EFFECT OF HARVEST SEASON AND TIME, RIPENING TEMPERATURE AND DAYS ON DE-SYNCHRONISATION OF 'HASS' AVOCADO FRUIT SKIN COLOUR CHANGE WITH SOFTENING DURING RIPENING

by

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DECLARATION

I, Zwoitwaho Maureen Nthai, hereby declare that this mini-dissertation submitted to the University of Limpopo, for the degree of Masters 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 in execution, and that all material contained herein has been fully acknowledged.

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Student: Ms ZM Nthai Date

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DEDICATION

I would like to dedicate this study to my loving and caring mother (Ms M.M. Phadziri), my handsome son (Dziphathutshedzo Nthai) and my loving siblings (Livhuwani and Denga)

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ABSTRACT

Avocado ‘Hass’ fruit characteristically change skin colour from green to deep purple or black during ripening. However, there is an ongoing debate about the use of ‘Hass’ avocado fruit skin colour change as an indicator for ripening and whether pre- and post-harvest factors can alter this relationship. Thus, the aim of the study was to investigate the effect of harvest season, harvest time, ripening temperature and ripening days on ‘Hass’ avocado fruit skin colour change during ripening. The experiment was carried out as 2 x 3 x 3 x 5 factorial with three replicates. The experiment consisted of four treatment factors: 2 x harvest season (2014 and 2015), 3 x harvest time (May-early, June-mid and July-late), 3 x ripening temperature (16, 21 and 25°C) and 5 x ripening days (0, 2, 4, 6 and 8). Fruit were stored at industry recommended temperature of 5.5°C. After storage, fruit were ripened at 16, 21 and 25°C, therefore, evaluated at 0, 2, 4, 6 and 8 days for subjective and objective skin colour, fruit firmness, ripening percentage and chilling injury. Harvest season, harvest time, ripening temperature and ripening days had a significant effect ($P<0.001$) on ‘Hass’ avocado fruit skin subjective colour development during ripening. However, amongst the treatment factors; harvest time, ripening temperature and ripening days were the predominant factors in skin subjective colour development variation. Hence, late harvest fruit showed an improved skin colour development at higher temperature (25°C) at day 4 to ripening when compared with early and mid-harvest fruit. Moreover, skin lightness showed a decreasing trend during all harvest time and ripening temperature throughout days to ripening during 2014 and 2015 harvest season. Furthermore, ripening at higher temperature (25°C) resulted in rapid decrease on fruit firmness when compared with lower temperature (16°C), irrespective of harvest season and harvest time. In addition, mid-season fruit showed significantly higher chilling damage during the 2014 harvest season. In conclusion, the study showed that harvest season, harvest time, ripening temperature and ripening days factors had a significant influence on ‘Hass’ avocado fruit skin colour development, firmness and susceptibility to chilling injury.

Keywords: Chilling injury, chroma (C*); firmness, hue angle (*h*°); lightness (L*)

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

The world avocado fruit market trends indicate an increasing demand for ‘Hass’. ‘Hass’ avocado is known internationally for its high vitamin content and versatility for use in different dishes. Physiologically, ‘Hass’ avocado fruit undergo a characteristic skin darkening during softening. This is commonly used by consumers as an indicator of fruit ripeness (Donetti and Terry, 2012). However, the reliability of ‘Hass’ avocado fruit skin colour change as an indicator of ripening has previously been questioned (Cox *et al.,* 2004). Moreover, higher ripening temperatures between (20-25°C) have been found to induce darker colouration when compared with fruit ripened at 15°C (Cox *et al.,* 2004). It has been suggested that ‘Hass’ avocado fruit can fully develop a dark or purple skin colour depending on the fruit growing area (Cox *et al.,* 2004). Currently, the export market is experiencing problems with ‘Hass’ avocado fruit skin colour not changing during ripening. There is also an ongoing debate on the use of skin colour change as an indicator of ‘Hass’ avocado fruit ripening and whether pre- and post-harvest factors can alter this relationship. According to Hewett (2006), post-harvest fruit quality can be determined by pre-harvest factor during development.

