EFFECT OF HARVEST SEASON AND RIPENING DURATION ON THE PHYSICO-CHEMICAL PROPERTIES OF NEW 'FUERTE-TYPE' AVOCADO FRUIT SELECTIONS DURING RIPENING

BY

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DECLARATION

I, declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Horticulture) has not previously been submitted by me for a degree at this or any university; that it is my work in design and in execution, and that all material contained herein has been duly acknowledged.

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Munzhedzi, M (Ms) Date

DEDICATION

This study is dedicated to my sweet and loving parents, Mr MA Munzhedzi and Mrs NS Munzhedzi.

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ABSTRACT

The Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) is continuously developing new avocado selections, in order for the South African Avocado Industry (SAAI) to remain competitive in various international avocado markets. However, information on the response of some of these selections, including 'Fuerte 2 and 4', 'BL1058' and 'H287' to low temperature storage and ripening physiology, has not been investigated. Thus, the objective of this study was to evaluate the effect of harvest season and ripening duration on the physico-chemical properties of newly developed 'Fuerte-type' avocado fruit selections during ripening. 'Fuerte-type' avocado fruit were indexed for maturity using moisture content, thereafter harvested and stored at 5.5°C for 28 days during the 2014 and 2015 harvest seasons. The experiment comprised five treatments: control (commercial 'Fuerte'), 'Fuerte 2 and 4', 'BL1058' and 'H287' arranged as a factorial in a completely randomised design (RCD) with 3 replicates. The treatment factors were: (i) 2 x harvest seasons, (ii) 5 x selections and (iii) 6 x ripening days. After withdrawal from low storage temperature, fruit were ripened at ambient temperature. During ripening, the following physico-chemical properties were evaluated; external chilling injury, electrolyte leakage, mass loss, firmness, respiration rate and peel colour. Results showed that selections and harvest seasons had no significant effect (P=0.668) on the moisture content of the evaluated 'Fuerte-type' avocado fruit. After withdrawal from low storage temperature, there was a significant interaction (P<0.05) between selections and harvest seasons on external chilling injury and electrolyte leakage. Results further showed that external chilling injury correlated with electrolyte leakage during both harvest seasons. Treatment factors had no significant effect (P=0.997) on mass loss. Similarly, treatment factors had no significant effect (P=0.139) on firmness. However, selection 'H287' had hard skin with an average firmness of 83.44 densimeter units during ripening in both harvest seasons. Treatment factors were highly significant (P<0.05) on respiration rate. Respiration rate followed a climacteric pattern and the magnitude of climacteric peak and day of occurrence varied amongst selections during both harvest seasons. Ripening percentage differed significantly (P<0.05) amongst harvest seasons, selections and ripening days. Treatment factors had no significant effect on lightness (P=0.711), chroma (P=0.378) and hue angle (P=0.536) skin colour parameters;

however, variations were recorded as a result of the cold damage black spots. The results indicated that the ‘Fuerte-type’ avocado selections had poor storage qualities. Further studies are required to evaluate physico-chemical properties during low storage temperature and the effect of season, production conditions and maturity level on development of chilling injury. In addition, studies on application of treatments to reduce chilling injury symptoms and analysis of bioactive compounds should be considered for conclusive recommendations. Thereafter, the selections can be planted in different production regions to assess and select the best producing and quality combinations for a given region as part of phase III of the project.

Keywords: *Electrolyte leakage*; *firmness*.; ‘*Fuerte-type’*; *mass loss*; *new selections*; *peel colour*; *physiological disorders*; *physico-chemical properties*; *respiration rate*

CHAPTER 1
INTRODUCTION

1.1 Background

Avocado (*Persea americana* Mill.) is a sub-tropical climacteric fruit belonging to the Lauraceae family (Eaks, 1980). In South Africa, ‘Hass’, ‘Fuerte’, ‘Ryan’ and ‘Pinkerton’ are the four main commercially grown cultivars (DAFF, 2014; FFED, 2015). Cultivar ‘Hass’ is predominantly of Guatemalan origin with some Mexican germplasm and ‘Pinkerton’ of Guatemalan origin, while ‘Fuerte’ and ‘Ryan’ are natural hybrids of the Guatemalan and Mexican races (Bergh, 1975).

The South African Avocado Industry (SAAI) is largely export orientated with 62% of the avocado production exported predominantly to the European markets (Potelwa and Ntombela, 2015). However, Japan and the USA have been new potential high paying markets. While the industry exploits new export opportunities, it faces competition from other Southern hemisphere countries such as; Peru, Chile and Australia (Witney, 2002). Furthermore, long-term low storage temperature is required for South African avocado fruit to reach overseas markets in order to preserve quality, with transit time of up to 4 weeks. However, low storage temperature may result in the development of various physiological disorders. In particular, 'Fuerte' cultivar is known to reach export markets with symptoms of physiological disorders (Bijzet, 1998). Moreover, importing countries increasingly expect guaranteed quality consistency throughout the year. Therefore, susceptibility of current avocado fruit to physiological disorders at low storage temperature makes it unfeasible to extend marketing distances and acquire new markets (Kruger and Mhlope, 2013).

To meet these challenges, the SAAI depends on continuous breeding of new improved cultivars with superior quality in order to increase variability and competitiveness. The Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) has developed new ‘Fuerte-type’ avocado selections namely; ‘Fuerte 2 and 4’, ‘H287’ and ‘BL1058’. Information on the effect of mandatory storage temperature and ripening properties of these selections is still lacking. Thus, the study proposed to evaluate the effect of harvest season and ripening duration on the physico-chemical properties of these avocado fruit selections during ripening after withdrawal from mandatory low storage temperature.

1.2 Problem statement

Most South African avocado cultivars are susceptible to physiological disorders during shipping to European markets, and ‘Fuerte’ is the most susceptible amongst the exported cultivars. Given the opportunities identified with regard to increasing demand and diversification of markets, which are no longer concentrated in Europe, the SAAI must continue selecting and breeding avocado varieties with superior characteristics. As one of the major stakeholders of SAAI, the ARC-ITSC is continuously developing new ‘Hass-type’ and ‘Fuerte-type’ avocado selections (Bijzet, 1998). As part of the evaluation process, information about the effect of harvest season and ripening duration on the physico-chemical properties of avocado fruit selections after withdrawal from a mandatory low storage temperature needs to be documented.

1.3 Motivation for the study

South African avocado industry needs to continue developing avocado cultivars with superior characteristics such as; attractive colour, and long storability in order to remain competitive in the global market (Cutting *et al*., 1992). In avocado, these parameters are maintained by low storage temperature, and low storage temperature may result in the development of various physiological disorders such as vascular straining and chilling damage. Evaluation on the effect of harvest season and ripening duration on the physico-chemical properties of avocado fruit selections after withdrawal from a mandatory low storage temperature would assist with their commercialisation and increase cultivar variability for the industry to remain competitive.

1.4 Aim and objectives of the study

1.4.1 Aim

The aim of this study was to evaluate the response of newly developed ‘Fuerte-type’ avocado fruit selections after withdrawal from industry recommended low storage temperature simulating export conditions.

1.4.2 Objectives of the study were to:

(i) Evaluate the effect of harvest season on the physico-chemical properties of newly developed ‘Fuerte-type’ avocado fruit selections during ripening.

(ii) Evaluate the effect of ripening duration on the physico-chemical properties of newly developed ‘Fuerte-type’ avocado fruit selections during ripening.

1.5 Hypotheses

(i) Harvest season would have no effect on the physico-chemical properties of newly developed ‘Fuerte-type’ avocado fruit selections during ripening.

(ii) Ripening duration would have no effect on the physico-chemical properties of newly developed ‘Fuerte-type’ avocado fruit selections during ripening.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The SAAI is export orientated and low storage temperature is a critical factor in maintaining fruit quality during shipping to long distance markets. Low storage temperature slows down the ripening physiology of avocado fruit to ensure arrival of fruit at good quality to designated markets. However, long exposure periods to low temperature during shipping may result in the development of physiological disorders (Lütge *et al*., 2010). Physiological disorders contribute to inconsistent avocado fruit quality. Avocado is a sub-tropical crop; therefore, exhibit marked physiological dysfunction when exposed to low but non-freezing temperatures ranging from 4-7°C (Hershkovitz *et al*., 2005). In particular, 'Fuerte' avocado cultivar is chilling susceptible and known to reach distant market with symptoms of physiological disorders (Bijzet, 1998). Successful storage of avocado fruit for extended periods is critical in ensuring maintenance of export of quality fruit. Therefore, the purpose of this study is to review background of ARC-ITSC avocado breeding program, maturity indexing, low storage temperature, chilling injury and electrolyte leakage as a result of low storage temperature, ripening and changes in physico-chemical properties during ripening.

2.2. ARC-ITSC avocado breeding program

In 1991, the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) initiated an avocado breeding program consisting of three phases, whereby, phase I entailed importing, grafting, evaluation and selection of improved scions and rootstocks (Bijzet *et al*., 1993). This phase has successfully been completed with various selections planted at Burgershall research farm in Hazyview (Bijzet *et al*., 1994). Pre-harvest characteristics of these selections have been studied and documented (Bijzet *et al*., 1994 and Sippel *et al*., 1994). However, information on the response of these new selections fruit to a mandatory low shipping temperature and ripening characteristics has not been documented. Such information will allow evaluation of the newly developed avocado selections in different production regions and bring phase II to completion. Thereafter, phase III (pre-commercial trials) can be carried out prior to selections being registered as export cultivars.

2.3 Avocado harvest maturity

Avocado fruit maturity refers to the stage of development at which the fruit, once detached from the tree, will ripen and results in a product desirable for eating (Kaiser *et al*., 1995). Avocado fruit must be harvested at a correct maturity for ripening process to commence without shrivelling. Harvesting avocado fruit prior to physiological maturity may result in uneven ripening, off-flavours and severe physiological disorders such as chilling injury and vascular staining (Zauberman *et al.*, 1977).

The SAAI uses fruit moisture content based maturity index (Eksteen, 2001). In avocado, fruit maturity is characterised by a decrease in moisture content, concomitantly, dry matter and oil content decreases (Bezuidenhout and Bezuidenhout, 2014). The maturity level at which an avocado fruit must be harvested depends on variety and intended market (Kruger *et al*., 2001). According to Mans *et al*. (1995), the maximum moisture content for 'Hass', 'Pinkerton', 'Fuerte', 'Ryan' and 'Lamb' and 'Maluma Hass' is 80, 77, 80, 80, 73 and 78%; respectively. In general, it is considered that moisture content levels of early-season, mid-season and late season fruit must be ±73, ±69, and ±66%; respectively (Roets *et al*., 2009; Van Rooyen, 2009).

2.4 Low storage temperature

Temperature is no doubt the single most influential factor in the maintenance of fruit quality during storage. Most biological processes are temperature controlled, therefore, it strongly affect fruit quality during storage (Workneh *et al*., 2011). According to Dixon *et al*. (2004), low storage temperature reduced the rate of respiration and ethylene production; and therefore, reduced metabolic rate, extended storage-life of fresh commodity. In 'Hass' avocado fruit from New Zealand an optimal quality was obtained during storage at 4-6°C for up to 4 weeks (Hopkirk *et al*., 1994). While, in 'Pinkerton' avocado fruit stored below the recommended temperature (5.5°C), the severity of mesocarp discolouration was reduced, while, storage temperatures above intensified the disorder (Van Rooyen and Bower, 2006). Zauberman *et al.* (1977) found the metabolic rate of 'Hass', 'Fuerte' and 'Naval' fruit to be reduced and ripening inhibited during storage at 6 and 8°C, and, fruit did not soften until transferred to 20°C. Low temperature reduced fruit ripening rate; therefore, preserving the overall avocado quality during storage.

2.4.1 Chilling injury

Low storage temperature is commonly used to extend the storage-life, and therefore, ensuring quality maintenance of fresh commodities. However, low storage temperature might result in chilling injury (Adams and Brown, 2007). Chilling injury refers to irreversible physiological damage to plant parts, particularly those of tropical and sub-tropical origin, as a result of exposure to low but non-freezing temperatures (Lyons and Breidenbach, 1987). A diversity of responses to low temperature stress exists, including alterations in ethylene biosynthesis, increased respiration rate and solute leakage, cessation of protoplasmic streaming, and uncoupling of oxidative phosphorylation (Hershkovitz *et al*., 2005). Ultimately, these various responses give rise to an array of physiological disorder visual symptoms (Hershkovitz *et al*., 2005). Avocado fruit can develop 2 types of chilling injury; internal (mesocarp) and external (pericarp) chilling damage. The main symptoms associated with chilling injury are black spots on the peel (pericarp) or grey or dark-brown discolouration of the mesocarp (Pesis *et al*., 1994).

Chilling injury can be detected approximately after 3 to 4 weeks of low storage temperature and most evident in softening or ripening fruit (Woolf *et al*., 2002). Furthermore, prolonged low storage temperature might increase manifestation severity of chilling injury symptoms (Crisosto *et al*., 2003). Eaks (1983) found that unripe 'Hass' avocado fruit stored at 0 and 5°C displayed chilling injury symptoms after 6 weeks when compared with 4 weeks storage period. A study by Forero (2007) confirmed the increased severity of chilling injury as a result of low storage temperature duration on 'Hass' avocado fruit stored at 7°C for 47 days.

