

EVALUATION OF COLD STORAGE POTENTIAL AND SHELF-LIFE OF NEW  
'HASS' TYPE AVOCADO SELECTIONS

BY

MACHIPYANE PHELADI BRIDGETTE

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SUPERVISOR: DR N MATHABA (ARC-ITSC)

CO-SUPERVISOR: PROF TP MAFEO (UL)

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## DECLARATION

I, Pheladi Bridgette Machipyane, 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 other University; that it is my work in design and execution, and that all material contained herein has been duly acknowledged.

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Student: Machipyane PB

.....

Date

.....

Supervisor: Dr N Mathaba

.....

Date

.....

Co-supervisor: Prof TP Mafeo

.....

Date

## **DEDICATION**

I would like to dedicate this paper to my grandmother, Ramaisela Elizabeth Phasha for her undying love, encouragement and dedication throughout the course of my study.

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## ABSTRACT

The current South African 'Hass' type avocado cultivars are inadequate to enhance competitiveness, cultivar diversity and profitability. In an effort to ensure competitiveness and maintain sustainability, the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) as one the South African Avocado Industry's (SAAI) main stakeholder, has bred and selected new superior 'Hass' type avocado selections. However, the cold storage potential and associated physico-chemical ripening properties of these selections ('Jalna', 'OA 184' and 'Balboa') have not been documented. Therefore, the objective of this study was to evaluate the effect of cold storage on internal and external physico-chemical ripening variables of the new 'Hass' type avocado selections. New 'Hass' type avocado fruit maturity was evaluated using moisture content, thereafter, harvested, sorted, graded and stored under two temperature regimes (2.0°C and 5.5°C) for 28 days to simulate export conditions. The experiment was a completely randomised factorial design with three treatment factors; temperature regimes (2.0°C and 5.5°C), days to ripening and 'Hass' type avocado selections fruit and control (commercial 'Hass') replicated three times. After withdrawal from cold storage, fruit were ripened at ambient temperature and evaluated for electrical conductivity, external chilling injury, fruit water loss, skin colour change, ripening percentage, firmness, respiration rate and seed:fruit weight ratio. Results indicated that treatment factors had no significant effect on moisture content ( $P=0.733$ ) and chilling injury ( $P=0.776$ ). Treatment factors had a significant effect on electrical conductivity ( $P=0.004$ ), skin colour parameters; eye colour ( $P<0.001$ ), hue angle ( $P<0.001$ ), lightness ( $P=0.011$ ) and chroma ( $P=0.042$ ). Selection 'Jalna' fruit started changing colour whilst in storage (2.0°C and 5.5°C). Furthermore, 'Hass' type avocado selection fruit followed a declining pattern for lightness, chroma and hue angle in agreement with commercial 'Hass'. Moreover, results indicated that treatment factors had a significant effect ( $P<0.001$ ) on respiration rate, fruit firmness, ripening percentage and seed:fruit weight ratio. Selection 'Jalna' and 'OA 184' desynchronised mesocarp softening with exocarp due to genetically non-softening exocarp. Treatment factors had no significant effect ( $P=0.998$ ) on fruit water loss during ripening. Selection 'OA 184' fruit showed export potential due to its good storage, ripening physico-chemical and shelf-life properties. Studies on cold sterilisation would add more value on generated scientific

information, as such would enable the SAAI to gain access to high paying export markets. In addition, the selections should be planted and evaluated in other avocado producing regions.

**Keywords:** Days to ripening; electrical conductivity; firmness; fruit water loss; physico-chemical variables; respiration rate; ripening percentage; seed:fruit weight ratio; skin colour change.

# CHAPTER 1 GENERAL INTRODUCTION

## 1.1 Background

The South African Avocado Industry (SAAI) has a reputation of producing good quality avocado fruit (Ramosodi, 2006). Currently, the industry is dominated by production and export of two main cultivars, 'Hass' and 'Fuerte' (DAFF, 2014). The existing South African 'Hass' type avocado cultivars are inadequate to enhance profitability and cultivar diversity for the avocado industry (Ramosodi, 2006). Therefore, the evaluation of new 'Hass' type avocado selections, with emphasis on improving commodity quality, earlier maturation, increased hardiness, extended storage and shelf-life remains the important strategic focus for SAAI (Vorster, 2004; Sippel *et al.*, 1994). As a result, the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) has developed new 'Hass' type avocado selections: 'Jalna', 'OA184' and 'Balboa' in an effort to expand cultivar diversity and consumer choice. Nonetheless, cold storage potential of these selections is not documented, therefore, should be assessed prior to registration and release. Thus, this study proposed to evaluate the response of these new 'Hass' type avocado selections to low temperature (2.0 and 5.5°C) and associated ripening physico-chemical properties.

## 1.2 Problem statement

The SAAI is predominantly export-orientated (Ramosodi, 2006) and earns revenue of up to 29% gross value per year (DAFF, 2014). The industry has traditional major importing countries. However, Japan and the USA have been the new potentially and highly profitable markets with stringent importing regulations (Vorster and Donkin, 2009). The stringent importing regulations set by these markets, pose challenges for the SAAI. Challenges faced by the SAAI are competition from other Southern hemisphere exporting countries, which have the same production season as South Africa. Therefore, in order to ensure competitiveness and maintain sustainability, the industry must continue selecting and breeding new superior selections (Van Rooyen, 2011). In response to the identified challenge, the ARC-ITSC has bred and selected new 'Hass type' avocado selections. As part of the evaluation process, information about the new 'Hass type' avocado selections

response to low temperature (2.0 and 5.5°C) and associated ripening physico-chemical properties need to be documented.

### 1.3 Motivation of the study

There is a need for continued development of more competitive new 'Hass' type avocado cultivars with respect to the following characteristics: attractive skin colour, early maturity, longer storability, less physiological and pathological disorders (Bijzet *et al.*, 1993). In avocado fruit, these characteristics are maintained by cold storage. However, cold storage might result in the development of various physiological and pathological disorders such as: anthracnose, chilling injury, stem end rot and vascular staining (Mhlophe and Kruger, 2012). The biochemical, internal and external quality characteristics of the new 'Hass' type avocado selections in response to cold storage is not yet documented. Hence, this study was undertaken to evaluate the response of these new 'Hass' type avocado selections to cold storage. Understanding the postharvest quality variables of these new 'Hass' type avocado in response to low temperature would enable full registration for export, and therefore, increase competitiveness, profitability and cultivar diversity of the industry.

### 1.4 Aim and objectives

#### 1.4.1 Aim

The aim of this study was to evaluate the response of newly developed 'Hass' type avocado selections to low storage temperature and associated ripening physico-chemical properties.

#### 1.4.2 Objectives of the study were to:

- i) Evaluate the internal and external fruit quality variables of the new 'Hass' type avocado selections in response to low storage temperature.
- ii) Evaluate the effect of cold storage on fruit ripening and shelf-life of the new 'Hass' type avocado selections.

### 1.5 Hypotheses

- i) The internal and external quality variables of the new 'Hass' type avocado selection fruit would not be affected by cold storage.

ii) Fruit ripening and shelf-life of the new 'Hass' type avocado selections would be affected by cold storage.

## CHAPTER 2 LITERATURE REVIEW

The major avocado cultivars in the South African value chain include: 'Hass', 'Fuerte', Ryan and Pinkerton. In South Africa, 'Hass' type avocado cultivars have over time seen a significant expansion comprising 33% of production, compared to 42% of 'Fuerte' production (DAFF, 2014). The existing 'Hass' type avocado cultivars are inadequate to enhance profitability, competitiveness and cultivar diversity in the avocado industry (Ramosodi, 2006). Thus, the ensuing review of literature focused on what has already been documented on the identified research problem, existing gaps and explanation on how the existing gaps were addressed.

### 2.1 Existing work done on the research

#### 2.1.1 South African avocado production trends

The South African subtropical fruit industry considers avocado as one of the most important commodity with up to 29% gross revenue annually (Bulagi *et al.*, 2015). The South African Avocado Industry (SAAI) produces up to 50 742 metric tonnes per annum with most of the production in the Northern parts of the country (DAFF, 2014). Limpopo is the leading province with 61% of total production, followed by Mpumalanga (30%), Kwa-Zulu Natal (8%) and Eastern Cape (1%) (DAFF, 2014). South Africa has almost a year round supply of avocado, which runs from February until November due to cultivar diversity and subtropical climatic conditions (Vorster, 2001). Avocado fruit are mainly supplied to the international markets.

The European Union (EU) is the major SAAI market, importing 40-45% of total production (Vorster, 2001). However, the industry continues to face competition from other avocado producing countries (Kremer-Kohne and Kohne, 1998). The South African avocado industry's main competitors in the Eurozone include Kenya, Spain, Mexico, Chile, Israel, Peru, Brazil and Venezuela (Dodd *et al.*, 2007; Vorster and Donkin, 2009). However, the SAAI has a reputation of producing good quality avocado fruit and export has increased (Ramosodi, 2006).

The major 'Hass' type avocado fruit produced in South Africa include 'Lamb Hass' and 'Maluma Hass' which are export cultivars, earning exponentially growing revenue. 'Maluma Hass' is more favourable in the United Kingdom (UK) markets,

earning significantly more export income compared to commercial 'Hass' (Ernst, 2007). Volumes of up to 800 tonnes of 'Maluma Hass' are currently exported to the European Union, with a potential to grow due to more plantings (Snijder, 2015). 'Lamb Hass' is an export cultivar with oversized fruit, however, export markets prefer small sized fruit.

### 2.1.2 South African 'Hass' type avocado breeding program

To ensure competitiveness in the export market, breeding and testing of new 'Hass' type avocado selections has long been identified as an important aspect for sustainability in the SAAI (Van Rooyen, 2011). The Agricultural Research Council, Westfalia Estate and Allesbeste nursery have been key players in the SAAI, selecting, breeding and releasing new 'Hass' type avocado selections.

The current study is part of the phase II evaluation process at the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) before the release of new material to the avocado industry (phase III and IV). The study selected scion and rootstock combinations less susceptible to alternate bearing, root disease, sun blotch viroid and exacerbated growth vigour (Sippel *et al.*, 1994). The study aimed to produce selections with higher yield, reduced physiological and pathological disorders, good storage and shelf-life potential (Bijzet *et al.*, 1993). While Allesbeste nursery released 'Maluma Hass' as an export cultivar (Ernst, 2007) which has been cultivated for 8 years, attributable to good storage potential and yield (Snijder, 2015). In addition, 'Lamb Hass' was released by the Westfalia Estate to extend the 'Hass' type avocado season (Kremer-Kohne and Kohne, 2001). Recently, 'Carmen Hass' and 'Gem Hass' cultivars were released by the Westfalia Estate with good yield and post-harvest quality properties (Van Rooyen, 2011). Kremer-Kohne and Mokgalabone (2003) also reported on a new 'Hass'-like cultivar 'Nobel', which matured simultaneously with commercial 'Hass', however, evaluation was discontinued due to large fruit size and low yields.

### 2.1.3 Avocado harvest index

Moisture content is used as a maturity index together with oil content, whereby, it is expected to decrease and oil content increases with avocado fruit maturity (Kruger *et al.*, 1995; Magwaza and Tesfay, 2015). A moisture content above 80% leads to



rubbery, shrivelled and watery avocado fruit, with the possibility of off-flavour development and reduced storage and shelf-life (Lee *et al.*, 1983; Mans *et al.*, 1995). However, avocado fruit with a low moisture content have a shorter shelf-life and require less time to ripen (Carvalho *et al.*, 2014). Therefore, moisture content is used to determine physiological maturity for exporting at different temperature regimes for 'Hass' type avocado fruit (Mans *et al.*, 1995). A previous study by Nelson *et al.*, (2000), reflected on immature and larger fruit being more vulnerable to external chilling injury. Over-mature fruit are susceptible to postharvest disorders such as grey pulp (Kruger *et al.*, 2004), whereas, immature fruit could either have uneven ripening or not ripen at all and as such, could be aggravated by extended cold storage (Pak *et al.*, 2003). The SAAI evaluates moisture content using an oven drying method, which is widely used and relatively cheap (Kruger *et al.*, 1995). Furthermore, oven drying has been used in various fruit, as a rapid and effective method, which uniformly dehydrates the fruit sample (Lee *et al.*, 1983). Moisture content varies with cultivars at harvest; 'Hass' (77.0%), 'Lamb Hass' (73.0%) and 'Maluma Hass' (78.0%) (Kassim *et al.*, 2013).

#### 2.1.4 Use of low storage temperature for avocado fruit

Low storage temperature has a beneficial effect on the maintenance of internal and external quality of avocado fruit (Bower and Magwaza, 2004). According to Blakey *et al.*, (2011), low storage temperature reduces respiration, ripening and preserves energy reserves. It enhances the storage-life of 'Hass' type avocado fruit by preserving fruit quality parameters such as colour, texture and flavour (Ferero, 2007). Moreover, storage temperature has the ability to lower respiration rate without inducing chilling injury (Blakey *et al.*, 2011).

Cold storage of 'Hass' avocado fruit at 1.0°C led to reduced enzyme activity and fruit softening, whereas, storage at 5.5°C resulted in increased fruit softening and enzyme activity (Kok *et al.*, 2013; Bower and Magwaza, 2004). However, prolonged storage at 1.0°C exacerbated chilling injury (Kok *et al.*, 2013). Storage of 'Hass' avocado fruit at 1.0°C suppressed the effects of cold chain breaks as it significantly improved fruit quality (Kok *et al.*, 2010). Furthermore, storage at 2.0°C had a positive effect on the preservation of 'Hass' avocado internal quality, while, storage at 5.5 and 8.0°C deteriorated fruit internal quality (Bower and Magwaza, 2004).