1.2 Problem statement

The ‘Hass’ avocado fruit skin colour change after harvest has been established as a ripening variable by growers, exporters and consumers. However, a few exporters are experiencing challenges with colour change of ‘Hass’ avocado fruit from South Africa, whereby, skin colour change is not synchronised with softening as expected (Newett *et al*., 2002; Ashton *et al.,* 2006). This problem has been found to be more prevalent with certain export consignments and that it varies with harvest time. Therefore, a request was made by South African Avocado Growers’ Association (SAAGA) to research the effect of harvest season, harvest time, ripening temperature and ripening days in variable colouring of ‘Hass’ avocado fruit.

1.3 Rationale

The colouration of ‘Hass’ avocado fruit has been confusing producers, marketers and customers as the fruit appears to be ripe, but is not soft. Further understanding of ‘Hass’ avocado fruit skin colour change with softening during ripening still need to be developed. This may lead to a better understanding phenomenon and ability of the industry to manipulate suitable conditions to co-ordinate softening and colour change. The current study was prompted by the knowledge that post-harvest fruit quality was determined during pre-harvest growth and development maintained by post-harvest technologies (Hewett, 2006). Furthermore, successful determination of factors causing ‘Hass’ avocado fruit skin colour not to change with softening during ripening could improve the status of ‘Hass’ avocado fruit exported from South Africa.

1.4 Aim and objective

1.4.1 Aim

The aim of this study was to investigate the effect of harvest season, harvest time, ripening temperatures and ripening days on ‘Hass’ avocado fruit skin colour change during ripening.

1.4.2 Objective

To investigate whether harvest season, harvest time, ripening temperatures and ripening days have effect on ‘Hass’ avocado fruit skin colour change with softening during ripening.

1.5 Hypothesis

Harvest season, harvest time, ripening temperatures and ripening days have no effect on ‘Hass’ avocado fruit skin colour change with softening during ripening.

CHAPTER 2
LITERATURE REVIEW

2.1 The effect of orchard management on fruit quality

Pre-harvest factors such as irrigation, pruning, thinning, soil type and climatic condition and crop management practices and water status influence fruit quality (Thompson, 2003). Previous studies argued that orchard and orientation management could optimise sunlight distribution, therefore, the amount of photosynthetically active radiation directed towards fruit quality improvement (Corelli-Grappadelli and Lakso, 2002). This was shown by Mowat (1992) who demonstrated that a series of climatic and orchard management practices were important in obtaining persimmon fruit quality such as fruit weight, soluble solids and soluble tannins during the growing season. Furthermore, good orchard management practices had also been found to positively influence 'Fuyu' persimmon fruit quality when compared with poor orchard management practices (Mowat and Chee, 2011). Crisosto *et al*. (1997) reported that good orchard management attributed to high fruit quality for stone fruit. Skin colour development of several fruit including avocado and apple fruit depends on environmental factors such as light intensity, orchard temperature, elevation, soil type and cultural practices (Chen *et al*., 2009; Lloyd and Farquhar, 2008). In apples, colour development was found to be influenced by both environmental and orchard management factors (Iglesias *et al*., 2002). Therefore, orchard management decision and application made during fruit production, production environment are vital for product quality which reaches the consumers (Kader, 1992). In avocado orchards, innovative technologies are required for adequate light management to enhance the rate of photosynthesis during the growing season. Consequently, such strategies could assist in minimising 'Hass' avocado fruit skin colour problem.

2.2 Effect of harvest time, ripening temperature and days on skin colour development

'Hass' avocado fruit skin colour is an important evolutionary trait used by both industry and consumers as an indicator of the ripening stage (Arzate-Vazquez *et al*.,

2011). Colour is measured either by objective means using a chromameter or using subjective means, eye colour rating. According to Barrett *et al*. (2010), fruits and vegetables skin colour is derived from the natural pigments which changes as the plant reaches maturation and ripening. In a previous study by Donetti (2011), 'Hass' avocado fruit skin colour showed similar trend on chroma, lightness and hue angle during early, mid-and late harvest time. Moreover, Osuna-Garcia *et al*. (2010) found 'Hass' avocado fruit pulp colour to be significantly affected by both harvest time and degree of blackened skin.