Apart from severity and duration of exposure to chilling temperatures, the nature and severity of chilling injury also depends on cultivar and fruit maturity (Kader, 2002; Kader and Rolle, 2004). Zauberman *et al.* (1973) found that 'Nabal' avocado fruit were more cold tolerant when compared with 'Ettinger' and 'Fuerte' when stored 0-

6°C for 6 weeks. Furthermore, Zauberman *et al.* (1977) showed that ‘Nabal’ and ‘Hass’ avocado fruit were more resistant to chilling injury when compared with ‘Fuerte’ at 6 and 8°C low storage temperature.

Previous studies have suggested that the sensitivity of avocado fruit to physiological disorders decrease as the harvest season progresses (Toerien, 1986). Early-season fruit with high moisture content were more susceptible to physiological disorders such as chilling injury when compared with more mature fruit (Kosiyachinda and Young, 1976). Vorster *et al*. (1987) found that early-season ‘Fuerte’ avocado fruit (oil content less than 14%) were more susceptible to chilling injury when compared with more mature fruit (oil content 14-20%) stored at a temperature regime of 5,5°C for up to 21 days. Similar results were reported by Dixon *et al*. (2008) on ‘Hass’ avocado, whereby, fruit harvested in February were more susceptible to chilling injury when compared with fruit harvested in April after 28 days of storage at 1-5°C. According to Dixon *et al*. (2004), incidences and severity of rots and chilling injury in ‘Hass’ avocado fruit decreased when fruit moisture content decreased from 76 to 64% over the harvest season.

2.4.2 Electrolyte leakage as an indication of chilling injury in avocado fruit

Chilling injury is the consequence of low temperatures disrupting the fluidity and order of the membrane lipids, affecting their function as semi-permeable barriers and interaction with associated enzymes (Lyons and Raison, 1970). As a result of low temperature, membrane lipids commonly undergo phase transitions, i.e., liquidcrystalin or fluid to gel or solid, which temporarily affect membrane permeability during short periods of temperature decrease (Leshem, 1992).

Electrolyte leakage refers to a measure of membrane integrity as a result of electrolytes dissociating into ions and leaking through membrane channels (McCollum and McDonald, 1991). In avocado fruit, electrolyte leakage is evaluated from electrical conductivity (EC) measurements and reflects on the biochemical changes occurring during storage and shelf-life. Increased rate of electrolyte leakage was shown to serve as a good indicator of membrane permeability on ‘Hass’ avocado fruit stored at 6°C, and highly correlated with manifestation of chilling injury symptoms (Montoya *et al*., 1994). Furthermore, Hershkovitz *et al*. (2009) showed on ‘Arad’ and ‘Ettinger’ avocado fruit stored at 5°C an increase in electrolyte leakage concurrent with chilling injury symptoms visible as mesocarp discolouration when transferred to 20°C.

2.5 Ripening physiology

Ripening refer to processes that cause a change in the taste, texture and /or colour, of fruit, making the fruit acceptable for consumption (Lee *et al*., 1983). Thus, ripening imparts on the quality of fruit as agricultural commodity. Ripening is the result of a number of complex physiological and physical changes reflected in cellular structural modification of cell and plasma membranes (Bower and Cutting, 1988). In avocado, ripening occurs between fruit maturity and senescence (Biale, 1975). Avocado fruit mature on the tree but does not ripen until detached from the tree (Woolf *et al*., 2005). Once ripening process has begun, it cannot be reversed, but only slowed by various methods. Plant growth regulators, ripening enzymes, mineral nutrition and water movement contribute to the ripening process (Van Rooyen and Bower, 2006).

2.6 Ripening temperature

Temperature is a critical component in the post-harvest life of fresh produce. Low temperatures are needed during the shipping of fruit to significantly reduce metabolic activity, particularly the ripening enzymes, ethylene production and respiration in order to extend shelf-life (Donkin, 1995). Metabolic activity is important not only in the storage and shipping of fruit, but also during the ripening process after shipping.

Optimal ripening temperature depends on the orchard temperature and storage temperature. Fruit grown at a lower temperature should be ripened at 15-18°C while those grown at a higher temperature can be ripened at 20-25°C to preserve quality (Hopkirk *et al*., 1994). In avocado, lower ripening temperature may result in the development of pathological disorders and delayed colour change in black cultivars, whereas, higher temperatures may also result in development of pathological disorders and mixed ripening (Eaks, 1978). Hofman *et al*. (2002) found 'Hass' fruit ripened at 17°C took longer to change colour when compared with those ripened at 24°C, and, fruit ripened at 17°C were over-ripe by the time they had developed the desired black colour. Therefore, it is essential to maintain optimal metabolic activity during ripening. Avocado fruit typically could ripen at ambient temperatures (18-25°C), after removal from low storage temperature (Hopkirk *et al.*, 1994).

2.7 Physico-chemical changes that occur during ripening of avocado fruit

Ripening fruit undergoes many physico-chemical changes that determine fruit quality. Physico-chemical properties are dependent on the joint action of both physical and chemical processes (Mooz *et al*., 2012). In avocado fruit, physico-chemical properties associated with quality could refer to changes in skin colour, firmness, pH, total titratable acid, mass loss and flavour (Kassim *et al*., 2013). Such properties play an important role on how the consumer may perceive the quality of a ripe avocado fruit in relation to appearance, flavour and texture (Mooz *et al*., 2012). Therefore, it is necessary to have an understanding of the complex physiological processes occurring during fruit ripening in order to improve practice towards increased storage and shelf-life (Bower and Cutting, 1988). For the purpose of this study, the physico-chemical properties reviewed would include firmness, respiration rate, and water loss and peel colour during ripening.

2.7.1 Firmness

Firmness is an important characteristic of avocado, and the most reliable method of determining if the fruit is ripe to eat. Fruit firmness differences are good predictors of the difference in ripening stages (Arzate-Vazquez *et al*., 2011). Firmness determines suitability for consumption and is important as physiological and pathological disorders develop rapidly during the latter stages of avocado ripening (Hopkirk *et al*., 1994).

Firmness could be described as the force necessary to attain a defined deformation during textural evaluation (Landahl *et al*., 2009). Previously, a range of different methods to assess firmness of avocado fruit have been used. For example; firmometer (Swarts, 1981), puncture tests using Effegi probes (Arpaia *et al*., 1987) and conical probes (Meir *et al*., 1995). The various methods used classified fruit firmness in the categories from very hard to soft, while, the SAAI utilises densimeter to measure firmness. Densimeter units converts to Newtons (N) on a scale of 85-90 (hard, unripe; ≡ 8.06 N) to <60 (soft, ready to eat; ≡ 5.05 N) (Köhne, 1998).

Holding avocado fruit at optimum ripening temperature effectively reduces firmness, allowing for normal ripening to commence (Abou-Aziz *et al*., 2005). Similarly, Cutting *et al*. (1992) found 'Fuerte' avocado fruit firmness to be reduced when transferred from 5.5 to 20°C. Zamorano *et al*. (1994) found 'Fuerte' and 'Hass' avocado fruit firmness declined from 10 N to 6-4 N after 1 week during ripening at 20°C following 39 days storage at 7°C. Similar results were observed on 'Hass' fruit held at 15°C, whereby, firmness values of 130.51, 54.62, 19.92 and 7.37 firmometer units were recorded on day 0, 4, 8 and 12; respectively (Villa-Rodriguez *et al*., 2011).

2.7.2 Water loss

Post-harvest water loss has been found to significantly affect fruit ripening and reduce shelf-life (Wills *et al*., 1998). The occurrence of water loss during ripening of avocado fruit is of major concern as water cannot be replaced after the fruit is detached from the tree. Water loss is necessary during ripening, however, should be minimised and managed during storage and ripening to reduce development of physiological disorders (Blakely, 2011).

Bower and Cutting (1987) showed that ripening rate and fruit quality were both affected by the rate of water loss during storage. Bower and Cutting (1988) suggested that this may be a result of ethylene production as a result of stress during ripening. Burdon (2005) reported that increased water loss is a result of ethylene biosynthesis during respiratory climacteric. Lallum *et al*. (2004) showed on 'Hass' avocado fruit held at 20°C that water loss during the initial stages of ripening acted through ethylene synthesis pathway and significantly affected the rate of ripening. (Blakely, 2011) suggested that water loss during storage and ripening of fruit should be limited to prevent the initiation of the climacteric response, as ethylene biosynthesis is stimulated by increased water loss.

2.7.3 Respiration rate

Avocado is a climacteric fruit, and show increased respiration during ripening, therefore, termed respiratory climacteric (Kadam and Salunkhe, 1995). Climacteric refers to fruit ripening stage associated with increased respiration rate and ethylene production (Rhodes, 1981). The climacteric pattern is divided into the following three stages; the pre-climacteric (low respiration); climacteric (maximum respiration) and post-climacteric stage (decline in respiration) (Kadam and Salunkhe, 1995). Yang (1981) considered ethylene formation to be essential in the ripening of climacteric fruit and its peak usually precedes the respiratory climacteric. Avocado fruit climacteric nature is characterised by a marked increase and decrease in respiration rate (CO_2 production) and ethylene production during fruit ripening (Wills *et al.*, 1998).

Fruit respiration describes a catabolic process of complex molecules into simpler molecules, yielding energy, water and carbon dioxide needed for cellular biochemical reactions. Thus, fruit respiration is measured using CO_2 production (Rhodes, 1981). Respiration rate of 'Hass' avocado fruit held at 20°C followed a climacteric pattern with maximum CO_2 production reached on the second day of ripening (176.17±15.98 mL/kg/h), decreased on the fourth day (90.4±22.88 mL/kg/h), and at the end of shelf-life (8 days) CO_2 production was less than 100 mL/kg/h (Perez *et al*., 2004). Cultivar 'Fuerte' avocado fruit reached climacteric peak on the third day at 17°C after withdrawal from low storage temperature at 2°C (Pesis *et al*., 1994). Eaks (1983) reported the maximum climacteric and ripening after 4 days for 'Hass' and 'Fuerte' avocado fruit when fruit were held at 20°C after five weeks storage at 5 and 10°C.

2.7.4 Peel colour

Colour is an important fruit quality parameter. It affects consumer acceptance, sweetness perception, flavour and can even evoke emotional feelings (Ornelas-Paz *et al*., 2008). During fruit ripening, colour development is important for industry and consumers as an indication of ripeness (Cox *et al*., 2004). According to Cox *et al*. (2004) 'Hass' avocado fruit skin colour changes from green to purple/black during ripening. However, peel colour of the 'Sharwil' avocado fruit does not darken during ripening (Chen *et al*., 2009). Similarly, peel colour of a 'Fuerte-type' avocado fruit remain green during ripening, therefore, does not undergo colour change (Dorria *et al*., 2010).

Objective colour is measured with a chromameter on the basis of the CIELAB colour system (L*, C*, a*, b*, and h°). In this system, L*, a* and b* describe a three dimensional space, whereby, L* (Lightness) is the vertical axis, with values varying from 100 for perfect white and 0 for black. Values of a* and b* specify the green-red and blue-yellow axis; respectively. Chroma (C*) describes the length of colour vector in the plane formed by the values of a* and b*. While Hue angle (h°) determines the position of such vector. Chroma and h◦ values are calculated based on a* and b* values according to the following equations: $C^* = [(a^*)^2 + (b^*)^2]$ 0.5 and $h° = 180 - \tan^{-1}(b^*/a^*)$ (McGuire, 1992).

In 'Fuerte' avocado fruit held at 20°C, hue angle (h°) decreased at the least rate, lightness (L) moderately, while chroma (C) changed at a more increased rate (Dorria *et al*., 2010). Similarly colour parameters (L*, C* and h°) significantly decreased during ripening of 'Hass' avocado fruit at 15, 20 and 25°C (Cox *et al*., 2004). Bender *et al*. (2000) found L*, a* and b* colour parameters to change at varying rates during ripening of 'Tommy Atkins and Haden' mango fruit stored at 2, 3, 4 or 5 kPa O_2 plus N_2 at 12°C for 14 days or 25 kPa CO_2 at 15°C for 21 days.

2.8 Addressing the identified gaps

The South African avocado industry is largely export orientated and exports to European markets by shipping under low storage temperature for up to 4 weeks. However, current avocado cultivars are susceptible to physiological disorders which have necessitated the need to breed and select new avocado selections with superior traits such as cold tolerance, disease resistance and improved storability and shelf-life. Information on the response of the new 'Fuerte-type' selections developed by the ARC-ITSC to mandatory low temperature storage and physico-chemical properties during ripening could assist in expanding cultivar variability of the industry and ensures global market competitiveness. Furthermore, in South Africa, 'Fuerte' still plays an important role as it comes onto the market 2-4 weeks earlier than 'Hass'; and therefore, assisting exporters in entering the export season early (Donkin, 2007). Registration of the newly developed 'Fuerte-type' avocado selections as commercial cultivars would enable the South African avocado industry to export fruit to the USA and Japan markets from mid-February through May.