## 2.1.5 Physico-chemical variables in avocado fruit

### **Electrical conductivity**

Electrical conductivity indicates intracellular ion leakage and cell membrane integrity (Ahmed *et al.*, 2010; Tesfay, 2009). Therefore, reduced electrical conductivity might be associated with low membrane permeability and delayed avocado fruit ripening (Woolf *et al.*, 2003). 'Hass' avocado cell plasma membranes destabilise when exposed to prolonged cold storage, leading to high electrical conductivity (Feng *et al.*, 2005). Electrical conductivity also evaluates biochemical browning mechanisms (vascular and mesocarp browning), which prevail in an oxygenated environment due to the combined effect of polyphenol oxidase and peroxidase (Montoya *et al.*, 1994b; Pathirana *et al.*, 2011). Additionally, high electrical conductivity during cold storage could lead to polyphenol oxidase activity, thereby enhancing cellular reservoir permeability, which is evident as mesocarp browning (HersHKovitz *et al.*, 2005; Van Rooyen and Bower, 2002). Furthermore, electrical conductivity reflects on the biochemical fluctuations during the ripening process and is expected to increase with chilling injury under low storage temperature in numerous fruit (Montoya *et al.*, 1994a). Contrarily, in 'Hass' avocado fruit, electrical conductivity did not indicate chilling injury but rather a change in cell permeability (Fuchs *et al.*, 1989).

### **Chilling injury**

Chilling injury is an irreversible physiological damage to plant cells and tissue due to low temperature stress (Kok *et al.*, 2010) and could prevail during short-term storage under poor storage conditions (Woolf *et al.*, 2005). In avocado fruit, chilling injury might manifest as pitting, pulp browning, development of off-flavour and vascular strands (Bill *et al.*, 2014). Additionally, external chilling injury manifests when 'Hass' avocado fruit are stored at low temperatures and is evident through internal degradation of cellular components (Woolf *et al.*, 2003). Furthermore, external chilling injury in 'Hass' type avocado fruit might lead to mesocarp darkening which manifests as small black, sunken lesions on the exocarp during prolonged storage at low temperature (Bower and Bertling, 2008). Moreover, chilling injury results in the interruption of fluidity and membrane lipid order, negatively impacting on the semi-permeable functionality of 'Hass' avocado cells (Vorster *et al.*, 1987). Chilling injury could result in the inability of 'Hass' avocado fruit to synthesise ethylene, leading to

uneven ripening (Eaks, 1983). Chilling injury is associated with water loss exacerbated by prolonged cold storage, enhancing fruit vulnerability to chilling damage (Woolf *et al.*, 2003; Kok *et al.*, 2011).

In 'Hass' type avocado fruit stored at 0 and 2.0°C, chilling injury manifested as black skin lesions and discolouration (Hopkirk *et al.*, 2010). Therefore, storage at such low temperatures might be ruled out, even though it results in decelerated ripening (Woolf *et al.*, 2005). However, 'Hass' avocado fruit change colour from green to purple/black during ripening, masking chilling injury, whereas it could be isolated as brown corky skin tissue (Yahia and Woolf, 2011). In 'Hass' avocado fruit, internal chilling injury manifests as greyish-brown flesh discolouration and could be detected 3-4 weeks after storage (Arpaia, 2005). 'Lamb Hass' avocado fruit should be stored at 7.0°C during late harvest to obviate chilling injury, as storage at 2.0°C results in diffuse discolouration (Dixon *et al.*, 2010).

### **Skin colour**

Skin colour is a key quality variable used to determine consumer product acceptance (Ahmed *et al.*, 2010). Colour could be measured using either subjective or objective colour parameters. Subjective colour change could be evaluated using eye colour rating by a qualified panel (White *et al.*, 2009). Qualified panelists use a rating scale from 1-5, which indicates the transit of 'Hass' avocado fruit skin colour from green to black, whereby, level 1 indicates emerald green, 2: forest green, 3: olive green, 4: purple and 5: black (Cox *et al.*, 2004). Objective colour parameters include: Lightness (L), Chroma (C) and Hue angle ( $h^\circ$ ) and can be evaluated using a chromameter. Lightness (L) indicates the lightness or brightness (Kassim *et al.*, 2013), hue angle ( $h^\circ$ ) evaluates the actual colour of fruit and chroma (C) measures colour intensity (Woolf *et al.*, 1999). During ripening lightness, chroma and hue angle decrease, concomitantly with eye colour rating increase as 'Hass' avocado fruit transit from green to purple/black (Villa-Rodriguez *et al.*, 2011). 'Hass' avocado skin colour change is also influenced by pigment differentiation.

Fruit pigments associated with colour change include: anthocyanin, chlorophylls and carotenoids (Lancaster and Lister, 1997; Ashton *et al.*, 2006). In 'Hass' type avocado fruit, skin colour change is attributed to a decrease in chlorophyll content and an increase in anthocyanins, mainly cyanidin 3-O-glucoside, during ripening (Villa-

Rodriguez *et al.*, 2011; Ernst, 2012). Storage temperature also influences skin colour changes in 'Hass' type avocado fruit.

'Hass' avocado fruit stored at 5.5°C led to enhanced colour changes (Maftoonazad and Ramaswamy, 2008). However, 'Lamb Hass' avocado fruit stored at 5.5°C did not synchronise colour change with fruit softening and ripening (Kremer-Kohne, 2000; Nelson *et al.*, 2001).

### **Respiration rate and ethylene production**

In avocado fruit, respiration resumes after harvest, whereby high CO<sub>2</sub> is produced and O<sub>2</sub> expended, resulting in the production of water and heat (Arpaia, 2005). Avocado fruit are climacteric and characterised by a high respiration rate and ethylene production during ripening (Milne, 1998). Furthermore, the fluctuation in ethylene production could be used to predict avocado ripening (Montoya *et al.*, 1994a). During ripening, avocado fruit's respiratory pattern should follow three ripening climacteric stages known as the: pre-climacteric stage (least respiration rate), climacteric stage (highest respiration rate) and post climacteric stage (reduced respiration rate) (Kassim *et al.*, 2013). According to Milne (1998), 'Hass' avocado fruit respiratory climacteric rise coincides with intense ethylene production and complex physiological and biochemical changes. 'Hass' type avocado fruit respiration rate is two folds that of a banana, and seven times that of an apple; and therefore, have a limited shelf-life (Ginsberg, 1985). During ripening, 'Hass' avocado fruit respire and produce a significant amount of water, leading to increased water loss (Blakey *et al.*, 2009; Workneh and Osthoff, 2010).

### **Firmness**

Firmness indicates resistance to penetration, ripening rate and is expected to decrease during 'Hass' avocado ripening (Kassim, 2012). Firmness reduction can be quantified using a densimeter at various ripening stages (Paull, 1999). Avocado fruit are eating ripe when the densimeter reading is at 65 and below, whereas 66-100 indicate unripe fruit (Kremer-Kohne and Kohne, 1998; Nelson *et al.*, 2000). In addition, firmness at which 'Hass' avocado fruit is considered eating ripe remains an important quality assessment, as rots and internal disorders occur during ripening (White *et al.*, 2009). Storage period and temperature also affect fruit firmness.

Storage time and temperature have an influence on 'Hass' avocado softening, ripening and is essential for shelf-life regulation (Blakey and Bower, 2009). Low storage temperature plays a significant role in retaining 'Hass' avocado fruit firmness (Mizrach *et al.*, 2000). 'Hass' avocado fruit stored at 2.0 and 4.0°C retained firmness and colour whereas, there was exacerbated skin colour changes and fruit softening after storage at 5.0, 6.0 and 8.0°C (Pinheiro *et al.*, 2009). Moreover, 'Hass' avocado fruit firmness could be maintained for 2-3 weeks when stored at 5.0°C (Abou-Aziz *et al.*, 2005). In addition, 'Lamb Hass' avocado fruit remained firm for 2 weeks when stored at 5.0°C due to reduced metabolic activities (Arpaia, 2005).

### **Fruit water loss**

Fruit water loss is an important factor in determining avocado shelf-life and quality (Yahia and Woolf, 2011). In avocado fruit, water loss is mainly due to temperature, respiration rate and postharvest handling effects (Dixon *et al.*, 2004). According to Perez *et al.*, (2004), 'Hass' avocado fruit water loss of 3-6% could lead to enhanced quality deterioration. Furthermore, water loss enhances polyphenol oxidase activity in 'Hass' avocado fruit, resulting in the presence and prevalence of physiological and pathological disorders (chilling injury, stem-end rot, vascular and cellular browning) (Woolf *et al.*, 2003; Kok *et al.*, 2013). Fruit water loss in avocado can be influenced by storage temperature, leading to physiological and biochemical changes.

'Hass' avocado fruit water loss was reduced during storage at 2.0°C, resulting in prolonged shelf-life, when compared to 5.5°C (Dixon *et al.*, 2004). There was increased water loss and reduced shelf-life in 'Hass' avocado fruit stored at 5.5 and 8.0°C, due to enhanced metabolic activities (Bower and Jackson, 2003; Lütge *et al.*, 2010).

### **2.2 Existing gaps and explanation on how these gaps were closed**

The current 'Hass' type avocado cultivars are inadequate for the export market, mainly due to reduced internal and external fruit quality during extended shipping periods. The response of new 'Hass' type avocado selection to cold storage temperature is not documented. Therefore, there is a need to investigate quality parameters of new 'Hass' type avocado selections in response to cold storage and associated physico-chemical ripening properties. The success of this study would

provide data for cold storage temperature requirements and physico-chemical ripening properties of the new 'Hass' type avocado selections for export, leading to their registration. Once registered, there would be cultivar diversity and enhanced consumer choices, resulting in improved competitiveness, sustainability and profitability in the SAAI.

## CHAPTER 3 RESEARCH METHODOLOGY

### 3.1 Experimental sites, design and treatments

Mature 'Hass' type avocado selection fruit ('Jalna', 'OA184' and 'Balboa') and a control (commercial 'Hass') were harvested from Burgershall Experimental Farm of the Agricultural Research Council-Institute of Tropical and Subtropical Crops (ARC-ITSC) (25° 35' 02" S, 31° 45' 07" E), Hazyview. Thereafter, fruit were transported to the ARC-ITSC post-harvest laboratory (25° 27' 04.6" S; 30° 58' 09.1" E), Nelspruit. The experiment was a completely randomised factorial design with three treatment factors, namely: temperature regimes (2.0 and 5.5°C), ripening days and 'Hass' type avocado selections ('Jalna', 'OA184', 'Balboa' and commercial 'Hass'). It was replicated three times, with 10 fruit per replication.

### 3.2 Experimental procedures

Twelve fruit were randomly harvested from three trees per selection, three fruit per selection were used for moisture content analysis to determine fruit maturity. Afterwards, the remaining fruit were sorted, graded, packed and stored at 2.0 and 5.5°C for 28 days to simulate export conditions. After withdrawal from cold storage, electrical conductivity was determined, using three fruit per selection. During days to ripening; chilling injury, respiration rate, mass loss, colour change, fruit firmness, ripening percentage and seed:fruit weight ratio were determined.

### 3.3 Determination of fruit maturity using moisture content

Moisture content was determined, based on the Swarts microwave oven method (Figure 3.1) using three fruit per selection, from week 2 to week 0 before harvest (Kruger *et al.*, 1995). A grated 10 g fruit sample was weighed and dried, using an oven (Model: 276, Ecotherm Labotec, Durban, South Africa) set at 30°C for 48 hours, and re-weighed after drying. Thereafter, moisture content was determined as follows:

$$\text{Moisture percentage (\%)} = [W_m - D_m / W_m] \times 100$$

Where  $W_m$  = Wet mass (g)

$D_m$  = Dry mass (g)



Figure 3.1 Determination of moisture content using the Swarts microwave oven method

### 3.4 Determination of physico-chemical variables

#### 3.4.1 Fruit water loss

Fruit were weighed before, after cold storage and during days to ripening, using a weighing scale (Figure 3.2) (Model: SBA 61, Scaltec, Hellingenstadt, Germany) to determine fruit water loss and weight expressed in kilograms. Fruit water loss was expressed, using the following equation:

$$\% \text{ Fruit water loss} = [M_1 - M_2 / M_1] \times 100$$

Where:  $M_1$  = Mass of fruit on day 0 after cold storage

$M_2$  = Mass of fruit during days to ripening





Figure 3.2 Weighing avocado fruit during evaluation for water loss

#### 3.4.2 Skin colour

Objective skin colour change [(L (Lightness), a (greenness/redness) and b (yellowness/blueness)] was measured using a Chroma Meter (Figure 3.3) (Model: CR-400, Kinoca Minolta Sensing Incorporation, Japan). A white calibration plate (Y= 87.00; x = 0.3146; y = 0.3215) was used to calibrate the chromameter and colour parameters were evaluated on a daily basis, at ambient temperature (Cox *et al.*, 2004). The colour parameters (L, a, b) were further used to calculate chroma (C) and hue angle (h) as follows:

$$C = [(a)^2 + (b)^2]^{(1/2)}$$

$$h^\circ = 180 + \text{degrees} [\text{atan}(a/b)]$$



Figure 3.3 Objective colour evaluation of avocado fruit (L, a, b) using a chromameter

Subjective colour change was evaluated according to Cox *et al*, (2004) using eye colour rating, which ranges from 1-5, whereby: 1= emerald green; 2 = forest green; 3= olive; 4= purple and 5 =black (Figure 3.4).