Furthermore, higher de-synchronised colouring was reported for early harvested 'Hass' avocado fruit from cooler parts of the orchard block with slope (Mathaba *et al*., 2015). Testoni (2002) stated that harvesting persimmons fruit early in the growing season may lead to pale orange colour with yellow discolourations when compared with the expected red colouration. Additionally, Kok *et al.* (2010) validated that early harvested 'Hass' avocado fruit were prone to uneven colour change when compared with later harvested fruit. According to Itoo (1986), colour development in persimmon fruit at late harvest maturity was due to increased carotenoids and xanthophylls with the total content of lycopene. Meanwhile, Cox *et al*. (2004) found skin colour change on late harvested 'Hass' avocado fruit to be as result of a decrease in chlorophyll content and an increase in cyanidin 3-*O*-glucoside.

Ripening temperature is considered as the main factor affecting fruit quality (Hopkirk *et al*., 1994). In black-skinned avocado cultivars such as 'Hass', there is often confusion between skin colour development and post-harvest disorders during ripening (Hofman *et al*., 2002). Blakey (2011) found that ripening of 'Hass' avocado fruit at low temperature (15°C) resulted in less ripe fruit with acceptable colour quality. Whereas, ripening at higher temperature (20°C), the percentage of ripe fruit with black skin colour increased when compared with lower temperature. Furthermore, Hofman *et al*. (2002) found that 'Hass' avocado fruit ripened at 17°C took longer to reach black skin colour when compared with fruit ripened 24°C. In a study conducted by Cox *et al*. (2004), 'Hass' avocado fruit ripened at 15°C failed to exhibit fully black colouration when compared with those held at 20 and 25°C

Chen *et al*. (2009) found a positive correlation (R^2=0.21) between skin colour of 'Sharwil' avocado fruit and days to ripening. Moreover, Cox *et al*. (2004) found initial colour change from "emerald green" to "darker green" within 3 days to ripening. Donetti and Terry (2012) reported that at 7 days to ripening, 'Hass' avocado fruit harvested in July showed a darker skin colour which was prominent by a lower hue angle when compared with fruit harvested in June. In addition, Woolf and Laing (1996) found chlorophyll concentration in 'Hass' avocado fruit skin to be stable at day 3 to ripening for both unripe and ripe fruit.

2.3 Effect of harvest time, ripening temperature and days on fruit firmness

Fruit firmness is an important determinant when assessing the degree of ripeness and has also been well documented that changes in cell wall are related to fruit firmness (Flitsanov *et al*., 2000). Firmness can be evaluated by hand tactile test, destructive methods (penetrometers) and Sinclair (Ochoa-Ascencio *et al*., 2009). Previously, Mizrach *et al*. (2000) found a strong correlation between 'Ettinger' avocado fruit firmness and maturity stage and expected storage time. In a previous study, late harvest 'Hass' avocado fruit showed the highest firmness faster when compared with mid-harvest (Kok, 2011). Furthermore, Zauberman *et al*. (1986) also found late harvest 'Fuerte' avocado fruit to soften at accelerated rate when compared with earlier harvest.

Blakey (2011) suggested that ripening temperature affects fruit metabolic rate by and hastening and reducing enzyme activities, which are known to occur at a certain temperature range. In a study by Zamorano *et al*. (1994), 'Fuerte' and 'Hass' avocado fruit firmness decreased rapidly within 7-9 days when ripened at 20°C when compared with fruit ripened at lower temperature (15°C). Additionally, higher ripening temperature (23°C) decreased 'Hass' avocado fruit firmness at an accelerated rate when compared with (18°C) (Donetti and Terry, 2012). Ahmad *et al*. (2001) found 'Cavendish' banana ripened at higher temperature (20°C) to have higher fruit firmness compared with that at lower temperature (16 and 18°C) at day 3 of ripening.