2.9 Summary of the gaps to be investigated

In an on-going attempt to overcome drawbacks of the physiological disorders resulting from low storage temperature on the existing commercial avocado cultivars, the ARC-ITSC has developed new ‘Fuerte-type’ avocado selections. However, effect of harvest season and ripening duration on the physico-chemical properties of these selections during ripening after withdrawal from need to be evaluated under commercial low temperature storage conditions need to be evaluated in order to be registered. Registration of these selections will enable the South African avocado industry to compete in lucrative global markets alongside Southern hemisphere avocado producing countries with overlapping season. Throughout the literature studied it was evident that the existing South African commercial avocado cultivars especially ‘Fuerte’ are susceptible to physiological disorders as a result of low storage temperature. This is a concern as the fruit reach distant markets with symptoms of physiological disorders and could discredit the SAAI among its competitors in the global market. Furthermore, the ripening process affects the physico-chemical properties of avocado fruit differently, attributing the overall quality and shelf-life of the fruit.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Experimental sites, design and treatments

Mature newly developed ‘Fuerte-type’ avocado fruit were harvested from a gene block at the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) Burgershall research farm in Hazyview (25°06'30.53"S, 31°05'04.75"E) and export grade ‘Fuerte’ avocado fruit were obtained from a commercial (Halls and Sons) farm in Nelspruit (25°27′07.18″S, 30°56′29.17″E). New ‘Fuerte-type’ avocado fruit (‘Fuerte 2 and 4’, ‘H287’ and ‘BL1058’) were randomly harvested from three trees per selection during the 2014 and 2015 seasons. Afterwards, fruit were transported to the ARC-ITSC post-harvest laboratory in Nelspruit (25°27′04.8″S, 30°58′09.75″E) for storage and laboratory analysis. In the laboratory, fruit were sorted, graded, weighed; and afterwards, packed in 3 boxes of 9 fruits each, replicated three times, making 27 fruits per selection and stored at industry recommended temperature (5.5°C) for 28 days. The experiment was laid out as a factorial arranged in a completely randomised design (CRD). The treatment factors were: (i) 2 x harvest seasons, (ii) 5 x selections and (iii) 6 x ripening days.

3.2 Data collection

3.2.1 Determination of fruit maturity

Moisture content at harvest was determined from three fruit for each selection. Each fruit was cut into halves with a fruit chopper; one half peeled, seed removed and then flesh grated using a kitchen grater. A sample of 10 g of the grated flesh from each fruit was weighed, oven dried at 30°C for 48 h (Model: 279, Ecotherm, Labotec, South Africa); and afterwards re-weighed to determine the moisture content. Thereafter, the moisture content % was determined as follows:

$$\text{Moisture content (\%)} = (M_0\text{-}M_1/M_0) \times 100$$

Where:

M_1 = Final mass of the dried sample

M_0 = Initial mass of the fresh sample

3.2.2 Determination of tissue electrolyte leakage

After withdrawal from low storage temperature, fruit were analysed for electrolyte leakage (Figure 3.1) according to the method of Montoya *et al*. (1994). Three avocado fruit from each selection were used for the determination of tissue electrolyte leakage after storage. A 10 mm in diameter cork borer was used to remove sample disks from the fruit. The initial electrolyte leakage (EC_1) was taken after shaking the disks in 10 mL ultra-pure water for 3h. The electrical conductivity was measured using an electrical conductivity meter (Model: Hi991301N, El- Hamma Instruments, Israel). Afterwards, samples were boiled in a hot water bath for 1 hour, and thereafter, allowed to cool to ambient temperature before the final electrolyte leakage was measured (EC_2). Electrolyte leakage was then expressed as percentage using the following formula: EC_1-(EC_2/EC_1) × 100.

3.2.3 Determination of external chilling injury

External chilling injury was visually assessed on skin lesions using a scale of 0 to 1 whereby, 0 indicated no chilling injury (A=0),) and 1 indicated chilling injury (B=1) (Figure 3.2) and expressed as a percentage chilling injury (Donkin and Cutting, 1994).

Figure 3.1 Measuring electrolyte leakage of the new ‘Fuerte-type’ avocado tissue

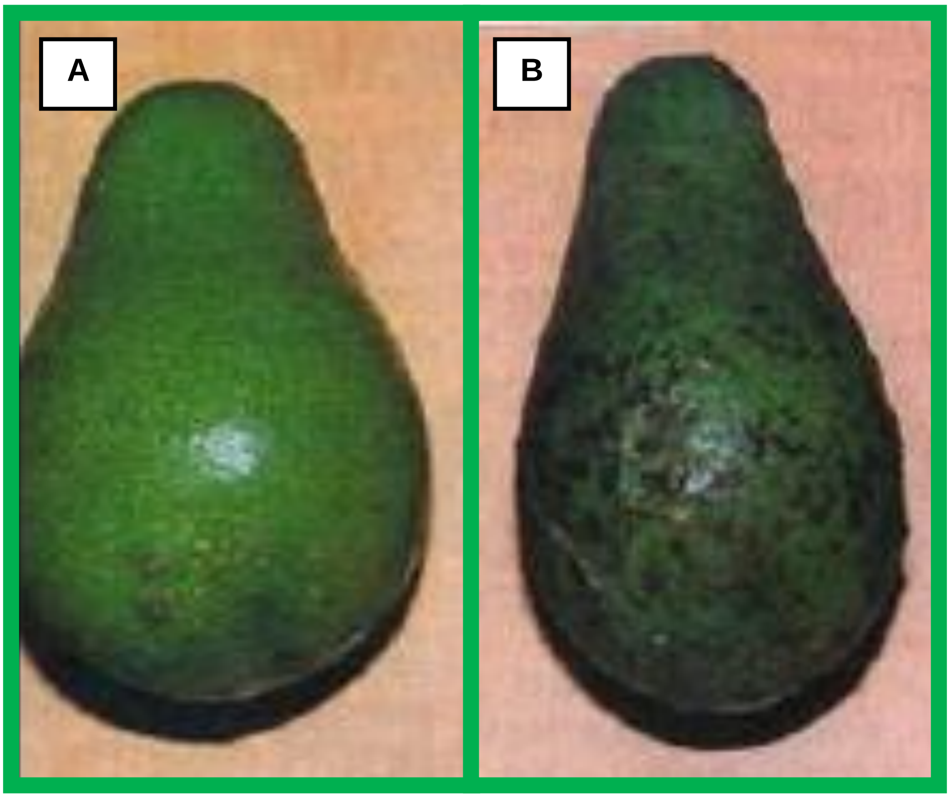

Figure 3.2 External chilling injury symptoms of ‘Fuerte’ fruit after withdrawal from low storage temperature

3.2.4 Determination of mass loss

Fruit mass loss was calculated as the difference in fruit mass before and after cold storage, and expressed as a percentage of the initial mass of each fruit. Fruit mass was measured using an electronic weighting scale (Scaltec instruments, Heiligenstadt-Germany) (Figure 3.3). Individual fruits were weighed prior to cold storage and after removal from cold storage on daily basis during ripening. Fruit mass loss was calculated as the difference in fruit mass before and after low storage temperature, and expressed as a percentage of the initial mass of each fruit as follows:

Mass loss = $(W_0\text{-}W_1/W_0)\times100$

Where:

W_1 = Mass of fruit on particular ripening day

W_0 = Mass of fruit before low storage temperature

Figure 3.3 Weighing new ‘Fuerte-type’ avocado fruit for mass loss

3.2.5 Determination of fruit firmness

Fruit firmness was determined by a non-destructive method according to Kohne *et al.* (1998) using a hand-held densimeter (Model: 53524, Bareiss, Oberdischingen, Germany) with a 5 mm tip was used to measure fruit firmness on a scale of 85-90 (hard, unripe; ≡ 8.06 N) to <60 (soft, ready to eat; ≡ 5.05 N) densimeter units. Three readings were taken around the circumference of each fruit and the average reading recorded. Firmness was expressed in densimeter units.

3.2.6 Determining ripening percentage

Ripening percentage was calculated as the number of fruit that reached ‘eating soft’ stage, which corresponded to an average densimeter reading of less than 60 (5.05 N) during shelf-life.

3.2.7 Determination of peel colour

The colour characteristics were assessed using a Minolta Chroma Meter (Model: CR-400, Minolta Corp, Ramsey, NJ, USA) to determine L value (lightness or brightness), a* value (redness or greenness) and b* value (yellowness or blueness) of the avocado fruit (Figure 3.4). The instrument was calibrated with a white standard tile: L=95, 87, a=0, 86 and b=2, 47.

The parameters relating to colour measurement were:
L = Lightness or brightness,
a = Redness or greenness and
b = Yellowness or blueness.
From these parameters the chroma (C) and hue angle were determined as explained by Maftoonazad and Ramaswamy (2008) using the formula:

$$C = \sqrt{a^2 + b^2}$$

$$hue\ angle = \tan^{-1}\left(\frac{a}{b}\right)$$

Figure 3.4 Measuring the colour parameters of the new 'Fuerte-type' avocado fruit

3.2.8 Determination of respiration rate

Each avocado fruit was placed in an airtight plastic container (1000 mL) with a sealed hole at the top for a minimum period of 30 minutes (Figure 3.5). A dual gas analyser (Model 250, International Controlled Atmosphere Ltd, Paddock Wood, Tonbridge, Kent, UK) was used to determine the CO_2 production after the set period. The headspace CO_2 concentration was converted to respiration rate using fruit mass, fruit volume, free space in the jar and the ambient CO_2 concentration and expressed as µmol CO_2 Kg^{-1} hr^{-1}.

Figure 3.5 Measuring the respiration of the new ‘Fuerte-type’ avocado fruit

3.3 Data analysis

Data was subjected to analysis of variance (ANOVA) using Genstat 16th version (VSN International Bioscience Software and Consultancy, 2014) and Duncan’s multiple range tests at P≤0.05 were used to compare the mean difference of the treatments.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Moisture content

Selection and harvest season had no significant effect (P=0.668) on the moisture content used to determine maturity of the evaluated ‘Fuerte-type’ selections (Appendix 1). Commercial ‘Fuerte’ was harvested at moisture content of 77.3% during both harvest seasons (Table 4.1). Selection ‘Fuerte 2’ fruit were harvested at a moisture content of 74.3 and 78.7% during the 2014 and 2015 harvest seasons; respectively. Selection ‘BL1058’ fruit were harvested at a moisture content of 73.3 and 75.3% during the 2014 and 2015 harvest seasons; respectively. Selection ‘Fuerte 4’ fruit were harvested at a moisture content of 73.3 and 74.0% during the 2014 and 2015 harvest seasons; respectively. Therefore, selection ‘Fuerte 4’ fruit were harvested at the highest maturity level during the 2014 and 2015 harvest seasons. While, selection ‘H287’ fruit were harvested at a moisture content of 77.7 and 80.3% during the 2014 and 2015 harvest seasons; respectively. Therefore, selection ‘H287’ fruit were harvested at the lowest maturity level during both harvest seasons. In addition, ‘H287’ fruit were the only selection fruit harvested at optimum maturity level, at moisture content of 80.3% amongst the evaluated selections.

Table 4.1 Means of maturity percent moisture content and their standard error of the evaluated ‘Fuerte-type’ avocado fruit during the 2014 and 2015 harvest seasons

Selections	**Moisture content (%)**	
	2014	2015
Commercial Fuerte	77.3±0.88	77.3±0.88
‘Fuerte 2’	74.3±0.67	78.7±0.88
‘Fuerte 4’	73.3±2.40	74.0±3.00
‘BL1058’	73.3±1.20	75.3±0.88
‘H287’	77.7±1.45	80.3±0.67

4.1.2 External chilling injury

There was a significant difference ($P<0.05$) in external chilling damage of the evaluated 'Fuerte-type' selections during the 2014 and 2015 harvest seasons (Appendix 2). All the evaluated new 'Fuerte-type' selections, including the commercial 'Fuerte' showed symptoms of external chilling injury during the 2014 and 2015 harvest seasons (Figure 4.1). However, selection 'Fuerte 4' fruit showed the lowest external chilling damage (8.3%) followed by 'H287' (83.3%) when compared with all other evaluated selections during the 2014 harvest season (Figure 4.2). Meanwhile, selection 'BL1058' fruit showed the lowest external chilling damage (86.7%) during the 2015 harvest season.