Figure 3.4 Subjective colour change for 'Hass' type avocado fruit (White *et al*, 2009).

### 3.4.3 Fruit firmness and ripening percentage

Fruit firmness was non-destructively measured during days to ripening, using a handheld densimeter (Model: 53254, Bareiss, Oberdischingen, Germany). The 5 mm fitted round tip densimeter was exposed to three cheeks of the new 'Hass' type avocado fruit (Figure 3.5) (Kok *et al.*, 2010). 'Hass' type avocado fruit were considered eating ripe when the densimeter reading was at 65 and below, whereas a reading of 66-100 indicated unripe fruit (Kohne *et al.*, 1998). Firmness values were further used to calculate ripening percentage. Ripening percentage was calculated daily, as follows:

$$\text{Ripening percentage (\%)} = [\text{Ripe fruit}/\text{Total number of fruit per replication}] \times 100$$



Figure 3.5 Measuring avocado fruit firmness, using a handheld densimeter

### 3.4.4 External chilling injury

External chilling injury was evaluated according to the method of Bower and Bertling (2008). It was assessed upon removal from cold storage using a rating scale of 0 (absent) to 1 (present) (Figure 3.6) and expressed as percentage of fruit with external chilling injury, using the following formula:

External chilling injury (%) = [Fruit with chilling injury/Total number of fruit per replication] ×100



Figure 3.6 Rating scale of external chilling injury in 'Hass' type avocado selection fruit (Bower and Bertling, 2008)

#### 3.4.5 Seed:fruit weight ratio

Seed weight was measured during trial termination. The fruit were cut, using a knife, the seed were then removed and weighed using a weighing scale (SBA 61, Scaltec, Hellingenstadt, Germany). The relationship between seed and fruit weight were further expressed, using the following equation:

$$\text{Seed:fruit weight ratio} = [\text{Seed weight (g)}/\text{Fruit weight (g)}] \times 100$$

#### 3.4.6 Determination of respiration rate using CO<sub>2</sub> evolution

Respiration rate was measured according to Blakey *et al*, (2012), using a dual gas analyser (Model: ICA 250, International Controlled Atmosphere Ltd, Kent, UK)

(Figure 3.7). Five fruit per selection were incubated daily in a 1.2 L container for 30 minutes at ambient temperature and CO<sub>2</sub> emission recorded and expressed as  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ .



Figure 3.7 Determination of the respiration rate of avocado fruit, using CO<sub>2</sub> evolution

#### 3.4.7 Electrical conductivity

Electrical conductivity (EC) was measured (Figure 3.8) according to van Rooyen and Bower, (2002). Three fruit per selection were used for the determination of electrical conductivity. A handheld cork borer with a diameter of 10 mm was used to take a sample from the whole fruit. The samples were placed in test tubes, rinsed three times with ultra-pure water and 20 ml ultra-pure water was added to the sample, prior shaking. The test tubes with samples were shaken for three hours and electrical conductivity evaluated, using an electrical conductivity meter (Model: HI 991301, Hanna Instruments, Johannesburg, South Africa). Electrical conductivity was then recorded as initial EC. Thereafter; samples were exposed to a hot water bath at 100°C for one hour. Afterwards, samples were cooled at ambient temperature and electrical conductivity measured (Final EC). Electrical conductivity was expressed as a percentage using the following formula: Electrical conductivity (EC) =  $[\text{Initial EC} / \text{Final EC}] \times 100$



Figure 3.8 Electrical conductivity evaluation of avocado fruit using an EC meter

### 3.5 Statistical analysis

Statistical analysis of the data was conducted, using Genstat 16<sup>th</sup> version (VSN International bioscience software and consultancy, 2014). Treatment means were separated, using Duncan's multiple range test (DMRT) at the 5% level of significance.

## CHAPTER 4 RESULTS AND DISCUSSION

### 4.1 Results

#### 4.1.1 Moisture content

There were no significant differences ( $P=0.733$ ) in moisture content between studied selections during fruit growth, development and maturity (Appendix 1). Fruit moisture content of 'Hass' type avocado selections followed a declining pattern as the season progressed, a common trend for commercial 'Hass' (control) (Figure 4.1). Selection 'OA 184' fruit showed a high moisture content, followed by 'Jalna', 'Balboa' and control fruit at harvest time. Fruit moisture content of selection 'OA 184' and 'Jalna' decreased from 80.33% to 78% as the season progressed, whereas, moisture content of control fruit decreased from 79.33% to 75%. Selection 'Balboa' fruit moisture content decreased from 76.67 to 75%.

#### 4.1.2 Electrical conductivity

After withdrawal from storage (2.0 and 5.5°C), there were no significant differences ( $P=0.004$ ) in electrical conductivity between evaluated 'Hass' type avocado selections (Appendix 2). However, electrical conductivity of selection 'Balboa', control, 'Jalna' and 'OA 184' fruit were slightly higher after withdrawal from storage at 2.0°C compared to 5.5°C (Figure 4.2). After withdrawal from storage at 2.0°C, the control, 'Balboa', 'OA 184' and 'Jalna' fruit had an electrical conductivity of 70.2%, 69.2%, 64.4% and 68.1%; respectively. After withdrawal from storage at 5.5°C, the control, 'Balboa', 'OA 184' and 'Jalna' fruit had an electrical conductivity of 37.9%, 56.2%, 55.9% and 64.7%; respectively.

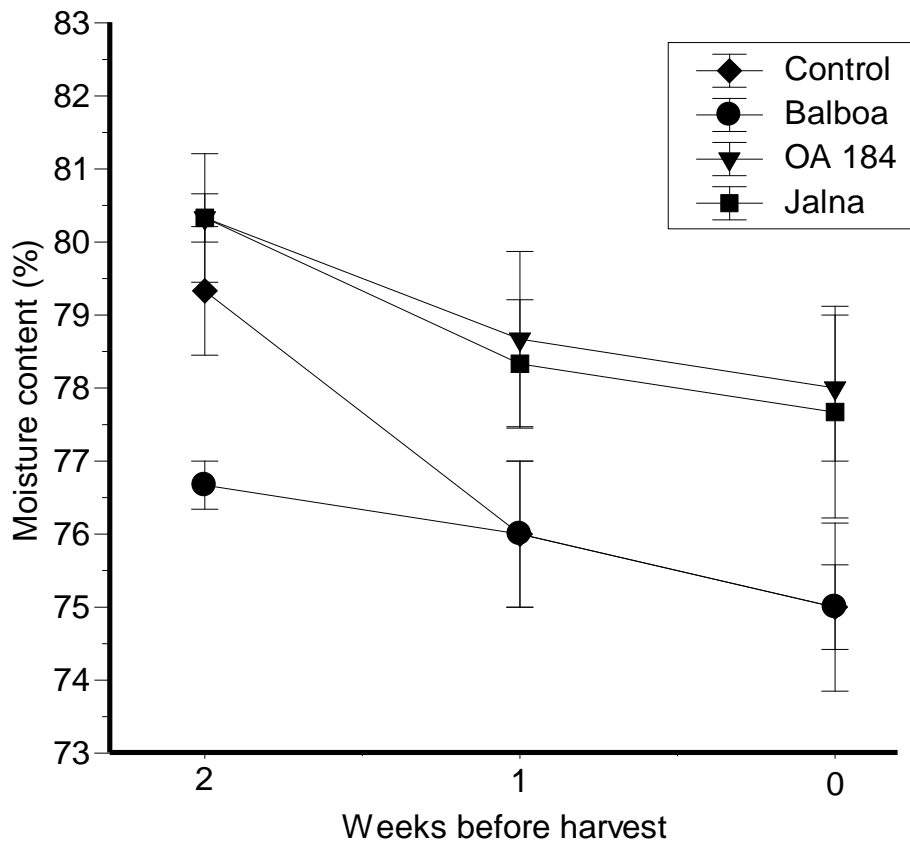


Figure 4.1 Moisture content of studied avocado selections during fruit growth, development and maturity. Vertical bars indicate the standard error of means



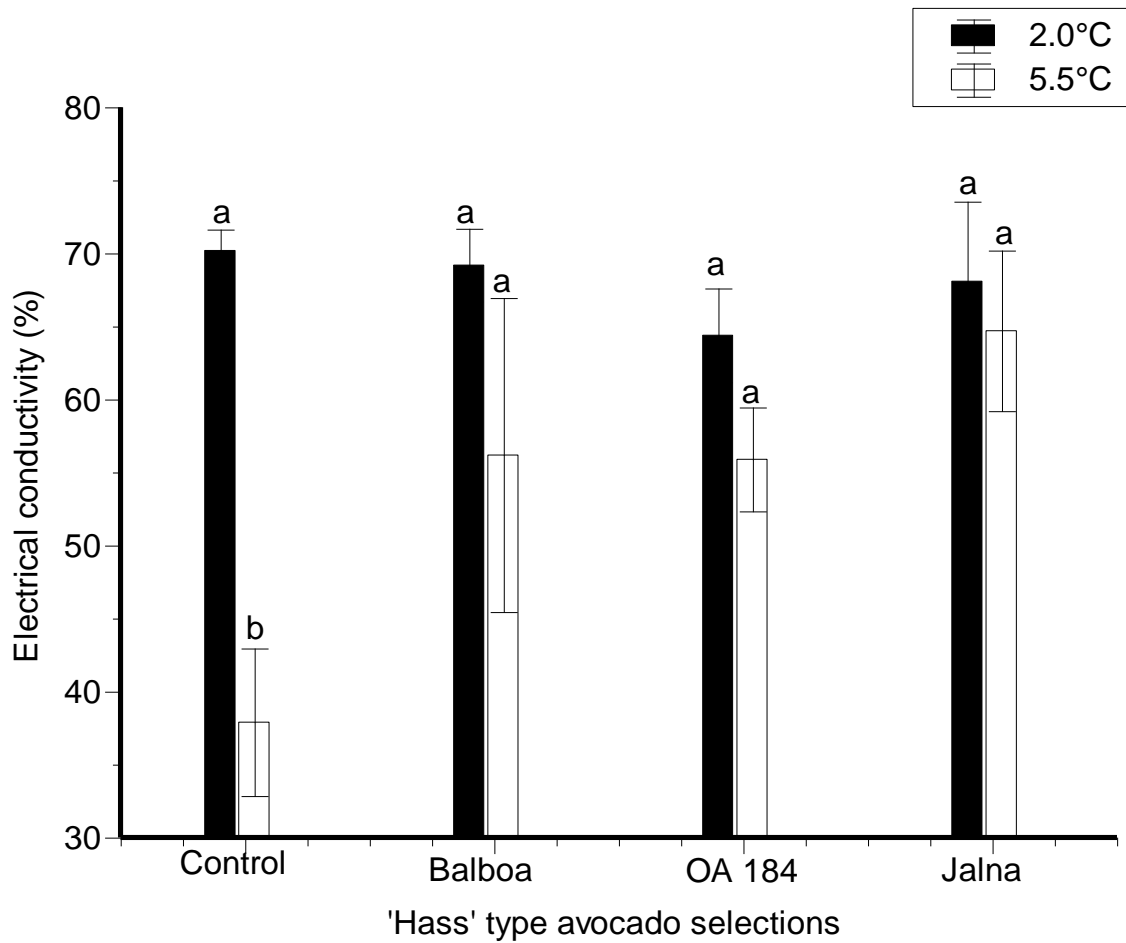


Figure 4.2 Effect of storage temperature on electrical conductivity in 'Hass' type selections. Vertical bars indicate the standard error of means

#### 4.1.3 External chilling injury

Storage temperature had no significant effect ( $P=0.776$ ) on external chilling injury of studied avocado selections during storage (Appendix 3). However, selection 'Jalna' fruit had the lowest chilling injury symptoms after withdrawal from storage at 2.0 and 5.5°C (Figure 4.3). Furthermore, selection 'OA 184' fruit showed lower chilling injury after withdrawal from storage at 5.5°C, compared to storage at 2.0°C. Upon removal from storage at 2.0 and 5.5°C, external chilling injury for selection 'Balboa', 'OA 184', 'Jalna' and control fruit was visible as dark colouration, but not exocarp collapse (Figure 3.6). The control and 'Balboa' fruit had 100% external chilling injury after withdrawal from storage at 2.0 and 5.5°C, whereas, after withdrawal from storage at 2.0°C, selection 'OA 184' and 'Jalna' fruit had external chilling injuries of 100% and

60%, respectively. However, after storage at 5.5°C, selection 'OA 184' and 'Jalna' fruit had chilling injury percentages of 83.3% and 53.3%, respectively.

