as time of fruit set, as avocado fruits sometimes have a long fruit set period and this, therefore, could cause a wide range of different maturity levels. Ferguson *et al.* (1999) also reported that avocado fruits on the outside of a tree are subjected to higher heat exposures during the day and are correspondingly less susceptible to CI than those on the inside. Ferguson *et al.* (1998) also discovered that the protein profile of the inside fruits differed from the outside and ascribed the increased resistance to CI to the formation of heat shock proteins in the fruits that had been exposed to the sun.

Based on the data obtained in Chapters 3 and 4 and the fruit maturity results of Kruger (1996) and Kruger & Claassens (1996a and 1996b), it appears as though the primary reason for the deviation was definitely the variation in fruit maturity. Thus because of the maturity variations within a consignment it could be difficult for a pack house to apply the technique developed and evaluated in this thesis.

Recommendations

The HST technique developed in this thesis has potential for use with certain cultivars like the South African 'Fuerte', 'Edranol' and 'Pinkerton' cultivars. This technique would be even more valuable if the fruits could be first sorted according to maturity levels. Maturity and, therefore, oil and moisture levels determine the heat transport through the fruit (Ferguson *et al.*, 1998) and thus different temperature and time duration schedules will be required. A thorough study of the relationship between oil/moisture content, firmness and internal browning must also be conducted. The investigation of a potential correlation between moisture content, oil, firmness and CI should also be evaluated, as well as the effect what the electronic sorting (Hall's Avoscan), the CA (controlled atmosphere) and HST has on the internal browning and CI in all the cultivars (Kruger & Rowell, 1998).

The results obtained in Chapters 3 and 4 showed in contrast with New Zealand results (Woolf *et al.*, 1996), that the HWHST worked effectively for South African 'Fuerte', but was ineffective for South African 'Hass'. This indicated that the South African avocados differ from the avocados of other countries and, therefore, new methods for each South African cultivar have to be developed. The results in Chapters 3 and 4 confirmed that HWHST was effective for 'Edranol'. 'Ryan' with its thick skin appeared to be less susceptible to CI and, therefore, HST appeared to be unnecessary. 'Pinkerton' still has a lot of factors that have to be investigated so no conclusions could be drawn for this cultivar and, therefore, further investigation is recommended.

Maturity levels clearly played an important role in the effectiveness of the HST technique and, therefore, resulted in different time and duration time schedules. This heat shock technique is, therefore, too specific and can not be justified as economical viable.

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