Figure 4.1 Chilling injury on the evaluated 'Fuerte-type' avocado fruit 28 days after low storage temperature

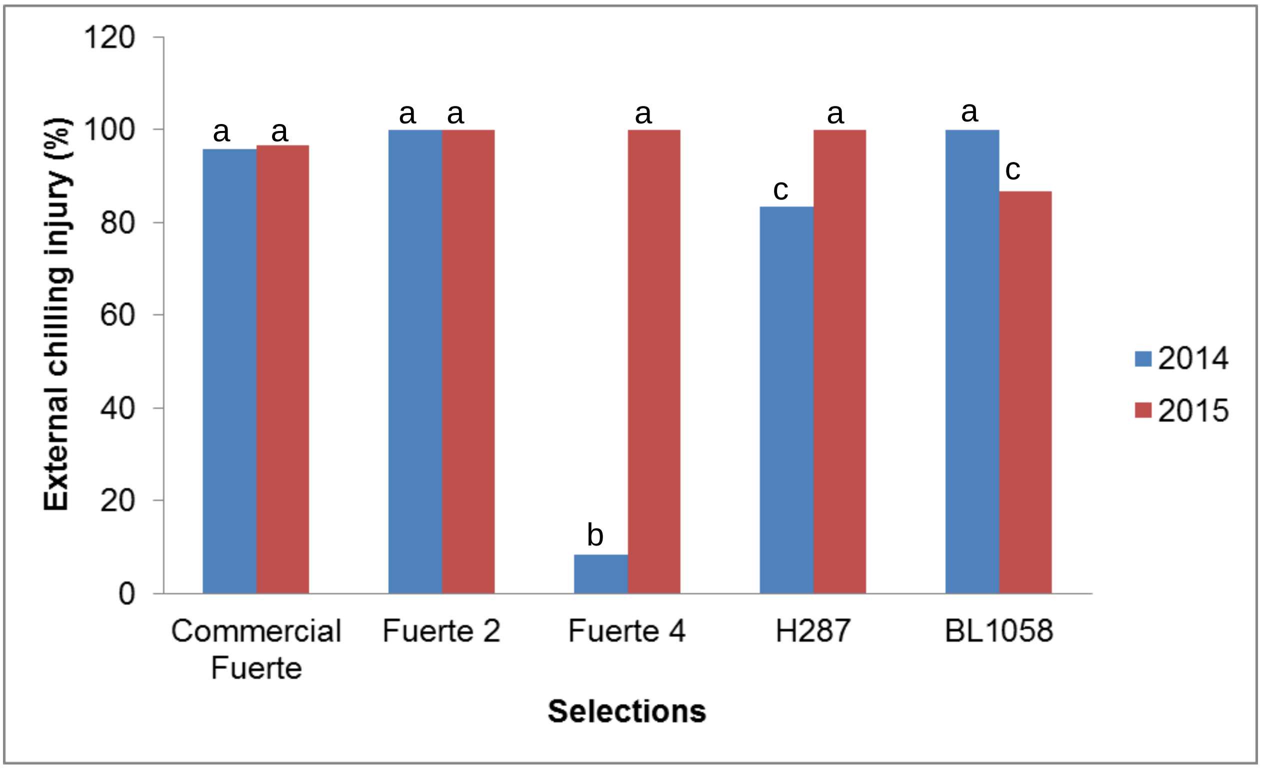

Figure 4.2 External chilling injury of selected 'Fuerte-type' selections recorded after low storage temperature withdrawal during 2014 and 2015 harvest season

4.1.3 Electrolyte leakage

After withdrawal from low storage temperature, there was significant interaction ($P<0.05$) between selections and harvest seasons on electrolyte leakage (Appendix 3). Electrolyte leakage of selection 'BL1058' and 'H287' fruit was 44.5 and 40.5% during the 2014 harvest season; respectively. While, electrolyte leakage of 'BL1058' and 'H287' fruit was 78.3%? and 80.2% during the 2015 harvest season; respectively (Figure 4.3). Therefore, indicating an almost two-fold increase in electrolyte leakage during the 2015 harvest season when compared with 2014 for 'BL 1058 and 'H287' fruit. Furthermore, increased electrolyte leakage for commercial 'Fuerte' fruit was observed during the 2015 harvest season when compared with 2014. However, an increased electrolyte leakage for commercial 'Fuerte' fruit was insignificant when compared with a two-fold increase observed with selection 'BL1058' and 'H287' fruit. In contrast, the electrolyte leakage of 'Fuerte 2 and 4' fruit decreased during the 2015 harvest season when compared with 2014. Electrolyte leakage and chilling injury symptoms highly correlated (*R*=0.84) during the 2014 harvest season (Figure 4.4). Contralily, a poor correlation (*R*=0.29) between electrolyte leakage and chilling injury was recorded during the 2015 harvest season (Figure 4.5).

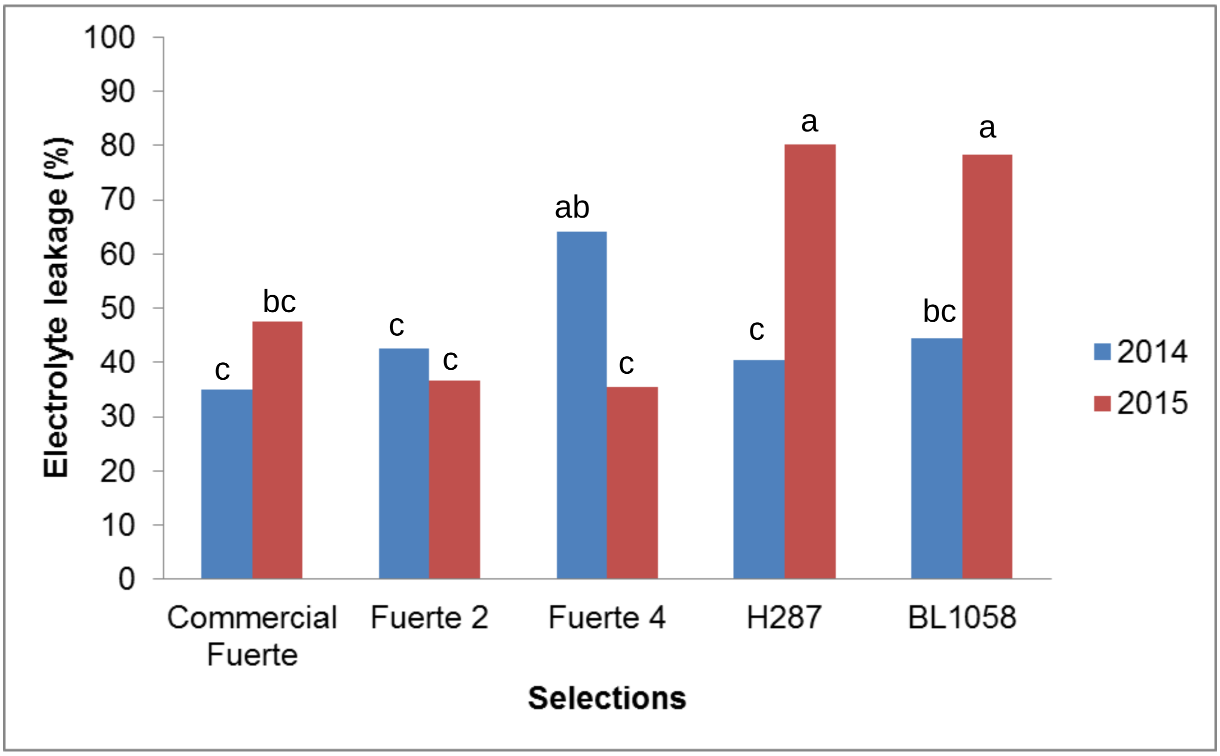

Figure 4.3 Electrolyte leakage of selected ‘Fuerte-type’ recorded after low storage temperature withdrawal during 2014 and 2015 harvest season

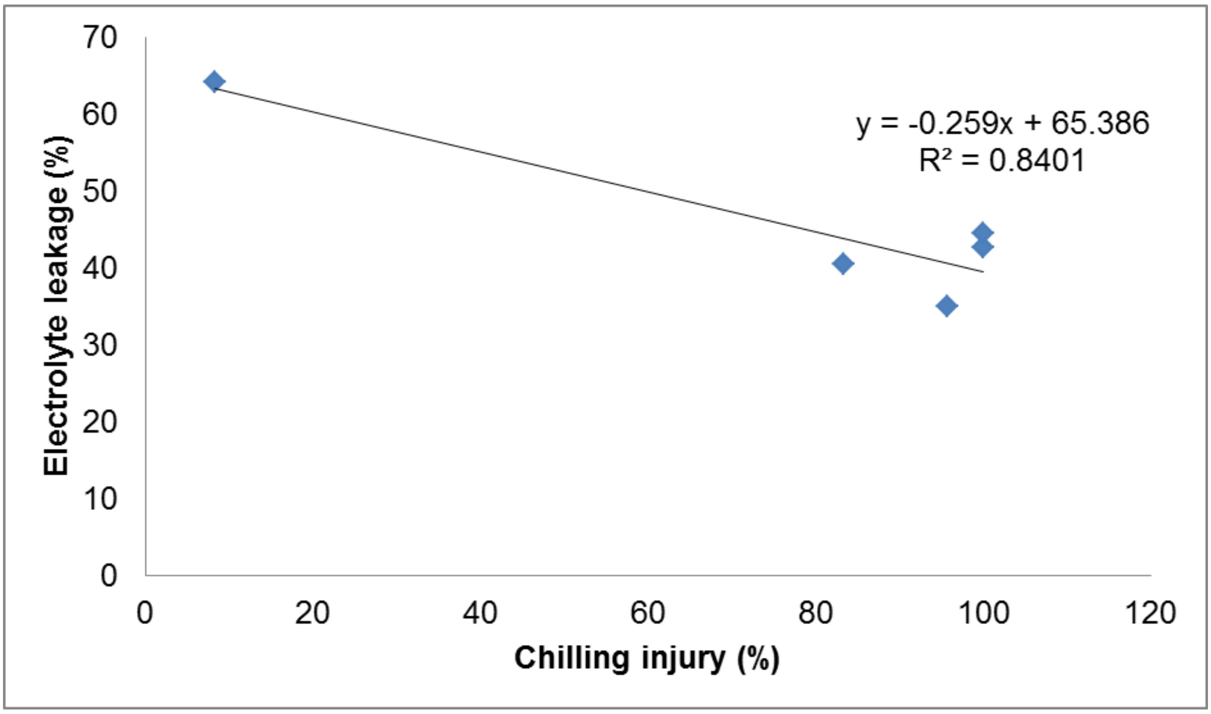

Figure 4.4 Correlation of electrolyte leakage and chilling injury symptoms of 'Fuerte-type' avocado fruit recorded after low storage temperature withdrawal during 2014 harvest season

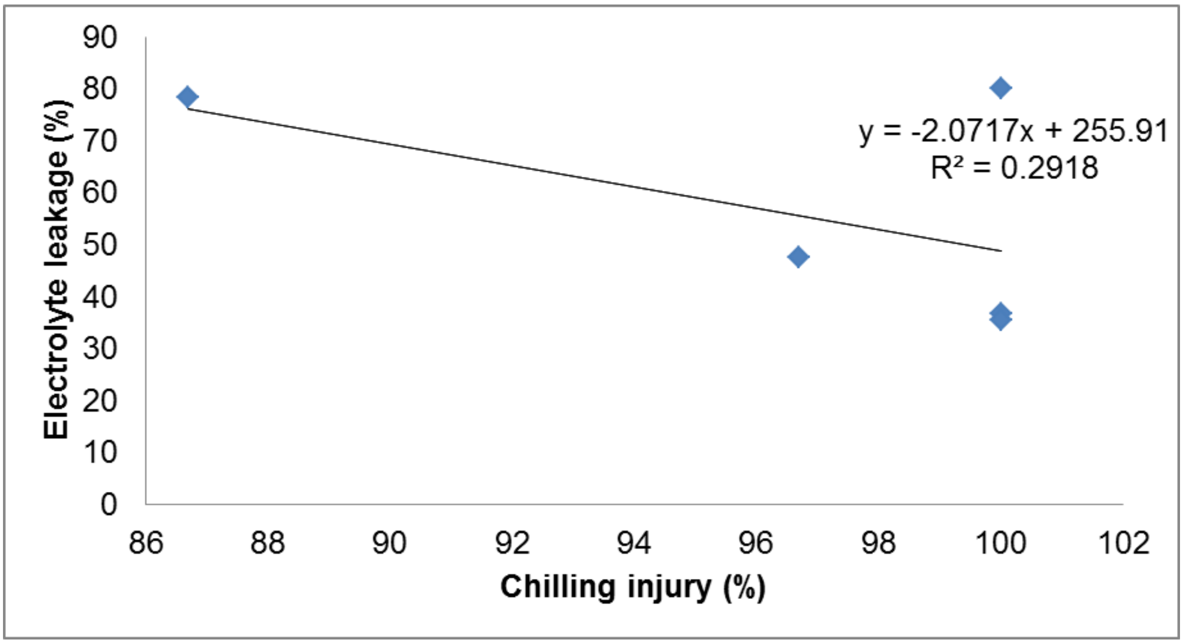

Figure 4.5 Correlation of electrolyte leakage and chilling injury symptoms of 'Fuerte-type' avocado fruit recorded after low storage temperature withdrawal during 2015 harvest season

4.1.4 Mass loss

Overall, harvest season, selection and ripening time had no significant effect (P=0.977) on mass loss of 'Fuerte-type' fruit during ripening (Appendix 4). However, mass loss of evaluated 'Fuerte-type' avocado selections increased as a function of shelf-life during the 2014 and 2015 harvest seasons (Figure 4.6). Selection 'BL1058' fruit had highest mass loss during 2014 (10.93%) and 2015 (10.87%) harvest seasons, recorded on day 4 during both harvest seasons. Interestingly, commercial 'Fuerte' was terminated at a mass loss of 8.77%, recorded on day 3 during 2015 harvest season. However, during the 2014 harvest season for commercial 'Fuerte' fruit were terminated at the lowest mass loss of 7.94%, recorded on day 4 when compared with all evaluated selections. Furthermore, 'Fuerte 2' was terminated at a high mass loss (9.19%), recorded on day 4 during the 2014 harvest season, however, significantly decreased to (5.99%), recorded on day 4 during the 2015 harvest season. In addition, 'Fuerte 4' fruit were terminated at the lowest mass loss of 5.82%, recorded on day 3 amongst evaluated selections during the 2015 harvest season.