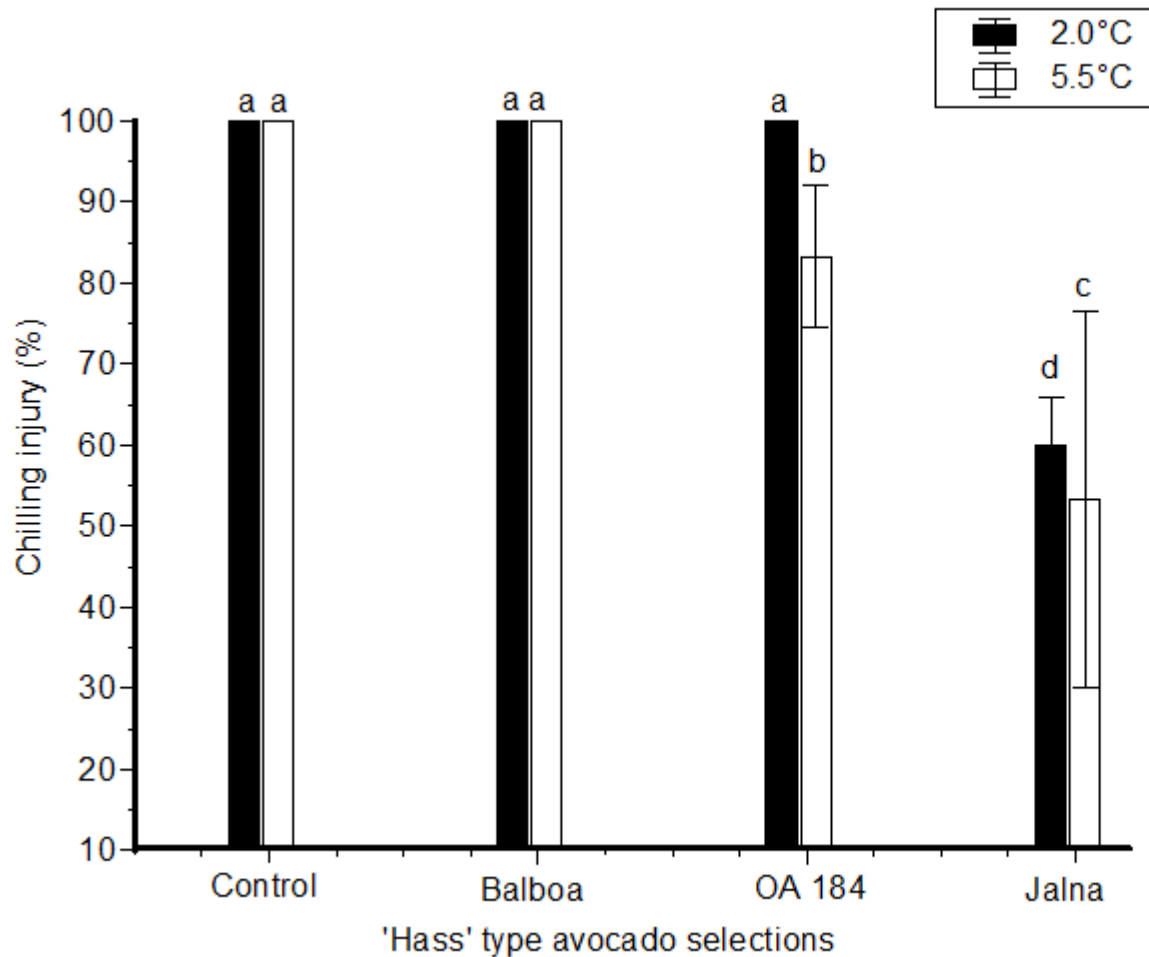


Figure 4.3 Effect of storage temperature on external chilling injury of the new 'Hass' type avocado selections. Vertical bars indicate the standard error of means

#### 4.1.4 Skin colour change

Skin colour change was evaluated, using subjective (eye colour rating) and objective colour parameters (lightness, chroma and hue angle).

##### Eye colour

There were significant differences ( $P < 0.001$ ) in skin eye colour rating for the evaluated 'Hass' type avocado selections after withdrawal from storage and during ripening (Appendix 4). Selection 'Jalna' fruit started changing eye colour within storage at 2.0 and 5.5°C, with skin eye colour ratings of 2.47 (forest green) and 3.43

(olive), respectively (Figure 4.4A and 4.5A). After withdrawal from storage at 2.0°C, the skin eye colour rating of control fruit increased from 1.0 (emerald green) to 4.23 (purple) during day 0 to 4 (Figure 4.4A). After storage at 5.5°C, the skin eye colour rating of control fruit also increased from 1.0 (emerald green) to 2.90 (olive) during day 0 to 3 (Figure 4.5A). After withdrawal from storage at 2.0 and 5.5°C, selection 'Balboa' fruit skin eye colour rating increased from 1.0 (emerald green) to 2.87 (olive) and 1.0 (emerald green) to 2.93 (olive) during 0 to 3 days to ripening, respectively. Selection 'OA 184' fruit skin eye colour rating increased from 1.0 (emerald green) to 4.35 (purple) between 0 to 6 days to ripening and from 1.0 (emerald green) to 3.87 (purple) during 0 to 5 days to ripening, after storage at 2.0 and 5.5°C, respectively. Selection 'Jalna' fruit skin eye colour rating increased from 2.47 (forest green) to 3.57 (olive) between day 0 to 3 and from 3.43 (olive) to 4.02 (purple) during 0 to 2 days to ripening, after storage at 2.0 and 5.5°C, respectively.

### **Hue angle (h°)**

Storage temperature and days to ripening had a significant effect ( $P < 0.001$ ) on fruit skin hue angle (h°) of the studied avocado selections (Appendix 5). Selection 'Balboa' and 'OA 184' fruit showed a significant decrease in skin hue angle (from 145.64 to 75.77 and 143.74 to 27.42) after withdrawal from storage at 2.0°C during 0 to 3 and 0 to 6 days to ripening, respectively (Figure 4.4B). After withdrawal from storage at 5.5°C, selection 'Balboa' and 'OA 184' fruit also showed a significant decrease in skin hue angle (from 146.34 to 77.04 and 143.36 to 83.68) during 0 to 3 and 0 to 5 days to ripening, respectively (Figure 4.5B). Selection 'Jalna' and control fruit showed a significant decrease in skin hue angle (from 94.46 to 60.19 and 141.32 to 74.96) during 0 to 3 and 0 to 4 days to ripening, after withdrawal from storage at 2.0°C, respectively. Selection 'Jalna' and control fruit skin hue angle also decreased (from 64.52 to 46.50 and 153.17 to 86.15) during 0 to 2 and 0 to 3 days to ripening after storage at 5.5°C, respectively.

### **Lightness (L)**

Storage temperature, selections and days to ripening had a significant effect ( $P = 0.011$ ) on fruit skin lightness (Appendix 6). After withdrawal from storage at 2.0°C, the fruit skin lightness of selection 'OA 184', followed a declining pattern (from 30.54, 30.37, 30.06, 30.01, 26.63, 25.57 to 24.52) during 0, 1, 2, 3, 4, 5 and 6 days

to ripening, respectively (Figure 4.4C). Skin lightness of control fruit also decreased (from 30.11, 29.41, 29.22, 28.79 to 27.27) during 0, 1, 2, 3 and 4 days to ripening after storage at 2.0°C, respectively. Selection 'Balboa' fruit skin lightness decreased (from 30.06, 29.66, 29.15 to 28.38) during 0 to 3 days ripening after storage at 2.0°C, respectively. Selection 'Jalna' fruit skin lightness decreased (from 34.73, 33.11, 31.85 to 31.07) during 0, 1, 2 and 3 days to ripening, after withdrawal from storage at 2.0°C.

After withdrawal from storage at 5.5°C, control fruit showed a decrease in skin lightness (from 29.71, 28.46, 26.67 to 25.39) during 0, 1, 2 and 3 days to ripening, respectively (Figure 4.5C). Selection 'Balboa' fruit also showed a decrease in skin lightness (from 30.35, 30.10, 28.76 to 28.08) during 0, 1, 2 and 3 days to ripening, after storage at 5.5°C. Whereas, selection 'OA 184' fruit skin lightness decreased (from 31.50, 31.26, 30.76, 30.20, 29.19 to 28.20) during 0, 1, 2, 3, 4 and 5 days to ripening after storage at 5.5°C. Selection 'Jalna' fruit also showed a decrease in skin lightness (from 30.85, 30.54 to 30.37) during 0, 1, 2 and 3 days to ripening, after withdrawal from storage at 5.5°C.

### **Chroma (C)**

After withdrawal from storage, there was a significant difference ( $P=0.042$ ) in skin chroma of the evaluated selections during days to ripening (Appendix 7). After withdrawal from storage at 2.0°C, fruit skin chroma for selections 'OA 184', control, 'Balboa' and 'Jalna' decreased (from 12.25 to 4.56; 13.93 to 7.16; 10.84 to 7.85 and 15.15 to 10.32) during 0 to 6, 0 to 4, 0 to 3 and 0 to 3 days to ripening, respectively (Figure 4.4D). After withdrawal from storage at 5.5°C, the control, 'Jalna', 'Balboa' and 'OA 184' fruit skin chroma also decreased (from 9.42 to 5.50; 10.90 to 7.31; 11.38 to 7.46 and 13.20 to 7.78) during 0 to 3, 0 to 2, 0 to 3 and 0 to 5 days to ripening, respectively (Figure 4.5D).

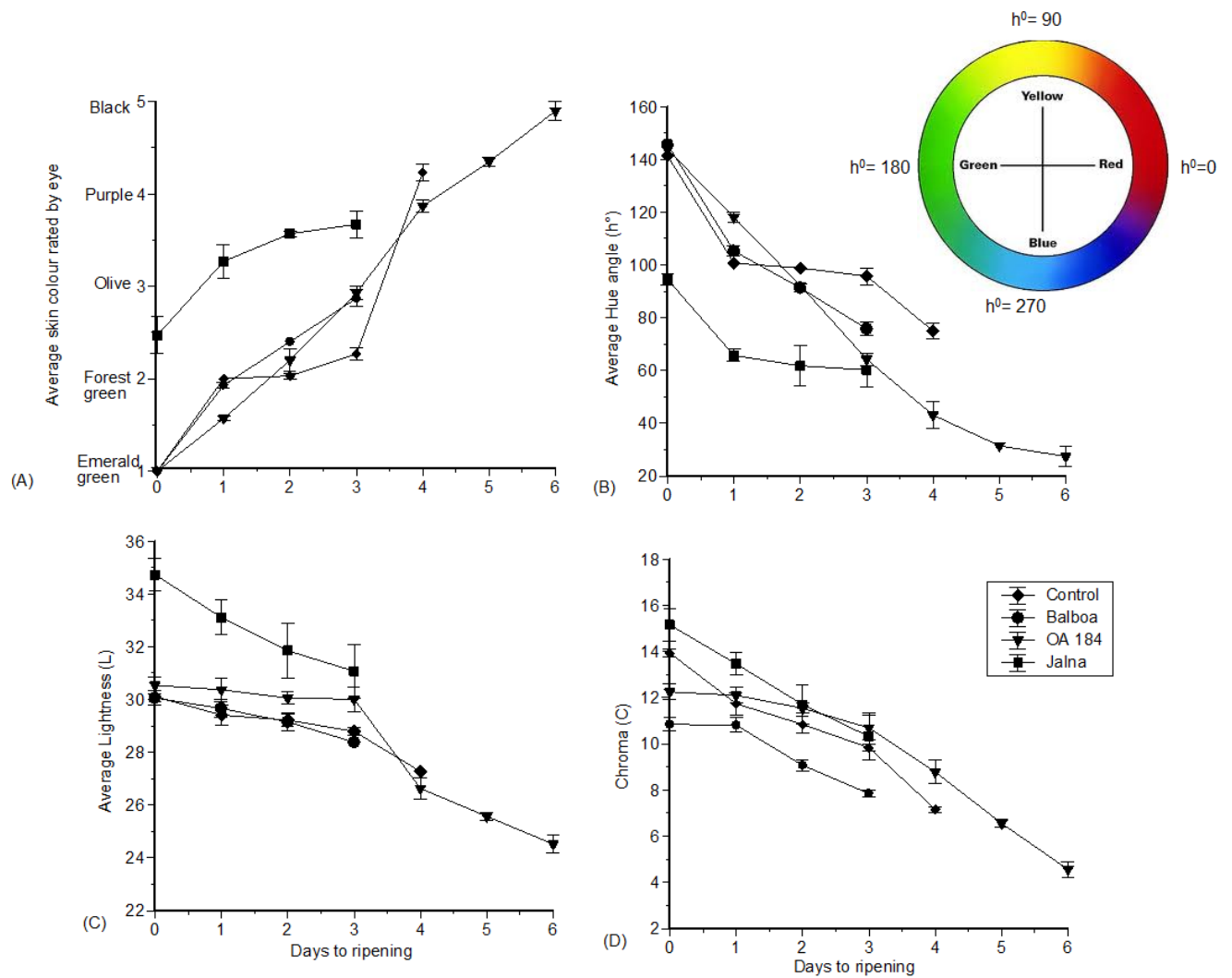


Figure 4.4 Subjective and objective colour changes for the new 'Hass' type avocado selections:(A) eye colour rating, (B) hue angle, (C) lightness and (D) chroma, after storage at 2.0°C during days to ripening. Vertical bars indicate the standard error of means