2.4 Effect of harvest time, ripening temperature and days on chilling injury

Kok (2011) defined chilling injury as an irreversible physiological damage to plant tissues, cell and organs which results from prolong exposure to temperatures belong critical threshold for that species or tissues. Therefore, avocado fruit 'Pinkerton' exposed to a chilling temperature below critical threshold for longer experiences high chilling damage (Van Rooyen, 2006). According to Wang (2010), chilling injury sensitivity may vary with development stage or maturity. Immature and begin ripe 'Tommy Atkins' mango fruit were highly susceptible to chilling injury when compared with early ripening stage (Mohammed and Brecht, 2002). Kok (2011) found early harvest fruit to be highly chilling susceptible when compared with mid-and late harvest 'Hass' avocado fruit. Also, Nilsen and Orcutt (1996) showed that lipid membranes saturation contributed to the development of chilling injury in early season fruit.

Chilling injury symptoms of 'Fuerte' avocado fruit does not manifest while fruit still under storage but become prevalent during ripening (Cantin *et al*., 2010). Moreover, ripening of 'Hass' avocado fruit at 15°C was able to reduce external chilling damage compared with 20 and 30°C (Woolf and Ferguson, 2000). Kane *et al*. (1982) also found ripening of 'Kent' mango fruit at 20°C reduced chilling injury symptoms.

2.5 Ripening physiology of 'Hass' avocado fruit

In avocado fruit, ripening takes place between maturity and senescence. Ripening is well known process associated with change in colour, taste and texture (Lütge, 2011). According to Lügte (2011), ripening can only be achieved when normal softening and certain maturity stage has been reached. Moreover, fruit softness during ripening was suggested to be related in loosening and degradation of cell wall (Kok, 2011). Lütge (2011) found that during ripening of 'Fuerte' avocado new pigments were associated with the anabolic process. Therefore, chloroplast thylakoids or cell wall breakdown was linked with catabolic processes (Bosse, 2012). In avocado fruit, enzyme active during the ripening process has been described (Kok *et al*., 2010). These include cellulase, poly galacturonase (PG) and pectin

methylesterase (PME). According to the Q_{10} principle, for a 10°C increase in temperature the rate of enzyme activities doubles (Wills *et al*., 2008). Therefore, the use of low storage temperature to minimise enzyme activities responsible for breaking down cell wall leading to fruit softening, while, extending fruit shelf-life is of paramount importance.

2.6 'Hass' avocado fruit skin colour development physiology

The pigment change during ripening contribute to skin colour change from green to purple to black in 'Hass' avocado fruit (Cox *et al*., 2014). During the ripening stage, chlorophyll decrease while anthocyanin (especially, cyanidin -*3*-*O*-glucoside) increase, therefore, responsible for purple colour (Ashton *et al*., 2006). Anthocyanins are a diverse group of water-soluble phenolic compounds with 15 carbon unit bound to sugars (Andersen, 2001). Furthermore, anthocyanins occur as glycoside of a glycone anthocyanidin chromophore with a sugar moiety attached at the third carbon ring position (Prior and Wu, 2006). Therefore, sugars regulate genes, enzymes (chalcone synthase – CHS and anthocyanin reductase - ANR) and proteins encoding and involved in the synthesis of anthocyanin (Mita *et al*., 2006).

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Experimental sites

Matured ‘Hass’ avocado fruit were harvested in May, June and July; during 2014 and 2015 harvest seasons in Nico Swart Estate (25°27′6845″S 30°58′337″E) near Kiepersol area, Mpumalanga Province. Afterwards, ‘Hass’ avocado fruit were taken to the Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC) post-harvest laboratory in Nelspruit (25°18′30″S 28°17′37″E) for storage and analysis.

3.2 Experimental design, treatments and procedures

The experiment was carried out as 2 x 3 x 3 x 5 factorial design with three replicates. The experiment consisted of four treatment factors: 2 x harvest season (2014 and 2015), 3 x harvest time (early, mid- and late), 3 x ripening temperature (16, 21 and 25°C) and 5 x ripening days (0, 2, 4, 6 and 8). After each harvest during the season ‘Hass’ avocado fruit were graded and packed in commercial cartons each with 30 fruit counts, thereafter, stored at recommended temperature of 5.5°C for 28 days. After storage, ‘Hass’ avocado fruit were ripened at 16, 21 and 25°C until fully ripe.

3.3 Data collection

3.3.1 Determination of subjective skin colour

Avocado ‘Hass’ fruit skin colour was measured after storage and every other day subjectively using; eye colour rating score (1-5) where; 1-emerald green; 2-forest green; 3-olive green; 4-purple; 5-black (Figure 3.1).