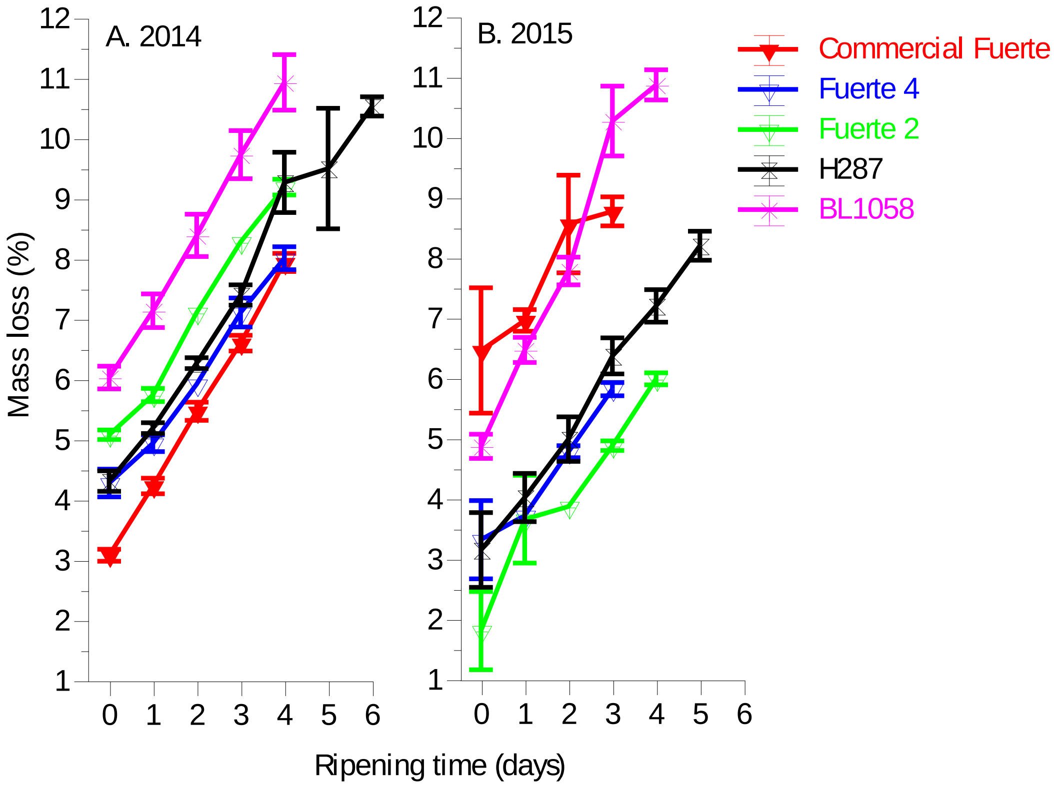

Figure 4.6 Mass loss of evaluated ‘Fuerte-type’ avocado fruit measured during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.1.5 Respiration rate

Overall, harvest season, selection and ripening time had a significant effect ($P<0.05$) on the respiration rate based on CO_2 production (µmol CO_2 Kg^{-1} hr^{-1}) of the evaluated ‘Fuerte-type’ avocado fruit (Appendix 5). Respiration rate of evaluated ‘Fuerte-type’ selections fruit followed a climacteric respiratory pattern during the 2014 and 2015 harvest seasons. Respiration rate increased, reached a maximum peak and decreased during both harvest seasons (Figure 4.7). However, the magnitude of the climacteric peak and day of occurrence varied amongst selections during both harvest seasons. ‘Fuerte-type’ avocado selection fruit reached respiratory peaks more rapidly during the 2015 season when compared with the 2014 harvest season. Commercial ‘Fuerte’ showed rapid respiration rate, reaching respiratory peaks of 1087 and 1377 µmol CO_2 Kg^{-1} hr^{-1} at day 1 when compared with all other evaluated selections during the 2014 and 2015 harvest seasons; respectively. However, commercial ‘Fuerte’ had the lowest respiratory peak (1087 µmol CO_2 Kg^{-1} hr^{-1}) during the 2014 harvest season. However, selection ‘BL1058’ had the lowest respiratory peak during ripening (1005 µmol CO_2 Kg^{-1} hr^{-1}) during the 2015 harvest season. Selection ‘Fuerte 2’ fruit reached the highest respiratory peak 1940 µmol CO_2 Kg^{-1} hr^{-1} on day 2 during the 2014 harvest season, whereas, the highest respiratory peak of 2333 µmol CO_2 Kg^{-1} hr^{-1} was recorded on day 2 for selection ‘Fuerte 4’ fruit during the 2015 harvest season.

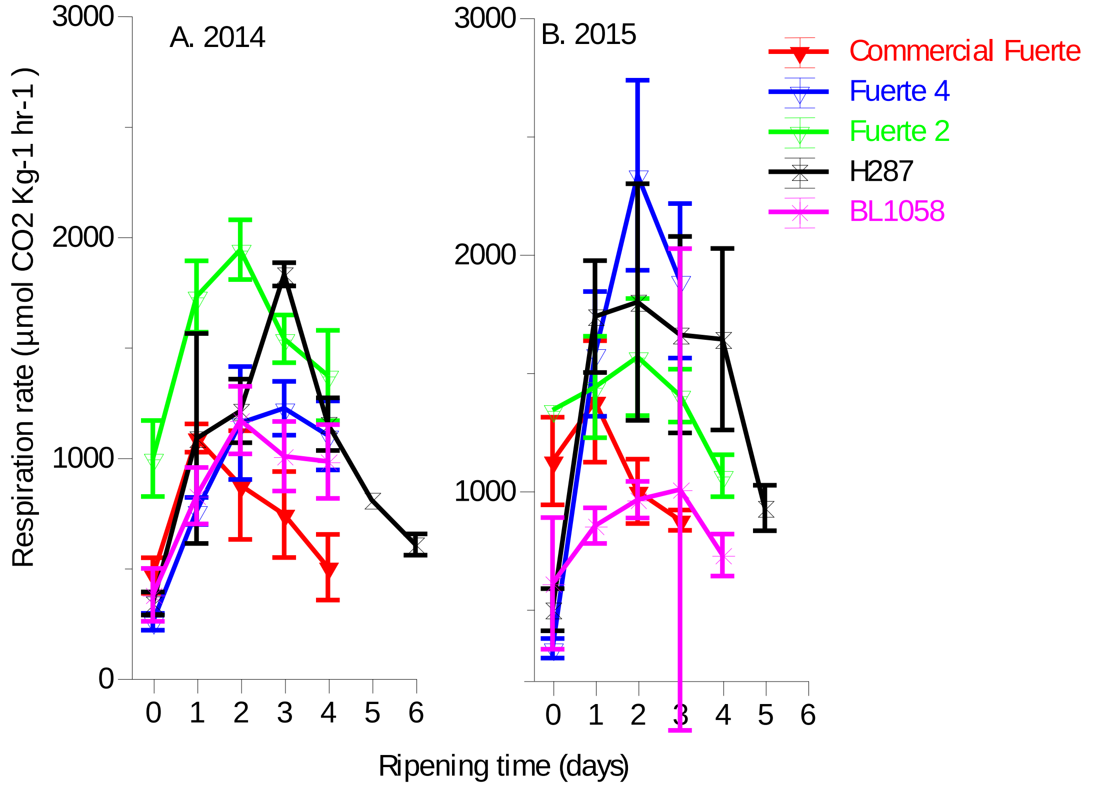

Figure 4.7 Respiration rate of new ‘Fuerte-type’ avocado fruit at shelf-life during ripening in the A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.1.6 Firmness

In general, harvest season, selection and ripening time had no significant effect (P=0.139) on fruit firmness during ripening of ‘Fuerte-type’ fruit (Appendix 6). A gradual decrease in firmness was observed after withdrawal from low storage temperature for all evaluated fruit during both harvest seasons (Figure 4.8). Firmness of ‘H287’ fruit remained high during both harvest seasons when compared with all tested selections during ripening. Furthermore, ‘H287’ fruit were terminated at a mean firmness value of 81.03 (day 6) and 75.4 (day 5) densimeter units during the 2014 and 2015 harvest season; respectively. Interestingly, selection ‘H287’ fruit firmness remained above 80 densimeter units when compared to all other evaluated selections and decreased by <1% with regard to ripening time during the 2014 harvest season. Commercial ‘Fuerte’ fruit were terminated at the lowest firmness value, 59.55 (day 4) and 49.57 (day 3) densimeter units when compared with the rest of the evaluated selections during 2014 and 2015 harvest seasons; respectively. Data collected for firmness was used to calculate the ripening percentage of the evaluated selections.

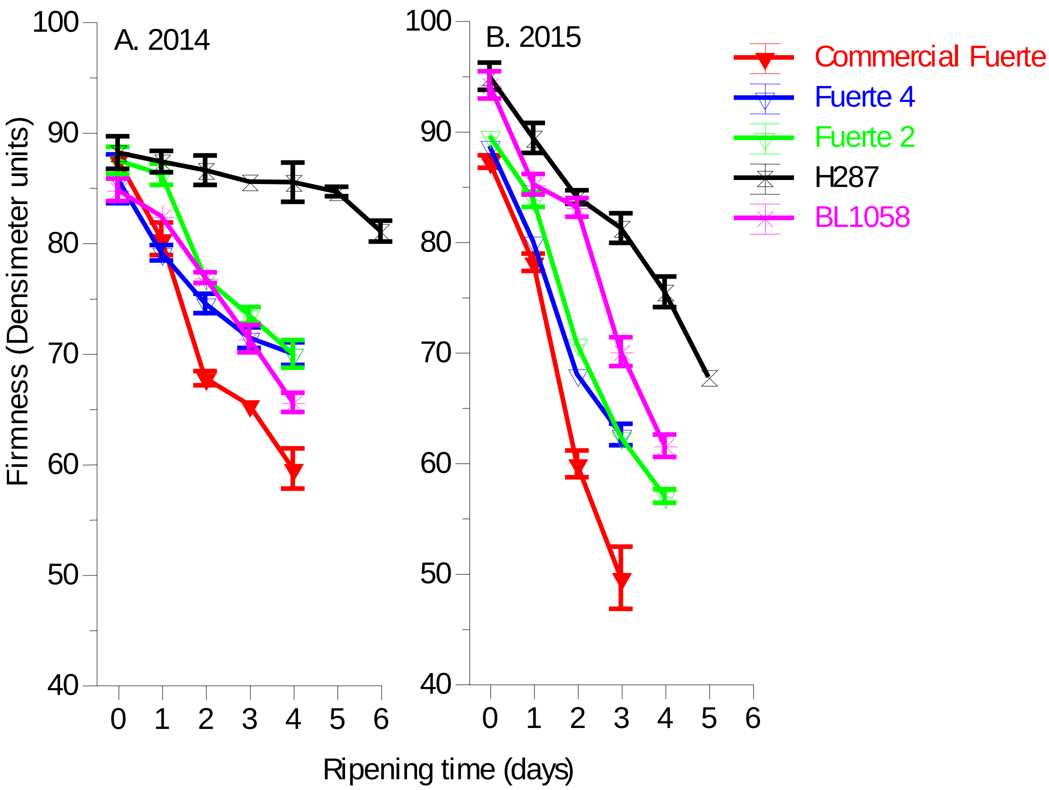

Figure 4.8 Loss of firmness of evaluated ‘Fuerte-type’ avocado fruit during ripening during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=84.1.6.2

4.1.7 Ripening percentage

Generally, harvest season, selection and ripening time had a significant effect ($P<0.05$) on the ripening pattern of 'Fuerte-type' fruit (Appendix 7). The maximum ripening period was shorter during the 2014 harvest season (5 days) when compared with the 2015 harvest season (6 days) (Figure 4.9). Commercial 'Fuerte' and 'Fuerte 4' ripened a day earlier (day 3) during the 2015 harvest season when compared with the 2014 harvest season (day 4). Ripening of 'Fuerte-type' fruit commenced on day 1 with 'Fuerte 2' fruit (4.33%) when compared to all evaluated selections which began to ripen on day 2, with the exception of 'H287' fruit which started ripening on day 5 (12.03%) during the 2014 harvest season. However, ripening of all evaluated 'Fuerte-type' fruit commenced on day 2 during the 2015 harvest season. Selection 'H287' fruit were terminated at the lowest ripening percentage of 24.70 and 54.50% during the 2014 and 2015 harvest seasons. Furthermore, selection 'H287' fruit were terminated at day 6 when compared with all other evaluated selections fruit, which were terminated at day 4 during the 2014 harvest season. In addition, 'H287' fruit were also terminated on the last day (day 5) during the 2015 harvest season. 'Fuerte 2' fruit showed the highest ripening percentage of 100 and 83.33% during the 2014 and 2015 harvest season; respectively. Furthermore, the highest ripening percentages for 'Fuerte 2' fruit were recorded on day 4 during both harvest seasons.