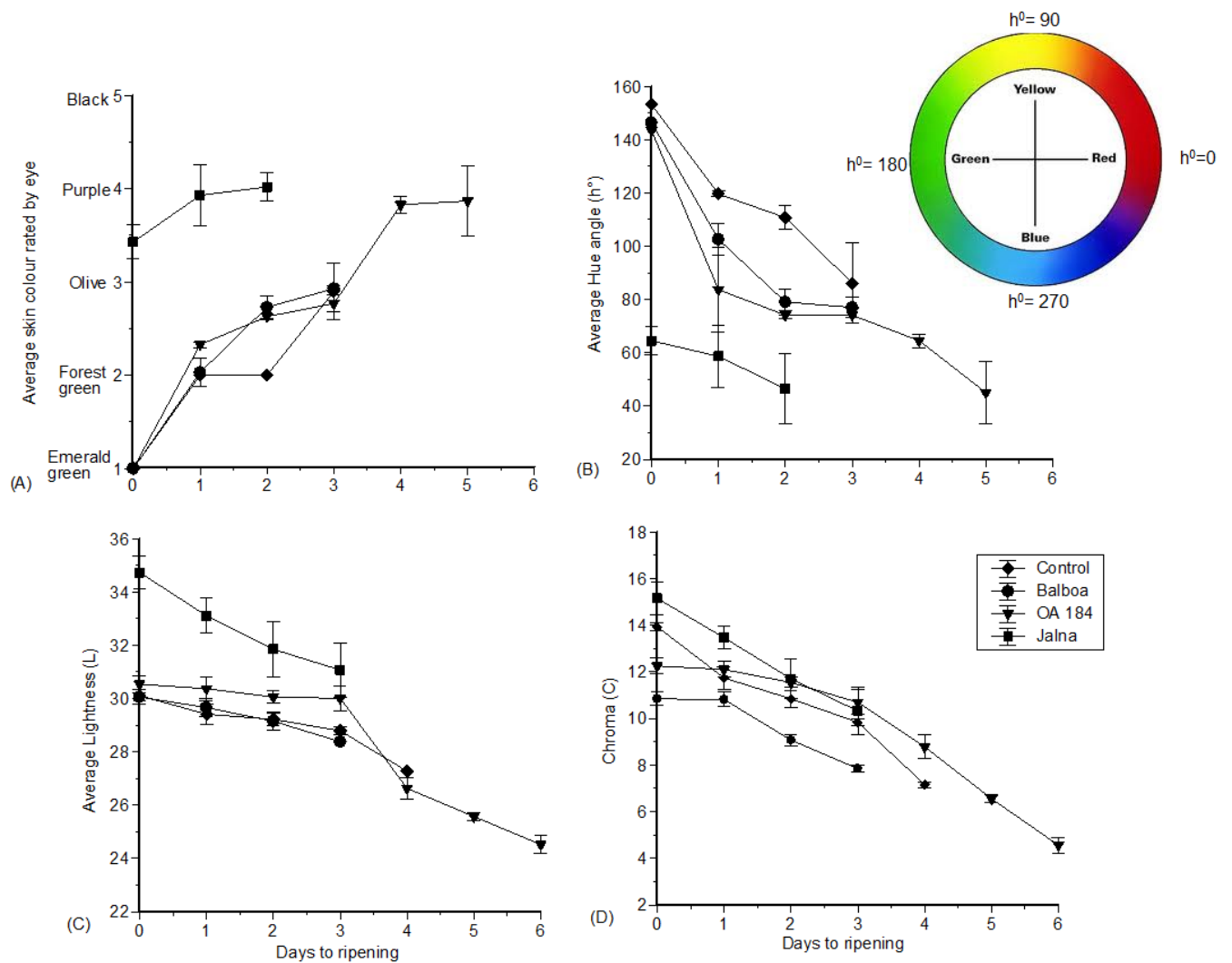


Figure 4.5 Subjective and objective colour changes for the new ‘Hass’ type avocado selections: (A) eye colour rating, (B) hue angle, (C) lightness and (D) chroma, after storage at 5.5°C during days to ripening. Vertical bars indicate the standard error of means

#### 4.1.5 Respiration rate

Storage temperature, evaluated selections and days to ripening had a significant effect ( $P < 0.001$ ) on respiration rate (Appendix 8). Selection ‘Balboa’ and ‘OA 184’ fruit had a higher respiration rate after withdrawal from storage at 2.0°C, compared to 5.5°C (Figure 4.6). However, selection ‘Jalna’ and control fruit had a lower respiration rate after storage at 2.0°C, compared to 5.5°C. The respiration rate for control fruit started at  $228 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at  $1421 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and declined to  $1083 \mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  between 0 to 4 days after withdrawal from storage at 2.0°C (Figure 4.6A). After storage at 5.5°C, the respiration rate of control

fruit started at 979  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1709  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and decreased to 1056  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during 0 to 3 days to ripening (Figure 4.6B).

After storage at 2.0°C, the respiration rate of selection 'Balboa' fruit started at 442  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1374  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and decreased to 1267  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  between 0 to 3 days to ripening. After withdrawal from storage at 5.5°C, 'Balboa' fruit's respiration rate started at 181  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1572  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and declined to 1206  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during 0 to 3 days to ripening.

After withdrawal from storage at 2.0°C, the respiration rate of selection 'OA 184' fruit started at 402  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 2784  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and decreased to 728  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during 0 to 6 days to ripening. Whereas, after withdrawal from storage at 5.5°C, respiration rate in selection 'OA 184' fruit started at 170  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1695  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and declined to 1325  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during 0 to 5 days to ripening.

The respiration rate for selection 'Jalna' fruit started at 96  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1019  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and decreased to 534  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  during 0 to 3 days to ripening, after withdrawal from storage at 2.0°C. While, it started at 210  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , reached a peak at 1619  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$  and decreased to 904  $\mu\text{mol CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ , for selection 'Jalna' fruit during 0 to 2 days to ripening, after withdrawal from storage at 5.5°C.

#### 4.1.6 Firmness

Storage temperature, studied selections and days to ripening had a significant effect ( $P < 0.001$ ) on fruit firmness (Appendix 9). After withdrawal from storage at both temperatures (2.0 and 5.5°C), selection 'Jalna' and 'OA 184' fruit retained high firmness values, compared to selection 'Balboa' and the control fruit (Figure 4.7). After withdrawal from storage at 2.0°C, selection 'Jalna' and 'OA 184' fruit showed a firmness decrease from 86.38 to 74.27 and 92.66 to 73.97 during 0 to 3 and 0 to 6 days to ripening (Figure 4.7A). While, the control and 'Balboa' fruit firmness decreased from 90.83 to 64.29 and 88.78 to 58.09 during 0 to 4 and 0 to 3 days to ripening, after withdrawal from storage at 2.0°C, respectively. After withdrawal from storage at 5.5°C, fruit firmness for selection 'OA 184' and 'Jalna' also decreased

from 91.71 to 72.93 and 79.90 to 68.24 during 0 to 5 and 0 to 2 days to ripening. Selection 'Balboa' and control fruit firmness decreased from 81.06 to 59.66 and 91.31 to 65.56 during 0 to 3 days to ripening, after withdrawal from storage at 5.5°C (Figure 4.7B).

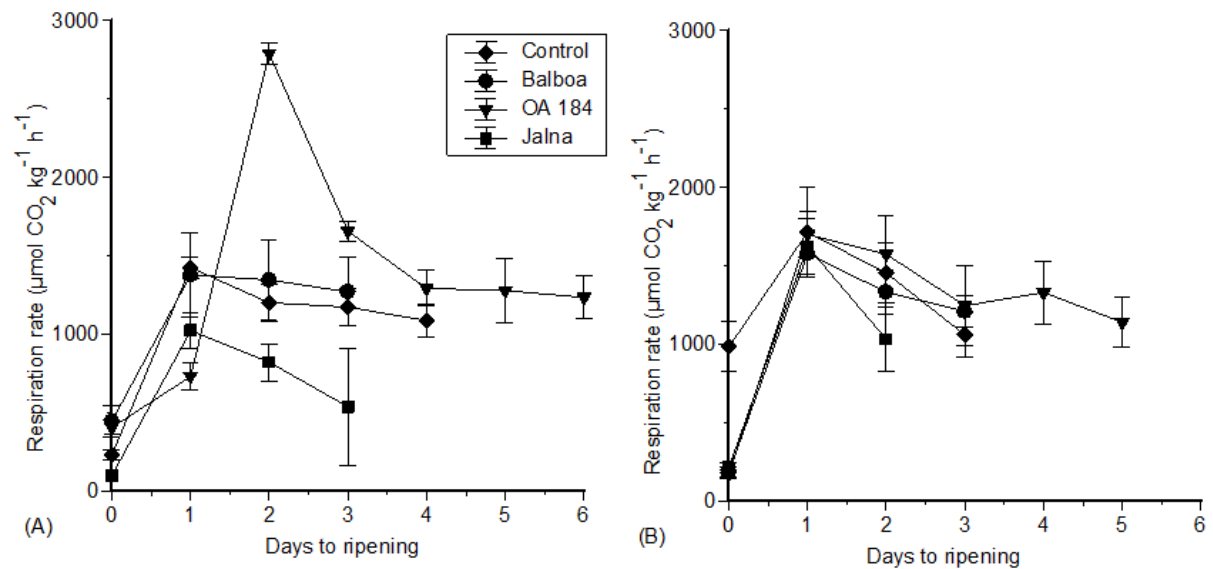


Figure 4.6 Carbon dioxide evolution in 'Hass' type avocado selections: (A) 2.0°C and (B) 5.5°C. Vertical bars indicate the standard error of means

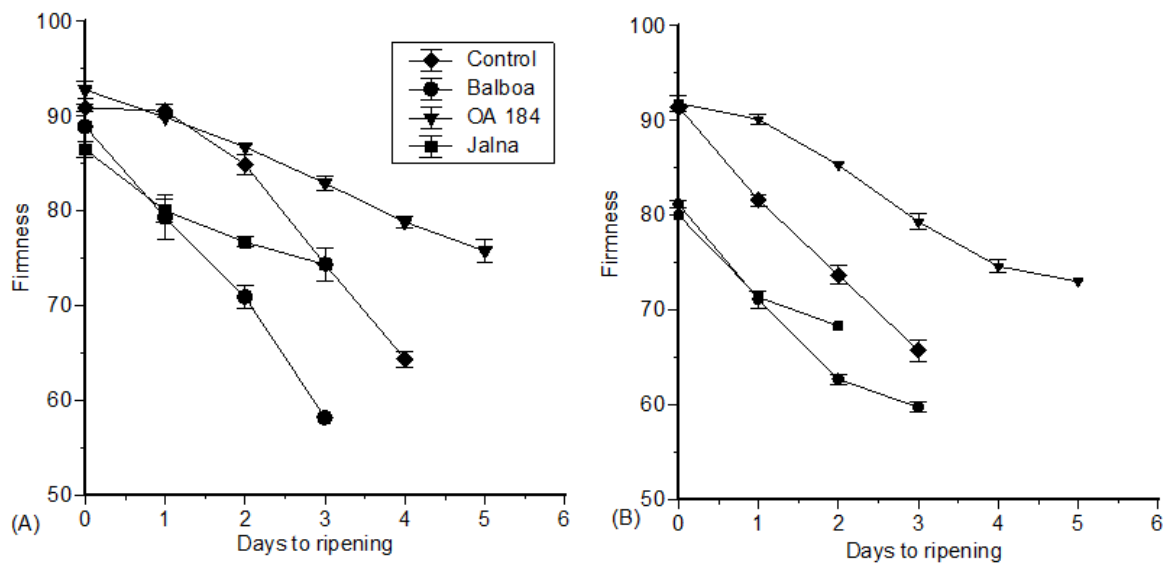


Figure 4.7 'Hass' type avocado selection fruit firmness in response to cold storage: (A) 2.0°C and (B) 5.5°C. Vertical bars indicate the standard error of means



#### 4.1.7 Ripening percentage

Evaluated selections, storage temperature and days to ripening had a significant effect ( $P < 0.001$ ) on ripening percentage (Appendix 10). Selection 'OA 184' fruit had a lower ripening percentage after storage at 2.0 and 5.5°C, compared to the control, 'Balboa' and 'Jalna' fruit (Figure 4.8). Selection 'Balboa' fruit reached 100% and 86.67% ripening after withdrawal from storage at 2.0 and 5.5°C, respectively. After withdrawal from storage at 2.0°C, the control, 'Jalna' and 'OA 184' fruit had ripening percentages of 66.67, 36.67 and 11.67% (Figure 4.8A). However, selection 'Jalna', the control and 'OA 184' fruit had ripening percentages of 76.67, 70.00 and 20.00% after withdrawal from storage at 5.5°C (Figure 4.8B).