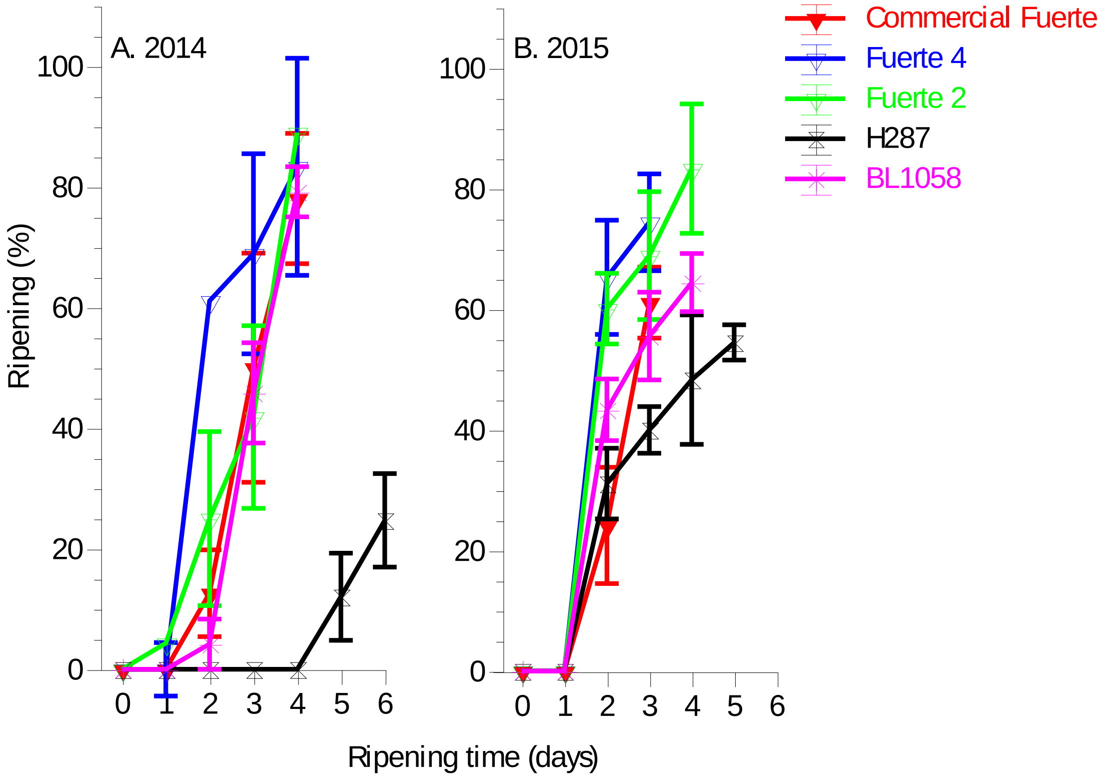

Figure 4.9 Ripening percentages of the evaluated ‘Fuerte-type’ avocado fruit at shelf-life during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.1.8 Peel colour

The evaluated fruit of ‘Fuerte-type’ avocado selections maintained a green colour during ripening. However, an insignificant variation in colour parameters (Lightness (L), Chroma (C) and Hue angle (h°)) for all evaluated selections was recorded. Black spots developed during and after low storage temperature (Figure 4.2).

4.1.7.1 Lightness

An interaction between harvest season, selection and ripening time had no significant effect (P=0.711) on the lightness value (Appendix 8). Overall, skin lightness (L) values of the evaluated fruit varied during ripening (Figure 4.10). Lightness values of selection ‘H287’ fruit showed a significant variation during the

2014 harvest season when compared with all evaluated selections. Furthermore, the highest (36.84) and lowest (23.65) lightness skin values were recorded for ‘H287’ during the 2014 harvest season. In addition, the highest and lowest lightness skin values for H287 were recorded on day 2 and 5 during the 2014 harvest season; respectively. Commercial ‘Fuerte’ and ‘H287’ fruit showed had the highest (33.70) and lowest (27.65) lightness skin values recorded on day 3 and 5 during the 2015 harvest season; respectively. Minimal variations were observed and recorded for ‘Fuerte 4’ during the 2014 harvest season.

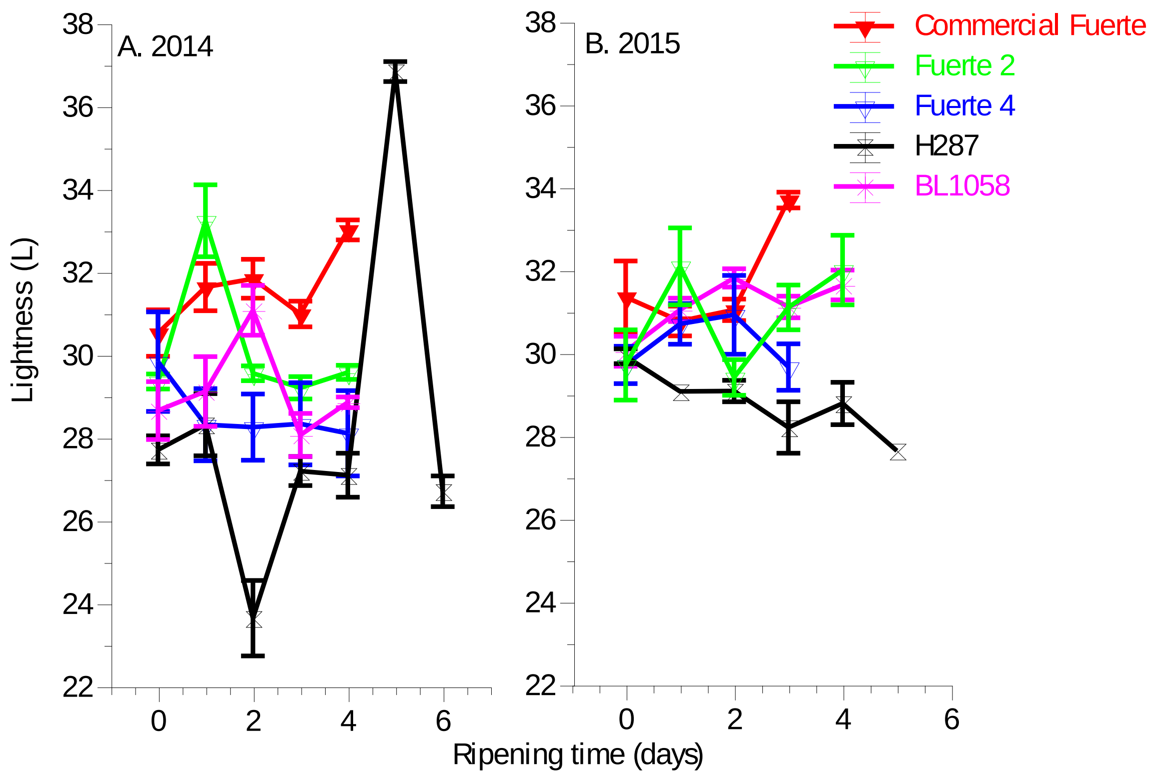

Figure 4.10 Lightness colour appearance parameter evaluated ‘Fuerte-type’ avocado fruit measured during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.1.7.2 Chroma

An interaction between harvest season, selection and ripening time had no significant effect (P=0.378) on the ‘Fuerte-type’ avocado fruit skin chroma value (Appendix 9). However, chroma values of the evaluated selections varied at a noticeable moderate rate (Figure 4.11). Selections ‘Fuerte 2 and 4’ fruit skin had higher chroma values when compared with commercial ‘Fuerte’, ‘H287’ and ‘BL1058’ fruit during the 2014 harvest season. Moreover, ‘Fuerte 2 and 4’ fruit had the highest chroma values of 26.12 and 23.91 recorded on day 1 and 0, during the 2014 harvest season; respectively. However, ‘Fuerte 2 and 4’ fruit had lower skin chroma values with during the 2015 harvest season when compared with the 2014 harvest season. Furthermore, minimal variations were recorded for ‘Fuerte 4’ fruit with regard to ripening time, during the 2015 harvest season. Selection ‘H287’ fruit had the lowest skin chroma values of 6.65 and 8.26 recorded on day 6 during the 2014 harvest season and day 5 during the 2015 harvest season; respectively.

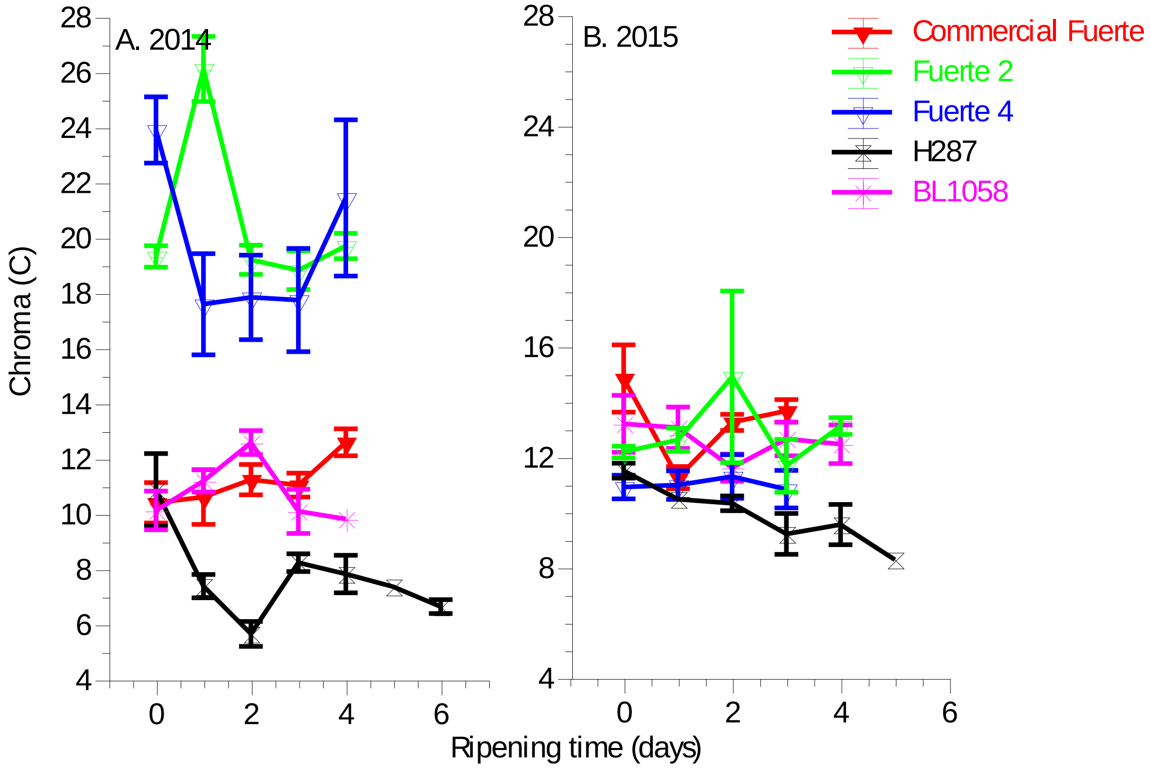

Figure 4.11 Chroma colour appearance parameter evaluated ‘Fuerte-type’ avocado fruit measured during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.1.7.3 Hue angle

An interaction between harvest season, selection and ripening time had no significant effect (P=0.536) on the skin hue angle of studied ‘Fuerte-type’ fruit during ripening (Appendix 10). However, skin hue angle (h°) values of the evaluated selections decreased at a noticeable slower rate (Figure 4.12). The lowest skin hue angle values were recorded for commercial ‘Fuerte’ (106.61) and ‘H287’ (106.73) fruit on day 3 and 5, when compared with the evaluated selections during the 2015 harvest seasons, respectively. Whereas, selection 'BL1058' fruit had the highest h° values of 128.57 and 134.89 recorded on day 0 during the 2014 and 2015 harvest seasons.

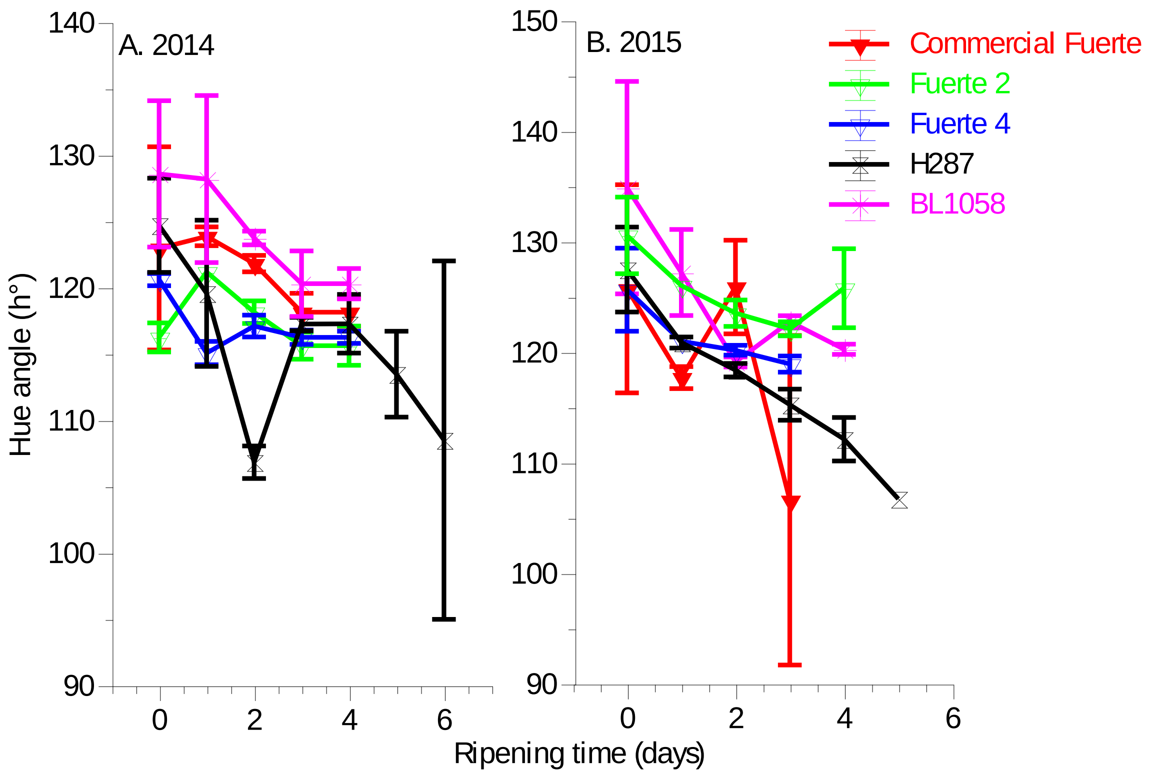

Figure 4.12 Hue angle colour appearance parameter evaluated 'Fuerte-type' avocado fruit measured during A. 2014 and B. 2015 harvest season. Vertical bars = SEM; n=8

4.2 DISCUSSION

4.2.1 Moisture content

Commercially, it is important to identify minimum fruit maturity index that ensures acceptable quality of an avocado fruit when ripe, but also allows early harvesting in order to obtain higher season prices (Abou-Aziz *et al.*, 2005). The Perishable Products Export Control Board (PPECB) along with the South African Avocado Growers' Association (SAAGA) have clearly defined the optimal fruit moisture content to assist growers and exporters in the correct application and interpretation of this maturity index (Eksteen, 2001). The maximum stipulated moisture content for ‘Fuerte’ is reported to be 80% (Eksteen, 1999).

In this study, avocado fruit were harvested below the stipulated harvest moisture content for ‘Fuerte’. Mans *et al*. (1995) reported similar results on ‘Fuerte’ avocado fruit, whereby, harvesting was done at a moisture content of 75% in the cooler production areas of KwaZulu-Natal and the fruit ripened to a desirable quality. Kruger *et al*. (2001) found 75-73% moisture content an appropriate maturity cut-off point for ‘Fuerte’ avocado fruit. Minimum harvest moisture content and cut-off point recommendations emanated from the above mentioned study and other studies by Bower *et al*. (2000); Penter and Stassen (2000) and Kruger *et al*. (2000) which were commissioned by SAAGA for based on cultivar, production area, harvest season and fruit mineral content. Therefore, moisture content of the evaluated ‘Fuerte-type’ avocado fruit were within acceptable range and had attained maturity stage when harvested. Early-season fruit with high water content were more susceptible to physiological disorders such as chilling injury (Kosiyachinda and Young, 1976) when compared with more mature fruit. High water content correlated with harvest maturity of the ‘Fuerte-type’ avocado fruit was thought to have an effect on the development of chilling injury symptoms.

4.2.2 External chilling injury

External chilling injury manifests as mesocarp discolouration and pitting after withdrawal from low storage temperature had developed on all ‘Fuerte-type’ avocado selections studied. This was in agreement with Van Rooyen (2005) and Woolf *et al*. (2002) who found that pitting, scalding and blackening were the most common external chilling injury symptoms in avocado fruit. Severity of chilling injury symptoms increased when the ‘Fuerte-type’ avocado fruit were held to ripen at ambient temperature. Eaks (1983) found that chilling injury symptoms severity increased when unripe ‘Hass’ avocado fruit were stored at 0 and 5°C for 4-6 weeks and ripened at ambient temperature. The degree of chilling injury incurred on avocado fruit has been found to heavily depend on the temperature which the fruit was exposed to and the duration of the exposure (Van Rooyen and Bower, 2006). In general, commodities exhibit increased chilling injury once removed from low storage temperature and the metabolic activity of the fruit increases (Crisosto *et al*., 2003).

4.2.3 Electrolyte leakage

Electrolyte leakage is inversely related to the integrity of the plasma membrane (Fergunson and Watkins, 1981). Previous studies have reported two independent causes of electrolyte leakage. The first was due to fruit ripening, whereby, electrolyte leakage increased to a maximum; and then decreased slightly. The second was an electrolyte leakage rise associated with the appearance of physiological disorders, either by senescence or chilling injury (McCollum and McDonald, 1991; Sarang *et al*., 2008).

In this study, increased electrolyte leakage was observed in all the evaluated 'Fuerte-type' avocado fruit tissue samples, indicating, the loss of plasma membranes integrity (Kays, 1991). Hershkovitz *et al*. (2009) found an increase in electrolyte leakage of 'Arad' and 'Ettinger' avocado fruit evaluated on various days during storage at 5°C and ripened at 20°C; and, which highly correlated with chilling injury symptoms. Similar results were reported by Dorria *et al*. (2010) on 'Fuerte' avocado fruit held at 20°C for 2 weeks, whereby, electrolyte leakage increased from 0.63 to 3.89 ml/mohs. Furthermore, Dorria *et al*. (2010) explained the increase in electrolyte leakage by an increase in the release of ions due to biochemical changes occurring during ripening. Electrolyte leakage recorded in the present study correlated with chilling injury; and thus, increased as a result of chilling injury manifestation when compared with the onset of ripening.

Montoya *et al*. (1994) defined an electrolyte leakage threshold value of 0.24S/m, whereby, avocado fruit could be transferred to non-injurious temperatures to ripen with minimal cold damage symptoms. In addition, Montoya *et al*. (1994) found that in

‘Hass’ avocado fruit stored at 4 and 6°C, evaluated on day 11, 19, 33 and 46 until transferal to 20°C, electrolyte leakage served as a good indicator of membrane permeability, and highly correlated with ethylene production, softening, and CI symptoms. Hershkovitz *et al*. (2005) reported that in ‘Hass’ avocado fruit the onset of a sharp rise in cellular electrolyte leakage was followed by a peak in ethylene production and softening, reflecting the beginning of the ripening process. In the present study, electrical conductivity of the ‘Fuerte-type’ avocado fruit seemed to reflect the biochemical changes occurring during low storage temperature. However, electrolyte leakage should have been recorded at various intervals during low storage temperature and shelf-life to note the threshold, and used it to identify the onset of ripening and quantify physico-chemical properties relationships during low storage temperature and ripening.

4.2.4 Electrolyte leakage and external chilling injury

Results in this study showed high correlation (R=0.84) between electrolyte leakage and chilling injury symptoms during the 2014 harvest season. On the contrary, a poor correlation (R=0.29) between electrolyte leakage and chilling injury was recorded during the 2015 harvest season. Low temperature exposure of commodities was considered to cause the cells in the skin to leak their contents, eventually the cellular activity ceases, shrink and then change colour (Platt-Aloia and Thomson, 1992). In this regard, electrolyte leakage is considered to serve as an indicator of membrane permeability and highly correlated with chilling injury symptoms (McCollum and McDonald, 1991). Overall, electrolyte leakage of the tissue did reflect the external chilling symptoms observed on the evaluated ‘Fuerte-type’ avocado fruit during both harvest seasons . According to Hershkovitz *et al*. (2009), increased ion mobility due to cell membrane damage is the source of rise in electrolyte leakage, resulting in increased occurrences of chilling injury. Montoya *et al.* (1994) reported high electrolyte leakage values on ‘Hass’ avocado fruit stored at 6°C, concomitantly with physiological disorders in the mesocarp discolouration manifestation. Increased electrolyte leakage might have result from breakdown in cellular compartmentation, thus mixing substrate and browning of enzymes such as polyphenoloxidase and peroxidase, initiating the browning reaction (Lyons, 1973).

This process explains the external chilling injury symptoms observed in the studied selections as a result of increased loss of membrane intergrity in relation to the electrolyte values recorded. Electrolyte leakage is thus a qualitative indicator of chilling injury symptoms (McCollum and McDonald, 1991).

4.2.5 Mass loss

As expected, evaluated ‘Fuerte-type’ avocado fruit had lost mass at withdrawal from low storage temperature. The rate of mass loss increased when fruit where held at ambient temperature during ripening. Similar results were reported by Abou-Aziz *et al*. (2005) on ‘Fuerte and ‘Hass’ avocado fruit, whereby, the rate of mass loss increased when fruit were transferred from 5 and 9°C storage temperatures to 20°C. Hardenburg *et al*. (1990) reported that mass loss increased rapidly with an increase in ripening temperature after withdrawal from low storage temperature. The transfer of ‘Fuerte-type’ avocado fruit from low storage temperature to ambient temperature affected the rate of mass loss.

Hardenburg *et al*. (1990) reported mass loss of 3 to 6% as enough to affect the quality of most fruit. Donkin and Cutting (1994); Bower and Magwaza (2004) found mass loss of 6 and 6-8% caused the development of chilling injury in avocado fruit stored at 2 and 5.5°C; respectively. This is a much greater mass loss than reported on the ‘Fuerte-type’ avocado fruit upon withdrawal from low storage temperature. In the current study, mass loss observed was assumed to not have affected the external quality of the evaluated fruit, and external chilling injury symptoms developed were not a consequent of mass loss during low storage temperature.

According to Lallu *et al*. (2003), post-harvest water loss, mass loss, had been found to significantly affect fruit ripening physiology. In fact, in avocado fruit high water loss could be expected to occur during the initial cooling of the fruit (Wills *et al*., 1998). Lallum *et al*. (2004) working on ‘Hass’ avocado fruit held at 20°C reported water loss during the initial stages of ripening affecting the rate of ripening and acts through the ethylene production pathway. Burdon *et al*. (2005) repeated Lallum *et al*. (2004) experiment over early, mid and late harvest season fruit and reported that the capacity of water loss during the initial stages of ripening accelerated fruit ripening in early season fruit only. Therefore, water loss during the initial stages of the evaluated 'Fuerte-type' avocado fruit of ripening might have accelerated ripening, and the effects of water loss were most likely acting through ethylene biosynthesis.

4.2.6 Respiration rate

Respiration of avocado fruit follows three characteristic climacteric stages *viz*. pre-climacteric minimum of increasing respiration, climacteric maximum of highest respiration and a post-climacteric stage synonymous with a decline in respiration (Kadam and Salunkhe, 1995). In the present study, the evaluated avocado selections followed a climacteric respiratory pattern, whereby carbon dioxide increased and reached a maximum; and thereafter, diminished until the end of shelf-life, during the 2014 and 2015 harvest seasons. Similar results were reported by Perez *et al*. (2004) on 'Hass' avocado fruit held at 20°C, where CO_2 increased and reached a maximum (176.17±15.98 mL CO_2 kg^{-1} h^{-1}) on the second day of shelf-life, thereafter decreased to 90.4±22.88 mL CO_2 kg^{-1} h^{-1} on the fourth day and was less than 10 mL CO_2 kg^{-1} h^{-1} at the end of shelf-life (day 8).

Magnitude of climacteric peak and day of occurrence varied amongst the evaluated 'Fuerte-type' avocado selections. In general, climacteric peak was detected on day 2 and 3 of shelf-life as previously reported by Donetti and Terry (2012) on 'Hass' avocado fruit held at 5-6 °C for 24-37 days and ripened at 18 or 23 °C. Interestingly, CO_2 production of commercial 'Fuerte' rapidly increased, and the climacteric peak was reached on day 1 of shelf-life (1087 and 1377 µmol CO_2 Kg^{-1} hr^{-1} during the 2014 and 2015 harvest seasons; respectively. High respiration rate based on CO_2 production was assumed to have affected the rate of mass loss and coincided with ethylene biosynthesis. Increase in respiration rate and ethylene biosynthesis is accompanied by complex biochemical changes including an increased cellulose activity resulting in fruit firmness degradation (Martinez-Romero *et al*., 2007).

4.2.7 Firmness

Fruit firmness is a good predictor of the differences in avocado ripening stage (Dopico *et al*., 1993). Firmness of all evaluated 'Fuerte-type' selections declined during low storage temperature. Furthermore, fruit showed a rapid decline in firmness when held at ambient temperature. Zauberman *et al*. (1977) reported that storage of 'Hass', 'Fuerte' and 'Naval' avocado fruit at 6 and 8°C reduced the metabolic activity of the fruit and inhibited ripening, and the fruit did not soften until they were transferred to a higher temperature. Villa- Rodriguez *et al*. (2011) found firmness of 'Hass' avocado fruit to decrease from 130.51 to 7.37 N at 15°C over a period of 12 days.

'Fuerte' avocado fruit have been reported to have a relatively thin skin (Donkin and Cutting, 1994). However, amongst the evaluated 'Fuerte-type' avocado fruit, selections 'H287' fruit were observed to have hard skin and retained high firmness during ripening. Vakis (1982) working on 'Ettinger', 'Fuerte' and 'Hass' avocado fruit reported that skin discolouration increased in some fruit following removal from low storage temperature, which appeared 'corky' in nature on ripe fruit and resulted in an increased hardening of the skin noted when fruit were cut (hard skin). This process might explain the hard skin observed on 'H287' fruit during ripening, however, the genetic composition of this newly developed selection could have constituted to the hard skin as it was observed during both harvest seasons.