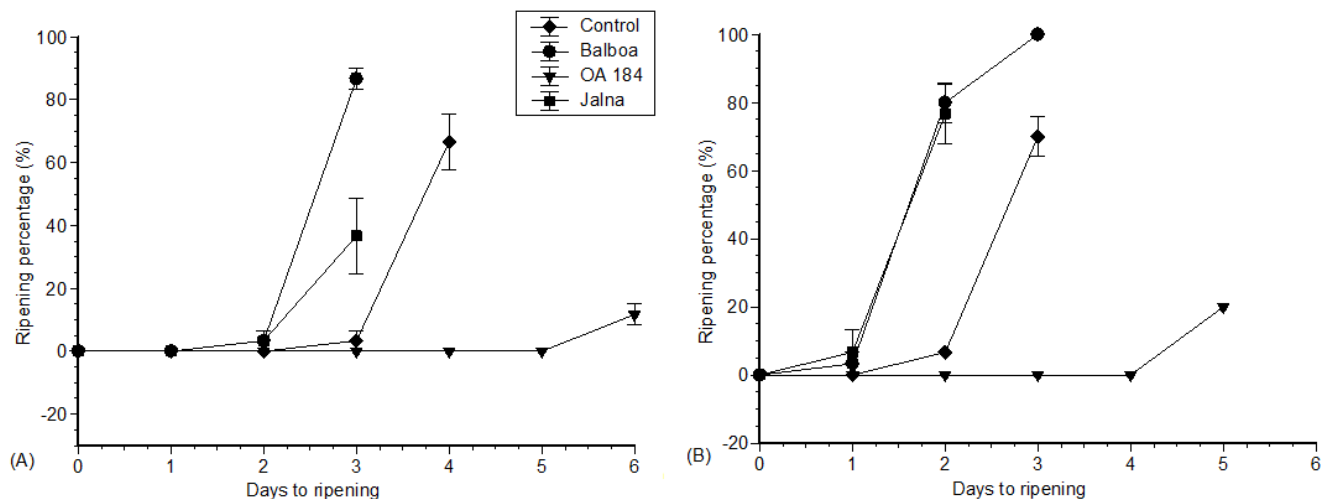


Figure 4.8 Ripening percentage in 'Hass' type avocado selections after storage at: (A) 2.0°C and (B) 5.5°C during days to ripening. Vertical bars indicate the standard error of means

#### 4.1.8 Fruit water loss

Selection, storage temperature and days to ripening had no significant effect ( $P = 0.998$ ) on fruit water loss (Appendix 11). However, selection 'Jalna' had higher water loss after storage at 2.0 and 5.5°C, compared to selection 'Balboa', 'OA 184' and the control fruit (Figure 4.9). After withdrawal from storage at 2.0°C, the control fruit water loss increased from 4.39% to 4.47%, between 0 to 4 days to ripening, whereas, after storage at 5.5°C, water loss for the control fruit increased from 6.14% to 6.26%, during 0 to 3 days to ripening.

After withdrawal from storage at 2.0°C, water loss for 'Balboa' fruit increased from 7.41% to 8.51%, during 0 to 3 days to ripening, while, after withdrawal from storage at 5.5°C, it increased from 5.09% to 5.85% during 0 to 3 days to ripening. Selection 'OA 184' fruit water loss increased from 5.00% to 7.65% during 0 to 6 days to ripening after storage at 2.0°C, while, after withdrawal from storage at 5.5°C, it increased from 6.46% to 6.71% between 0 to 5 days to ripening. Water loss in 'Jalna' fruit increased from 9.36% to 9.98% during 0 to 3 days to ripening, after storage at 2.0°C. However, after withdrawal from storage at 5.5°C, it increased from 8.17% to 8.46% between 0 to 2 days to ripening.

#### 4.1.9 Seed:fruit weight ratio

Selections had a significant effect ( $P < 0.001$ ) on seed:fruit weight ratio (Appendix 12). Furthermore, selection 'Balboa' accounted for 21.48, 'OA 184' for 15.65 and 'Jalna' for 30.81 compared to the control fruit, which accounted for 19.56 in seed: fruit weight ratio (Figure 4.10).

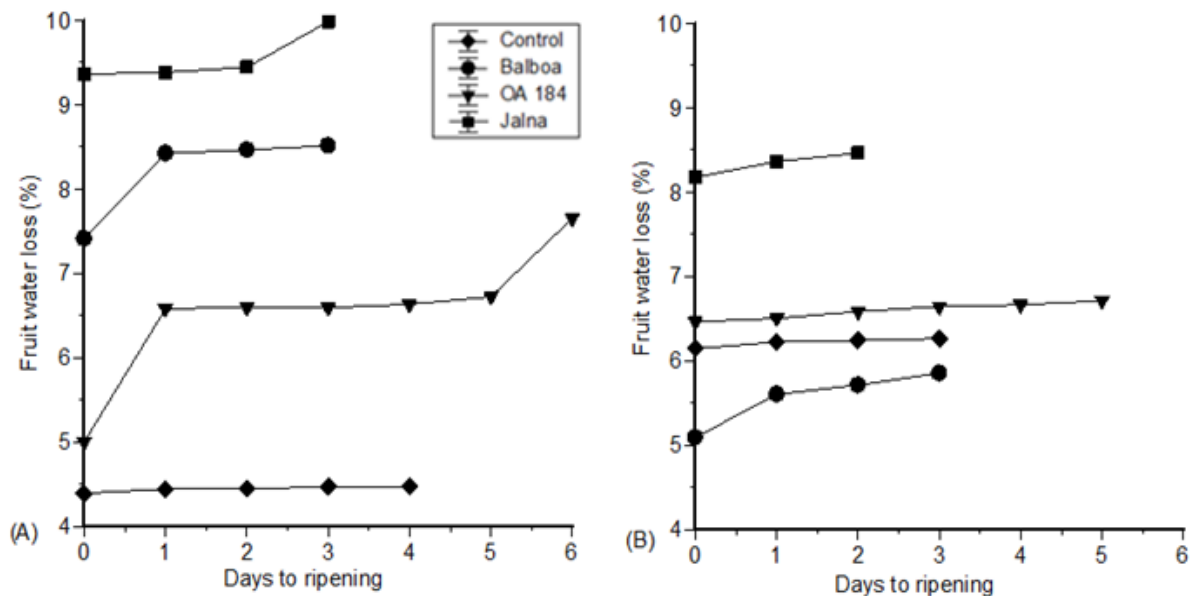


Figure 4.9 Selections water loss (%) in response to cold storage: (A) 2.0°C and (B) 5.5°C during days to ripening

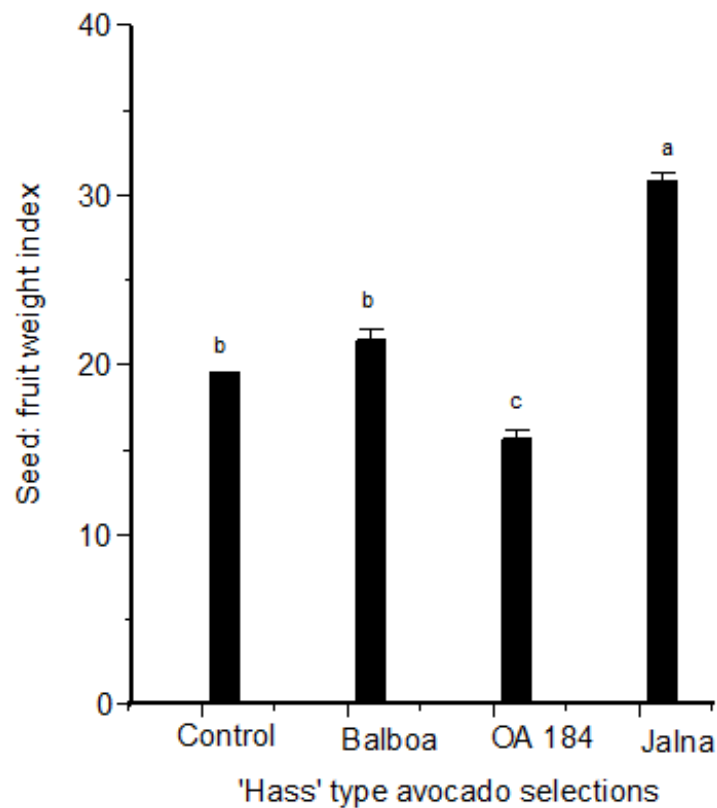


Figure 4.10 Seed:fruit weight ratio in evaluated selections. Vertical bars indicate the standard error of means

#### 4.1.10 Correlations between parameters

Selection 'Balboa' had a fair positive correlation between skin eye colour and ripening percentage after storage at 2.0°C ( $r^2=0.49$ ) (Table 4.1), while, selection 'OA 184' fruit had a weak positive correlation between skin eye colour and ripening percentage, after storage at 2.0 and 5.5°C ( $r^2 =0.11$  and 0.39). After storage at 2.0°C, selection 'Jalna' and 'Balboa' fruit had a fair positive correlation between skin eye colour and ripening percentage ( $r^2 =0.34$  and 0.49), whereas, selection 'OA 184' fruit had a weak positive correlation between skin eye colour and ripening percentage, after storage at 2.0 and 5.5°C ( $r^2 =0.11$  and 0.39). After storage at 2.0 and 5.5°C selection 'OA 184' fruit had a weak negative correlation between firmness and ripening percentage ( $r^2 =-0.12$  and  $-0.57$ ), whereas, the control fruit had a fair negative correlation between firmness and ripening percentage, after storage at 5.5°C ( $r^2=-0.34$ ).

Table 4.1 Correlation between eye colour, lightness, chroma, hue angle, firmness and ripening percentage for ‘Hass-type’ avocado fruit ripening after storage at 2.0°C and 5.5°C

Storage temperature (°C)											
		2.0°C					5.5°C				
Selection	Parameter	Eye colour	Lightness	Chroma	Hue angle	Firmness	Eye colour	Lightness	Chroma	Hue angle	Firmness
Control	Eye colour	1					1				
	Lightness	-0.91	1				-0.74	1			
	Chroma	-0.90	0.93	1			-0.81	0.96	1		
	Hue angle	-0.99	0.89	0.89	1		-0.95	0.75	0.81	1	
	Firmness	-0.86	0.86	0.88	0.75	1	-0.93	0.93	0.91	0.85	1
	Ripening percentage	0.82	-0.72	-0.62	-0.79	-0.66	0.62	-0.68	-0.78	-0.53	-0.57
‘Balboa’	Eye colour	1					1				
	Lightness	-0.87	1				-0.87	1			
	Chroma	-0.87	0.94	1			-0.84	0.98	1		
	Hue angle	-0.99	0.85	0.83	1		-0.98	0.84	0.79	1	

	Firmness	-0.92	0.92	0.96	0.89	1	-0.94	0.94	0.94	0.89	1
	Ripening percentage	0.49	-0.58	-0.67	-0.43	-0.69	0.78	-0.89	-0.95	-0.71	-0.89
'OA 184'	Eye colour	1					1				
	Lightness	-0.87	1				-0.74	1			
	Chroma	-0.90	0.99	1			-0.82	0.80	1		
	Hue angle	-0.97	0.76	0.80	1	1	-0.95	0.60	0.69	1	
	Firmness	0.87	0.87	0.90	0.96		-0.70	0.70	0.88	0.73	1
	Ripening percentage	0.11	-0.21	-0.17	-0.07	-0.12	0.39	-0.77	-0.45	-0.30	-0.33
'Jalna'	Eye colour	1					1				
	Lightness	-0.79	1				-0.94	1			
	Chroma	-0.88	0.94	1			-0.93	0.99	1		
	Hue angle	-0.93	0.78	0.90	1		-0.88	0.91	0.91		
	Firmness	-0.90	0.90	0.93	0.89	1	-0.95	0.94	0.96	0.89	1
	Ripening percentage	0.34	-0.70	-0.61	-0.40	-0.67	0.82	-0.70	-0.73	-0.72	-0.82

## 4.2 Discussion

### 4.2.1 Moisture content

In avocado fruit, physiological maturity is determined using a moisture content decline to less than 80% (Mans *et al.*, 1995). In this study, 'Hass' type avocado selection fruit were harvested when the moisture content decline was between 75% to 80%, indicating an acceptable physiological maturity. Characteristically, the moisture content decline for 'Hass' type avocado selection fruit was attributable to oil content increases during physiological maturity (Kruger *et al.*, 1995).

The moisture content at time of avocado harvest affects fruit shelf-life and quality. A low moisture content for selection 'Balboa' fruit led to a short shelf-life (3 days) after storage (2.0 and 5.5°C). These findings were in agreement with Chen *et al.*, (2009), who showed that a low moisture content at harvest time reduced 'Hass' avocado fruit's shelf-life. This was attributed to peak ethylene synthesis and increased cellular activity, leading to rapid fruit ripening. However, selection 'Jalna' fruit had a higher pre-harvest moisture content and a contrarily short shelf-life of 3 and 2 days after withdrawal from storage at 2.0°C and 5.5°C. Therefore, this study demonstrated that the relationship between moisture content and subsequent avocado fruit shelf-life is selection dependant. Hence, selection 'OA 184' had the same moisture content as 'Jalna' fruit at time of harvest, but a longer shelf-life of 6 and 5 days after storage at 2.0°C and 5.5°C. A longer shelf-life for selection 'OA 184' fruit was attributed to reduced metabolic activities (softening, colour change and ripening).

### 4.2.2 Electrical conductivity

Generally, 'Hass' type avocado selection fruit showed increased electrical conductivity after withdrawal from storage at a lower temperature (2.0°C), compared to a higher storage temperature (5.5°C). The results agreed with findings of Feng *et al.*, (2005), where, high electrical conductivity was observed at a lower temperature (2.0°C) due to severe cold stress, leading to accelerated cell membrane damage. The current study contradicted Woolf *et al.*, (2003), as 'Hass' type avocado selection fruit had low electrical conductivity, after withdrawal from a higher storage temperature but rapid ripening. The studied 'Hass' type avocado selection fruit

ripened rapidly, due to increased cell plasma membrane disturbance and ion mobility.

#### 4.2.3 External chilling injury

External chilling injury was high for selection 'Balboa', 'OA 184' and the control fruit after withdrawal from storage at 2.0°C and 5.5°C. This contradicted previous findings by Bower and Bertling (2008), who observed high chilling injury in 'Hass' avocado fruit, after storage at a lower temperature (2.0°C). Accelerated chilling injury was expected after withdrawal from storage at a lower temperature due to increased cellular permeability (ion and solute leakage) and glycolysis intermediate accumulation (glyceraldehyde 3-phosphate, pyruvate and dihydroxy-acetone phosphate), leading to membrane integrity loss (Fuchs *et al.*, 1989; Chaplin *et al.*, 1982).