4.2.8 Ripening percentage

In the present study, effect of increased temperature was evident when fruit were held at ambient temperature, ripening after 4-6 and 3-5 days during the 2014 and 2015 harvest seasons; respectively. This was expected due to the lower enzyme activity, respiration and ethylene production during low storage temperature (Brady, 1987). Zauberman *et al*. (1977) found that storage of 'Hass', 'Fuerte' and 'Naval' avocado fruit at 6 and 8°C reduced the metabolic activity and inhibited ripening; and fruit did not soften until transferred to a higher temperature. Storage of the 3 cultivars at 6 and 8°C did not cause any chilling injury for 6 weeks. Generally, at the recommended ripening temperature of 18-21°C, ripening period should be 4-7 days (Wills *et al*., 1989).

Early-season mature avocado fruit might take 10 to 12 days to ripen, whereas, mature late-season fruit might ripen within 5 to 6 days at 20°C (Bower and Cutting., 1988). Evaluated 'Fuerte-type' selections ripened at a period shorter than that stipulated for early season mature avocado fruit. Zauberman *et al*. (1977) reported that shelf-life at 25°C after storage at 6 and 8°C was shorter than for fruit without previous low storage temperature exposure. Therefore, water loss recorded on the 'Fuerte-type' avocado fruit after storage was suggested to have affected the shelf-life of the fruit and low storage temperature advanced the ripening process.

Temperature quotient (Q_{10}) reported for most fruit and vegetables vary at 25±2/3°C temperature range (Labuza and Breene, 1989). Common storage temperatures for avocado fruit are 7 and 25°C, and, using the reported Q_{10} value, the predicted shelf-life of avocado at these temperatures were 29.8 and 4.8 days, respectively. Perez *et al*. (2004) found that shelf-life of fruit harvested from different orchards and seasons were predicted with 20% underestimation. Although the evaluated 'Fuerte-type' avocado fruit shelf-life was in agreement with the predicted shelf-life of avocado fruit, no attempts to calculate the Q_{10} of the fruit was made in the present study. Therefore, Q_{10} calculation should have been considered in order to accurately predict shelf-life.

4.2.9 Peel colour

In this study, colour evaluations were affected by the severe external chilling injury symptoms developed during storage at low temperature (Figure 4.3). Black spots developed during low storage temperature and manifested during shelf-life. Woolf (1997) showed that after withdrawal from low storage temperature (5.5°C) of up to

28 days, ‘Hass’ avocado fruit appeared black/green as a result of external chilling injury symptoms. The fruit developed a brown/black appearance, rather than purple/black of normal ripening. Meanwhile, Vakis (1982) explained variations on ‘Hass’ avocado colour development by an increase in skin discolouration which developed as a result of cold damage following removal from low storage temperature, and affected colour parameters during ripening.

Peel colour was subjectively evaluated based on the lightness (L value), green/red (a value), blue/yellow components (b value). Recorded green/red (a value), blue/yellow (b value) were used to calculate chroma (C value) and hue angle (h° value). The lightness (L value) of the evaluated ‘Fuerte-type’ fruit decreased during ripening (Figure 4.10). The lightness (L value) is a measure of the colour in the light-dark axis; and therefore, the decreased L value indicated that the fruit peel was turning less bright. Chroma and hue angle values decreased at a moderate and slower rate; respectively. The decrease in colour parameters (lightness (L), chroma (C), and hue angle (h°) at various rates was reported on ‘Hass’ avocado fruit ripened at 15-25°C (Cox *et al*., 2004) and ‘Fuerte’ avocado fruit ripened at 20°C (Dorria *et al*., 2010).

Commercial ‘Fuerte’, ‘H287’ and ‘BL1058’ had lower chroma values when compared with ‘Fuerte 2 and 4’ fruit during the 2014 harvest season. McGuire (1992) on ‘Hass’ avocado fruit held at 20 °C reported that a more negative a* value showed a more predominant greenness in avocado fruit peel. This could imply that commercial ‘Fuerte’, ‘H287’ and ‘BL1058’ fruit retained a greener colour when compared with ‘Fuerte 2 and 4’ during ripening.

CHAPTER 5
SUMMARY, FUTURE RESEARCH AND CONCLUSIONS

5.1 Summary

The studied ‘Fuerte-type’ avocado selections showed significantly different ripening physico-chemical properties in response to harvesting seasons and ripening days. All the evaluated selections were highly susceptible to external chilling injury, and external chilling injury correlated with electrolyte leakage, which was used as a measure of membrane integrity. Peel colour evaluations were affected by the black spots that developed as a result of severe external chilling injury. Rate of mass loss, respiration rate and firmness degradation were accelerated when fruit were held at ambient temperature, irrespective of selection. Respiration rate and firmness degradation were the main indicators of avocado fruit ripening stage when compared with other recorded physico-chemical properties.

5.2 Recommended future research

This study was part of the big project of selecting the best ‘Fuerte-type’ avocado selections for registration and commercialisation; thus, the following were identified as outstanding research areas:

- Planting and evaluating the selections in different production regions as part of Phase III of the project.
- Physico-chemical properties evaluations at various intervals during low storage temperature to determine response of these new selections to low temperature.
- Application of post-harvest techniques to alleviate external chilling injury such as pre-conditioning, heat treatments, intermittent warming, controlled atmosphere storage.
- Effect of season, production conditions and maturity level seems necessary in order to determine the cause of the severe external chilling injury recorded.
- Physiological analysis of sugars associated with avocado fruit ability to resist cold stress and other bioactive compounds with antioxidant capacities which play a pivotal role in avocado quality.

5.3 Conclusions

Evaluated selections including the commercial ‘Fuerte’ fruit had poor storage qualities. Overall, results were preliminary; however, provided initial characterisation of the newly developed ‘Fuerte-type’ avocado fruit’s storage potential. Phase III of the project entailing planting and evaluating the selections in different production regions should commence.

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APPENDICES

Appendix 1: ANOVA table for the influence of seasons on the moisture content as maturity index of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	2.867	1.433	0.20	
Replication.*Units* stratum					
Selections	4	114.667	28.667	3.93	0.018
Seasons	1	28.033	28.033	3.85	0.065
Selections x Seasons	4	17.467	4.367	0.60	0.668
Residual	18	131.133	7.285		
Total	29	294.167			

Appendix 2: ANOVA table for the influence of seasons on chilling injury symptoms developed on 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	90.42	45.21	0.48	
Replication*Units* stratum					
Selections	4	8366.67	2091.67	22.24	<.001
Seasons	1	2755.21	2755.21	29.29	<.001
Selections x Seasons	4	10533.33	2633.33	28.00	<.001
Residual	18	1692.92	94.05		
Total	29	23438.54			

Appendix 3: ANOVA table for the influence of seasons on electrolyte leakage of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	165.0	82.5	0.71	
Replication.*Units* stratum					
Selections	4	2537.5	634.4	5.47	0.005
Seasons	1	786.1	786.1	6.78	0.018
Selections x Seasons	4	4787.8	1196.9	10.33	<.001
Residual	18	2086.4	115.9		
Total 29 10362.8					

Appendix 4: ANOVA table for the influence of seasons and ripening day on mass loss changes of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		300.7	150.3	1.27	
Replication*Units* stratum						
Selections	4		356.9	89.2	0.75	0.558
Seasons	1		5084.7	5084.7	42.96	<.001
Ripening days	6		22729.5	3788.2	32.00	<.001
Selections x Seasons	4		482.6	120.7	1.02	0.401
Selections x Ripening days	16	(8)	1211.0	75.7	0.64	0.845
Seasons x Ripening days	5	(1)	20907.0	4181.4	35.32	<.001
Selections x Seasons x Ripening days						
	14	(10)	635.8	45.4	0.38	0.977
Residual	100	(38)	11837.2	118.4		
Total	152	(57)	32003.7			

Appendix 5: ANOVA table for the influence of seasons and ripening day on respiration changes of ‘Fuerte-type’ avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		179332	89666	0.48	
Replication.*Units* stratum						
Selection	4		7187224	1796806	9.69	<.001
Season	1		1832170	1832170	9.88	0.002
Ripening days	6		13926903	2321151	12.52	<.001
Selection x Seasons	4		4707664	1176916	6.35	<.001
Selections x Ripening days	16	(8)	9457773	591111	3.19	<.001
Seasons x Ripening days	5	(1)	1139414	227883	1.23	0.301
Selections x Seasons x Ripening days						
	14	(10)	5157997	368428	1.99	0.026
Residual	100	(38)	18545992	185460		
Total	152	(57)	55744535			

Appendix 6: ANOVA table for the influence of seasons and ripening day on firmness changes of ‘Fuerte-type’ avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		57.58	28.79	1.10	
Replication.*Units* stratum						
Selections	4		4836.23	1209.06	46.10	<.001
Seasons	1		1182.40	1182.40	45.09	<.001
Ripening days	6		10490.15	1748.36	66.67	<.001
Selections x Seasons	4		763.29	190.82	7.28	<.001
Selections x Ripening days	16	(8)	1753.23	109.58	4.18	<.001
Seasons x Ripening days	5	(1)	1534.63	306.93	11.70	<.001
Selections x Seasons x Ripening days						
	14	(10)	537.41	38.39	1.46	0.139
Residual	100	(38)	2622.55	26.23		
Total	152	(57)	19619.44			

Appendix 7: ANOVA table for the influence of seasons and ripening day on ripening of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		169.2	84.6	0.61	
Replication*Units* stratum						
Selections	4		16140.3	4035.1	28.91	<.001
Seasons	1		5599.5	5599.5	40.11	<.001
Ripening days	6		113825.1	18970.8	135.90	<.001
Selections x Seasons	4		6061.8	1515.5	10.86	<.001
Selections x Ripening days	16	(8)	16216.8	1013.6	7.26	<.001
Seasons x Ripening days	6		11689.3	1948.2	13.96	<.001
Selections x Seasons x Ripening days						
	13	(11)	7155.9	550.5	3.94	<.001
Residual	100	(38)	13959.0	139.6		
Total	152	(57)	153327.9			

Appendix 8: ANOVA table for the influence of seasons and ripening day on lightness colour changes of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		0.886	0.443	0.07	
Replication.*Units* stratum						
Selections	4		347.063	86.766	13.83	<.001
Seasons	1		4.742	4.742	0.76	0.387
Ripening days	6		165.969	27.661	4.41	<.001
Selections x Seasons	4		11.843	2.961	0.47	0.756
Selections x Ripening days	16	(8)	127.262	7.954	1.27	0.233
Seasons x Ripening days	5	(1)	415.987	83.197	13.26	<.001
Selections x Seasons x Ripening days						
	14	(10)	66.583	4.756	0.76	0.711
Residual	100	(38)	627.364	6.274		
Total	152	(57)	1346.739			

Appendix 9: ANOVA table for the influence of seasons and ripening day on chroma colour changes of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		88.48	44.24	0.74	
Replication.*Units* stratum						
Selections	4		1482.01	370.50	6.21	<.001
Seasons	1		43.38	43.38	0.73	0.396
Ripening days	6		312.10	52.02	0.87	0.518
Selections x Seasons	4		2255.29	563.82	9.46	<.001
Selections x Ripening days	16	(8)	1427.25	89.20	1.50	0.116
Seasons x Ripening days	5	(1)	323.81	64.76	1.09	0.373
Selections x Seasons x Ripening days						
	14	(10)	907.47	64.82	1.09	0.378
Residual	100	(38)	5961.71	59.62		
Total	152	(57)	11883.83			

Appendix 10: ANOVA table for the influence of seasons and ripening day on hue angle colour changes of 'Fuerte-type' avocado fruit during 2014 and 2015

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2		169.21	84.60	1.80	
Replication.*Units* stratum						
Selections	4		941.98	235.49	5.01	0.001
Seasons	1		261.56	261.56	5.56	0.020
Ripening days	6		12886.40	2147.73	45.68	<.001
Selections x Seasons	4		587.44	146.86	3.12	0.018
Selections x Ripening days	16	(8)	1078.87	67.43	1.43	0.141
Seasons x Ripening days	5	(1)	271.84	54.37	1.16	0.336
Selections x Seasons x Ripening days						
	14	(10)	608.11	43.44	0.92	0.536
Residual	100	(38)	4702.20	47.02		
Total	152	(57)	11728.72			

Appendix 11: Papers presented at local, national and international conferences as part of this research project

a) **Oral presentation** - M Munzhedzi, N Mathaba, TP Mafeo and J Mlimi, "Ripening physcochemical properties of new 'Fuerte-type' avocado selections, *5th University of Limpopo Faculty of Science and Agriculture Research Day*, 3-4 October 2015, Polokwane, Limpopo, South Africa**.**

b) **Poster presentation** - M Munzhedzi, TP Mafeo, MR Masevhe, N Mathaba, J Mlimi and MJ Ntandane, "Evaluation of post-harvest storage temperature (5.5°c) and shelf-life of newly developed 'Fuerte-type' avocado selections 'ITSC selection', 'Calshad', 'BL1058' and 'Wurtz'", *Combined congress*, 19-22 January 2015, George, Western Cape, South Africa.

c) **Oral presentation** - T.P. Mafeo, M Munzhedzi, M.R. Masevhe, N Mathaba, J Mlimi and M.J. Ntandane, "Responses of newly developed South African 'Fuerte-type' avocado selections to post-harvest storage temperature and shelf-life", *VIII World Avocado Congress* 13-18 September 2015, Lima, Peru**.**