Selection 'Jalna' fruit's skin colour changed from olive green to purple, thereby masking chilling injury blemishes after withdrawal from storage at a higher temperature. These findings were in agreement with Kok *et al.*, (2010), where, 'Hass' avocado skin colour changed from green to purple/black during ripening, therefore concealing external chilling injury blemishes. Purple colour development in selection 'Jalna' fruit was attributed to anthocyanin accumulation and chlorophyll degradation during ripening.

#### 4.2.4 Skin colour change

Skin colour change, as measured by eye colour rating for selection 'OA 184' and the control fruit, was not affected by storage temperature, as purple skin colour development was observed after withdrawal from a lower storage temperature. However, selection 'Balboa' fruit skin colour development reached olive green after withdrawal from storage (2.0°C and 5.5°C). These results, contradicted Cox *et al.*, (2004), as 'Hass' avocado skin colouration was reduced after withdrawal from a lower storage temperature. Skin colour change should be accelerated after withdrawal from storage at a higher storage or ripening temperature attributed to increased metabolic activities (Ashton *et al.*, 2006). Furthermore, withdrawal of 'Balboa', 'OA 184' and the control fruit from a higher temperature significantly reduced skin hue angle decline, attributed to anthocyanin accumulation during

ripening. Declining patterns in hue angle led to reduced skin colour change from emerald green to purple for selection 'Balboa' and 'Jalna' fruit after withdrawal from a lower storage temperature. Moreover, selection 'Balboa', 'Jalna' and the control fruit had rapid skin lightness decline after withdrawal from storage at a higher temperature compared to a lower temperature. These trends were in line with Villa-Rodriguez *et al.*, (2011) who showed that accelerated skin lightness decline for 'Hass' avocado fruit was due to increased metabolic activities and anthocyanin production (cyanidin 3-O-glucoside). In addition, withdrawal of selection 'Balboa', 'Jalna' and the control fruit from a higher temperature led to exacerbated skin chroma decline resulting in colour intensity. Colour intensity for selection 'Balboa', 'Jalna' and the control fruit, after storage at a higher temperature was due to increased cell metabolism. The declining trend in lightness, hue angle and chroma concomitantly with eye colour rating increase indicated skin colour changes in 'Hass' type avocado selections, attributed to cyanidin 3-O-glucoside accumulation (Cox *et al.*, 2004).

Selection 'Jalna' fruit started changing colour while in storage (2.0°C and 5.5°C). These findings were in agreement with Bower and Bertling (2008), where, storage of 'Hass' avocado fruit at 1.0°C and 5.5°C, resulted in skin colour change as a defence mechanism against cold stress. Skin colour changes in 'Jalna' fruit was assumedly due to increased anthocyanin synthesis, acting as an antioxidant and free radical scavenger to alleviate tissue damage against increased cold stress (Kok *et al.*, 2010).

During days to ripening, skin colour development increased with a declining pattern in lightness, chroma, hue angle, concurrently with eye colour rating increase for 'Hass' type avocado selections. In 'Hass' type avocado fruit, rapid skin chroma and lightness decline indicated skin darkening as fruit changed colour during days to ripening. These findings were in agreement with Cox *et al.*, (2004), where, lightness, chroma and hue angle decreased for 'Hass' avocado fruit during days to ripening, accompanied by skin colour changes. 'Hass' type avocado fruit followed a declining pattern for hue angle during days to ripening. However, selection 'Balboa' and 'Jalna' fruit reached olive green skin colour development during days to ripening after withdrawal from a lower storage temperature, assumedly due to reduced cyanidin 3-



O-glucoside accumulation (responsible for purple colour development) (Cox *et al.*, 2004).

#### 4.2.5 Respiration rate

Selection 'Jalna' and the control fruit had low respiration rates, after storage at a lower temperature assumedly due to reduced cellular metabolism. This study was in line with Blakey and Bower (2009), where withdrawal of 'Hass' avocado fruit from storage at 1.0°C resulted in low respiration rates due to reduced cellular activity, compared to storage at a higher temperature (5.5°C). Therefore, higher temperature induced rapid respiratory responses due to increased cellular activity.

Climacteric maximum was higher for selection 'Balboa', 'Jalna' and the control fruit after storage at a higher temperature. Such findings were in agreement with Lallum *et al.*, (2004), who indicated that respiratory climacteric increased with temperature and concomitant with ethylene synthesis, leading to loss of membrane integrity (increased respiration rate, softening and ripening). Moreover, during respiratory climacteric, solute leakage increased exponentially due to mitochondrial exposure to harmful substances (organic acid release) and tissue pH changes, damaging metabolic pathways (Van Rooyen 2005).

The current study, assumed high energy reserve depletion which led to earlier ripening for selection 'Balboa' and 'Jalna' fruit during days to ripening, and therefore, a short shelf-life after storage (2.0°C and 5.5°C). These findings were in line with Blakey and Bower (2009), as energy reserves are used throughout respiration in 'Hass' avocado fruit. Moreover, 'Hass' type avocado selection fruit ripening was assumedly related to carbohydrate (heptose sugars) depletion, leading to decreased membrane integrity during days to ripening (Eaks, 1978).

#### 4.2.6 Firmness

The current study observed that storage at a higher temperature resulted in rapid firmness decline for selection 'Balboa' and the control fruit compared to a lower storage temperature, and therefore, accelerated ripening. These findings were in agreement with Zauberman and Jobin-Décor (1995), where, 'Hass' avocado fruit showed rapid ripening after storage at a higher temperature (5.0°C), attributed to enhanced metabolic activities. Accelerated ripening for selection 'Balboa' and the

control fruit was attributed to increased cell wall degradation (Pesis *et al.*, 1978). However, storage temperature had no effect on selection 'Jalna' and 'OA 184', as fruit retained high firmness values due to a genetically non-softening exocarp, although the mesocarp softened during days to ripening. In 'Hass' avocado fruit, non-softening of the exocarp was attributed to inhibition of polygalacturonase and polyuronide expression in the cell wall, leading to absence of turgor loss (Pesis *et al.*, 1978). Therefore, the ripening rate for selection 'Jalna' and 'OA 184' fruit was determined, using the correlation between eye colour rating and firmness decline ( $r^2 = 0.89, 0.89, 0.96$  and  $0.73$ , respectively).

The studied 'Hass' type avocado selection ripening ranged between 2-6 days, as indicated by fruit firmness decline. However, the normal ripening period for 'Hass' avocado fruit is between 7-10 days, depending on harvest time and treatment factors (Bower and Bertling, 2008). 'Hass' type avocado selection fruit rapidly ripened, assumedly due to increased production of cell wall degrading enzymes (polygalacturonase and pectin methyl esterase), leading to decreased cell wall and membrane integrity during days to ripening, in agreement with Blakey *et al.*, (2014). Selection 'Balboa' and control fruit were terminated at firmness values below 65, indicating fruit softening and ripening after withdrawal from storage (2.0°C and 5.5°C). This trend was in line with Kohne *et al.*, (1998), as 'Hass' avocado fruit densimeter values decreased to  $\leq 65$  during days to ripening, indicating eating ripeness. However, during days to ripening, selection 'OA 184' and 'Jalna' fruit were terminated at values above 65 due to a non-softening exocarp. Non-softening of the 'Hass' avocado exocarp was attributed to inactivation of polygalacturonase gene expression in the cell wall (Tucker and Grierson, 2013; Pesis *et al.*, 1978).

#### 4.2.7 Ripening percentage

Ripening percentage indicated the proportion of ripe to unripe 'Hass' type avocado selection fruit and increased with firmness decline. In this study, ripening percentage significantly increased for selection 'Balboa' and the control fruit after storage at a higher and lower temperature. Ripening percentage for selection 'Jalna' and 'OA 184' fruit steadily increased during days to ripening due to a genetically non-softening exocarp. Therefore, selection 'OA 184' and 'Jalna' fruit were terminated, using the correlation between firmness and skin eye colour change ( $r^2 = 0.89, 0.89,$

0.96 and 0.73, respectively). The rate of ripening for selection 'Balboa' and the control fruit was indicated by a firmness decline during days to ripening. These findings were in line with Paull (1999), as the ripening rate for 'Hass' avocado fruit was indicated by a fruit firmness decline and skin colour development during days to ripening. Accelerated ripening percentages for selection 'Balboa' and the control fruit was due to increased metabolic activities (accelerated ethylene synthesis, respiration rate and ripening) (Flitsanov *et al.*, 2000).

#### 4.2.8 Fruit water loss

'Hass' type avocado selections showed no significant differences in fruit water loss after storage at a higher and lower temperature. Contrarily, 'Hass' avocado fruit stored at a higher temperature (5.5°C) had higher water loss, compared to storage at a lower temperature (2.0°C), attributed to enhanced climacteric processes (Lallum *et al.*, 2004). In avocado fruit, lower water loss was associated with decreased epidermal cell collapse, resulting in decreased biochemical and physiological modifications, leading to decreased fatty acid saturation (Perez *et al.*, 2004).

In the current study, days to ripening had an insignificant effect on water loss for 'Hass' type avocado selection fruit. These findings were in contrast with Yahia and Woolf (2011), as 'Hass' avocado fruit's water loss increased during days to ripening and determined commodity quality. According to Lutge *et al.*, (2010), insignificant 'Hass' avocado fruit water loss was attributed to reduced ethylene synthesis stimulation, leading to decreased respiration rate and fruit softening. During days to ripening, reduced water loss for selection 'OA 184' fruit, after withdrawal from storage (2.0°C and 5.5°C), was able to prolong shelf-life and commodity quality to 6 and 5 days, respectively. Prolonged shelf-life for selection 'OA 184' fruit was assumedly attributed to reduced ethylene biosynthesis stimulation, leading to reduced cellular activity (abscisic acid signalling and fruit softening) and increased fruit quality, in agreement with Kok *et al.*, (2010).

#### 4.2.9 Seed:fruit weight ratio

Seed:fruit weight ratio indicated the proportion of the edible part compared to the non-edible seed portion in 'Hass' type avocado selections. Selection 'Balboa' had bigger fruit but a low seed:fruit weight ratio, compared to selection 'OA 184', 'Jalna'

and the control fruit. However, selection 'Jalna' had a bigger seed:fruit weight ratio, while it had a lower fruit weight, compared to selection 'Balboa'. This study illustrated that the relationship between seed and fruit weight was selection based.

## CHAPTER 5

### SUMMARY, FUTURE RESEARCH AND CONCLUSION

#### 5.1 Summary

In this study, electrical conductivity was not a reliable indicator of the presence and prevalence of chilling injury. Fruit firmness for selection 'Jalna' and 'OA 184' fruit did not serve as an accurate indicator of ripening, due to a genetically non-softening exocarp. Selection 'Jalna' fruit started changing colour whilst in storage (2.0°C and 5.5°C) and rapidly ripened during days to ripening. It was evident that storage at a lower temperature extended shelf life in selection 'OA 184' and 'Jalna' fruit, compared to storage at a higher temperature.

#### 5.2 Recommended future research

The current study was part of the ARC-ITSC phase II 'Hass' type avocado selection evaluation for registration and export. Therefore, the following were identified for future research:

- Evaluating selection 'OA 184', 'Jalna' and 'Balboa' in other South African avocado producing regions.
- Physiological (phenolics, sugars, vitamin A and E, antioxidants and lipid content) mechanism analysis, as avocado fruit quality is highly prioritised in the international trade.
- Evaluating the effect of cold sterilisation (1.0°C) on internal and external quality variables of the new 'Hass' type avocado selections to enable the South African avocado industry to gain access to high paying markets (USA, China and Japan), which require cold sterilisation against *Bactrocera dorsalis*, as a phytosanitary requirement.
- Ripening of selection 'OA 184', 'Jalna' and 'Balboa' fruit at various temperature regimes (16.0°C, 21.0°C and 25.0°C) to validate variability in fruit ripening, skin colour development and respiration rate.
- Conducting a consumer hedonic research to relate consumer acceptability with organoleptic variables, to recommend the selections to the avocado industry.

### 5.3 Conclusion

Selection 'OA 184' was found to be promising with improved physico-chemical and shelf-life properties, warranting further research. Results provided cold storage potential and physico-chemical ripening properties of the new 'Hass' type avocado selection fruit. Semi-commercial trials should be conducted to validate production regions and seasonal variation in 'Hass' type avocado selection fruit.

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## APPENDICES

Appendix 1 Analysis of variance for effect of maturity time and selection on fruit moisture content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	1.056	0.528	0.18	
Selection	3	62.556	20.852	7.21	0.002
Time (weeks)	2	45.056	22.528	7.79	0.003
Selectionx time (weeks)	6	10.278	1.713	0.59	0.733
Error	22	63.611	2.891		
Total	35	182.556			

Appendix 2 Analysis of variance for effect of storage temperature and selection on electrical conductivity

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	152.21	76.11	0.86	
Selection	3	488.72	162.91	1.84	0.187
Storage temperature	1	75.81	75.81	0.86	0.371
Selection x storage temperature	3	1862.80	620.93	7.01	0.004
Error	14	1240.93	88.64		
Total	23	3820.48			

Appendix 3 Analysis of variance for effect of storage temperature and selection fruit external chilling injury

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.04083	0.02042	0.81	
Selection	3	0.76792	0.25597	10.17	<0.001
Storage temperature	1	0.02042	0.02042	0.81	0.383
Selectionx storage temperature	3	0.02792	0.00931	0.37	0.776
Error	14	0.35250	0.02518		
Total	23	1.20958			

Appendix 4 Analysis of variance for effect of selection, storage temperature and ripening days on skin eye colour rating

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	0.23514	0.11757	1.97	
Selection	3	57.11681	19.03894	319.40	<0.001
Storage temperature	1	0.30201	0.30201	5.07	0.027
Ripening days	6	132.94611	22.15768	371.72	<0.001
Selection x storage temperature	3	1.44664	0.48221	8.09	<0.001
Selection x ripening days	10 (8)	14.61575	1.46157	24.52	<0.001
Storage temperature x ripening days	5 (1)	2.87518	0.57504	9.65	<0.001
Selection x storage temperature x ripening days	8 (10)	1.88906	0.23613	3.96	<0.001
Error	72 (38)	4.29187	0.05961		
Total	110 (57)	131.11465			

Appendix 5 Analysis of variance for effect of selection, storage temperature and ripening days on skin hue angle ( $h^\circ$ )

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	605.6	302.8	3.03	
Selection	3	47910.4	15970.1	159.69	<0.001
Storage temperature	1	846.2	846.2	8.46	0.005
Ripening days	6	121441.9	20240.3	202.40	<0.001
Selection x storage temperature	3	2376.4	792.1	7.92	<0.001
Selection x ripening days	10 (8)	14971.5	1497.1	14.97	<0.001
Storage temperature x ripening days	5 (1)	7047.2	1409.4	14.09	<0.001
Selection x storage temperature x ripening days	8 (10)	4038.6	504.8	5.05	<0.001
Error	72 (38)	7200.3	100.0		
Total	110 (57)	143867.8			

Appendix 6 Analysis of variance for effect of selection, storage temperature and ripening days on skin lightness (L)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	3.3459	1.6730	2.36	
Selection	3	118.7258	39.5753	55.75	<0.001
Storage temperature	1	26.5279	26.5279	37.37	<0.001
Ripening days	6	333.5661	55.5943	78.31	<0.001
Selection × storage temperature	3	120.2574	40.0858	56.47	<0.001
Selection × ripening days	10 (8)	55.4591	5.5459	7.81	<0.001
Storage temperature × ripening days	5 (1)	30.7255	6.1451	8.66	<0.001
Selection × storage temperature × ripening days	8 (10)	15.4641	1.9330	2.72	0.011
Error	72 (38)	51.1131	0.7099		
Total	110 (57)	467.9551			

Appendix 7 Analysis of variance for effect of selection, storage temperature and ripening days on skin chroma (C)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	1.6264	0.8132	0.84	
Selection	3	81.4845	27.1615	27.90	<0.001
Storage temperature	1	76.1706	76.1706	78.25	<0.001
Ripening days	6	653.1678	108.8613	111.83	<0.001
Selection × storage temperature	3	175.6537	58.5512	60.15	<0.001
Selection × ripening days	10 (8)	108.3834	10.8383	11.13	<0.001
Storage temperature × ripening days	5 (1)	11.1359	2.2272	2.29	0.055
Selection × storage temperature × ripening days	8 (10)	16.7288	2.0911	2.15	0.042
Error	72 (38)	70.0855	0.9734		
Total	110 (57)	739.6135			

Appendix 8 Analysis of variance for effect of selection, storage temperature and ripening days on fruit respiration rate

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	3886341.	1943170.	40.77	
Selection	3	4400538.	1466846.	30.78	<0.001
Storage temperature	1	788156.	788156.	16.54	<0.001
Ripening days	6	16113865.	2685644.	56.35	<0.001
Selectionx storage temperature	3	1813426.	604475.	12.68	<0.001
Selectionx ripening days	10 (8)	6257900.	625790.	13.13	<0.001
Storage temperaturex ripening days	5 (1)	3758597.	751719.	15.77	<0.001
Selectionx storage temperaturex ripening days	8 (10)	3834472.	479309.	10.06	<0.001
Error	72 (38)	3431612.	47661.		
Total	110 (57)	39108578.			

Appendix 9 Analysis of variance for effect of selection, storage temperature and ripening days on fruit firmness

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	2.702	1.351	0.53	
Selection	3	5291.331	1763.777	693.65	<0.001
Storage temperature	1	1131.943	1131.943	445.16	<0.001
Ripening days	6	8277.524	1379.587	542.56	<0.001
Selectionx storage temperature	3	193.332	64.444	25.34	<0.001
Selectionx ripening days	10 (8)	1539.016	153.902	60.53	<0.001
Storage temperaturex ripening days	5 (1)	303.778	60.756	23.89	<0.001
Selectionx storage temperaturex ripening days	8 (10)	202.186	25.273	9.94	<0.001
Error	72 (38)	183.078	2.543		
Total	110 (57)	12664.047			

Appendix 10 Analysis of variance for effect of selection, storage temperature and ripening days on fruit ripening percentage

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	57.26	28.63	0.73	
Selection	3	24913.81	8304.60	211.37	<0.001
Storage temperature	1	7375.53	7375.53	187.72	<0.001
Ripening days	6	38594.65	6432.44	163.72	<0.001
Selectionx storage temperature	3	1812.56	604.19	15.38	<0.001
Selectionx ripening days	10 (8)	27590.91	2759.09	70.23	<0.001
Storage temperaturex ripening days	5 (1)	8476.14	1695.23	43.15	<0.001
Selectionx storage temperaturex ripening days	8 (10)	10877.83	1359.73	34.61	<0.001
Error	72 (38)	2828.83	39.29		
Total	110 (57)	100372.97			

Appendix 11 Analysis of variance for effect of selection, storage temperature and ripening days on fruit water loss

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	1488.156	744.078	198.24	
Selection	3	277.660	92.553	24.66	<0.001
Storage temperature	1	1.465	1.465	0.39	0.534
Ripening days	6	13.579	2.263	0.60	0.727
Selectionx storage temperature	3	111.054	37.018	9.86	<0.001
Selectionx ripening days	10 (8)	3.666	0.367	0.10	1.000
Storage temperaturex ripening days	5 (1)	16.233	3.247	0.86	0.509
Selectionx storage temperaturex ripening days	8 (10)	3.523	0.440	0.12	0.998
Error	72 (38)	270.247	3.753		
Total	110 (57)	1511.754			

Appendix 12 Analysis of variance for effect of selection on seed: fruit weight ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	4.1213	2.0606	3.49	
Storage temperature	1	3.5971	3.5971	6.10	0.027
Selection	3	744.7269	248.2423	420.94	<0.001
Storage temperaturex selection	3	19.2033	6.4011	10.85	<0.001
Error	14	8.2562	0.5897		
Total	23	779.9047			

Appendix 13 Abstract submitted and presented as a poster at the combined congress, George, South Africa, 19-23 January 2015

## **EVALUATION OF COLD STORAGE POTENTIAL AND SHELF-LIFE OF NEW 'HASS' TYPE AVOCADO SELECTIONS**

**P.B. Machipyane<sup>1,2</sup>, T.P. Mafeo<sup>1</sup>, N Mathaba<sup>2</sup> and J Mlimi<sup>2</sup>**

<sup>1</sup>University of Limpopo-Turfloop Campus, Plant Science, Sovenga, 0727

<sup>2</sup>Agricultural Research Council-Institute for Tropical and Subtropical Crops, Private Bag x 11208, Nelspruit, 1200

**Keywords:** Avocado fruit; colour parameters; days to ripening; electrical conductivity; firmness; respiration

### **INTRODUCTION**

Cold storage can prolong storage life and quality of avocado as it reduces ethylene production, respiration rate and ripening. However, it can lead to reduced firmness and physiological disorders such as vascular staining, stem-end rot, chilling injury and anthracnose. There is a need for continued development of more competitive, viable avocado cultivars with superior characteristics to enhance competitiveness and profitability in the South African avocado industry. In response to these challenges, the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) has bred and selected new superior 'Hass' type avocado selections. Therefore, the aim of this study was to determine the response of some of these new 'Hass' type avocado selections to the mandatory low temperature required for prolonged storage.

### **MATERIALS AND METHODS**

Mature new 'Hass' type avocado fruit ('Jalna', 'S-SS2') were harvested from the Burgershall, ARC-ITSC gene block and transported to the ARC-ITSC Post harvest laboratory in Nelspruit for analysis. At the laboratory the fruit were sorted, graded and stored at 5.5°C for up to 28 days. Ten fruit per tree, per selection were harvested. The experiment was a factorial, arranged in a completely randomised design (CRD) which consisted of three boxes with nine fruit as one of the fruit was

destructively used for moisture analysis, replicated three times (27 fruit). After cold storage, fruit were kept at ambient temperature to ripen. During ripening fruit were evaluated for fruit water loss, firmness, colour change (Lightness, chroma, hue) and respiration rate (CO<sub>2</sub>).

## **RESULTS AND DISCUSSION**

According to Cutting *et al.*, (1988), during avocado ripening respiration increases, culminating in reduced fruit quality and shelf-life. Selections under investigation showed a decrease in colour parameters (lightness, chroma, hue), a characteristic of commercial 'Hass' (Cox *et al.*, 2004). 'S-SS2' showed increased chilling injury, electrolyte leakage, respiration rate and reduced synchronisation of flesh softening and firmness (fruit ripened from the inside out). Furthermore, 'Jalna' had accelerated respiration rate (CO<sub>2</sub>), water loss and therefore, reduced shelf-life.

## **CONCLUSION**

'S-SS2' appeared to have export potential due to reduced ripening rate, prolonged shelf-life, however, its downfall is predominantly chilling injury.

Appendix 14 Abstract submitted and presented as oral at the 6<sup>th</sup> Faculty of Science and Agriculture Research Day, Bolivia lodge, Polokwane, South Africa, 1-2 October 2015

## **EVALUATION OF COLD STORAGE POTENTIAL AND SHELF-LIFE OF NEW 'HASS' TYPE AVOCADO SELECTIONS**

**P.B. Machipyane<sup>1,2</sup>, T.P. Mafeo<sup>1</sup>, N. Mathaba<sup>2</sup> and T.J. Mlimi<sup>2</sup>**

<sup>1</sup>University of Limpopo-Turfloop Campus, Plant Science, Sovenga, 0727

<sup>2</sup>Agricultural Research Council-Institute for Tropical and Subtropical Crops, Private Bag x 11208, Nelspruit, 1200

### **INTRODUCTION**

The existing South African 'Hass' type avocado cultivars are inadequate to enhance competitiveness in export markets (Ramosodi, 2006). As a result, the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) has developed new 'Hass' type selections: 'Jalna', 'OA184' and 'Balboa', in an effort to mitigate potential competition challenges and to expand consumer choice. Cold storage can extend the storage-life of an avocado fruit as it reduces ethylene production, colour change, ripening and respiration rate (Blakey *et al.*, 2014; Perez *et al.*, 2004). However, cold storage can lead to reduced firmness and chilling injury (Zagory and Kader, 1988). Nonetheless, cold storage potential of these selections is not yet apprehended nor documented and as such should be assessed prior registration and release to avocado growers. Therefore, the aim of this study was to determine the response of these new 'Hass type' avocado selections to low temperature (2.0°C and 5.5°C) for prolonged storage.

**Keywords:** 'Balboa'; cold storage; colour change; days to ripening; firmness; 'Jalna'; 'OA184'; Respiration rate.

### **MATERIALS AND METHODS**

Mature 'Hass' type avocado fruit ('Jalna', 'OA184' and 'Balboa') and the control (commercial 'Hass') were harvested from Burgershall Experimental Farm ARC-ITSC gene block and transported to the ARC-ITSC Post harvest laboratory in Nelspruit. Fruit were harvested from three trees per selection and the experiment was a



completely randomised factorial design with two temperature regimes (2.0°C and 5.5°C) × three selections ('Jalna', 'OA184' and 'Balboa') and the control (commercial 'Hass') replicated three times. In the laboratory, fruit were sorted, graded and stored under two temperature regimes (2.0 and 5.5°C) for 28 days. After withdrawal from cold storage, fruit were kept at ambient temperature to ripen. During ripening, fruit were assessed for the following physico-chemical properties: fruit water loss, skin colour (objective skin colour changes (lightness, chroma, hue angle) and subjective colour changes (eye colour rating), fruit firmness, respiration rate (CO<sub>2</sub>), chilling injury and seed mass.

## **RESULTS AND DISCUSSION**

According to Bower and Jackson (2003), low temperature affects mass loss of avocado fruit. The current findings showed that storage at 2.0°C and 5.5°C had no significant effect on fruit water loss, as 'Hass' type avocado fruit had no significant difference in water loss, during days to ripening. 'Hass' type avocado selections had significantly higher cell damage indicated by electrical conductivity at after withdrawal from storage at 2.0°C compared to 5.5°C, primarily due to cold stress at lower temperature. Fruit firmness was not a reliable indicator of fruit ripening for 'OA 184' and 'Jalna' rather respiration, which decreased with fruit ripening. During ripening, selections under investigation showed a decrease in colour parameters (lightness, chroma, hue angle), a characteristic of commercial 'Hass' (Cox *et al.*, 2004). Stress imposed by low temperature storage triggered a colour response as a defense mechanism in 'Jalna' fruit, which were already changing colour while in storage (2.0°C and 5.5°C). In 'Hass' avocado, anthocyanin acts as an antioxidant and releases free radical scavengers which alleviate tissue damage under cold stress triggering fruit colour change (Bower and Bertling, 2008).

## **CONCLUSION**

'OA 184' is a potential selection as it had longer shelf-life and improved physico-chemical properties, during days to ripening.