Figure 3.1 Eye skin colour rating using a score plate 1-5 (Mathaba *et al*., 2015)

Objective skin colour parameter lightness (L*), a* value (redness or greenness) and b* value (yellowness or blueness) were measured using; Minolta chromameter (model; CR-400, Minolta Corp, Ramsey, N. USA) (Figure 3.2). The value of hue angle (h^0) and chroma (C*) was calculated from a* and b* using the formula: $\tan^{-1}$ (a*/b*) and C*= (a*^^{2} + b*^^{2})$^{1/2}$ as previously described by McGuire (1992).

Figure 3.2 Chromameter used to measure 'Hass' avocado fruit skin colour parameters (lightness (L*), a* and b* values)

3.3.2 Determination of fruit firmness

Fruit firmness was measured from 30 fruit after removal from cold storage on every other day using a non-destructive automated Sinclair IQ^{TM} desktop firmness machine (Model: 51DFTB, International LTD, Jorrold, Bowthorpa, Nonwich, NR5, 9.D, England) (Figure 3.3), by taking four measurements along the equatorial parts of fruit and the results were recorded in Sinclair units (SU). Thereafter, fruit were used to measure days to ripening. The same fruits were measured every other day of ripening period until full ripening is reached at equivalent to 25 Sinclair units (SU).

Figure 3.3 Sinclair IQ™ automated desktop firmness machine used to measure firmness of 'Hass' avocado fruit

3.3.3 Determination of ripening percentage

Ripening percentage was calculated from fruit firmness using the following formula: Ripening % = [(number of fully ripe fruit ÷ total number of fruit evaluated) *100].

3.3.4 Determination of external chilling injury

Avocado fruit 'Hass' skin chilling injury was visually assessed and the results expressed using chilling injury % = [(number of fruit with chilling injury symptoms ÷ total number of fruit evaluated) *100]

3.3.5 Data analysis

Statistical analyses were carried out using a windows software GenStat® version 16th (VSN International, Hemel Hempstead, UK, 2014). Analysis of variance (ANOVA) for subjective skin colour, lightness (L*), a* value (redness or greenness) and b* value (yellowness or blueness), fruit firmness, ripening percentage and chilling injury was done followed by mean comparison using Duncan Multiple Range Test at $P \leq 0.01$ or $P \leq 0.05$.

CHAPTER 4
RESULTS

Only third order interaction was used to explain the interactive effect of harvest time, ripening temperature and ripening days for all measured variables and second order interaction for chilling injury.

Subjective skin colour

Interaction between harvest time, ripening temperature and ripening days had a significant effect ($P<0.001$) on 'Hass' avocado fruit skin eye colour development during both 2014 and 2015 season (Appendix 1 and 2). There were no mean significant differences on subjective skin colour development for all harvesting times when fruit ripened at 25, 21 and 16°C up to day 4, 6 and 8 during both seasons, respectively (Table 4.1 and 4.2). In both harvesting seasons, skin colour development synchronised with ripening at day 4 under 25°C during all harvesting time (Figure 4.1-4.2). At lower temperature (16°C), skin colour development synchronisation was extended to 8 days during all harvesting time.

Objective skin colour

Lightness (L*)

Interaction between harvest time, ripening temperature and ripening days had no significant effect ($P=0.356$) on 'Hass' avocado fruit skin L* during ripening (Appendix 3 and 4). There were mean significant differences on L* for all harvesting times when fruit ripened at 25, 21 and 16°C up to day 4, 6 and 8 during both seasons, respectively (Table 4.1 and 4.2). In all harvesting times, fruit ripened at 25 and 16°C showed the lowest and highest L* means at day 4 for both seasons, respectively.

Chroma (C*)

Interaction between harvest time, ripening temperature and ripening days had no significant effect ($P=0.809$ and $P=0.815$) on 'Hass' avocado fruit skin C* during

ripening (Appendix 5 and 6). During the 2014 and 2015 season, late and mid-harvest fruit ripened at 25°C showed the highest (18.12±0.57) and lowest (7.13±0.26) at day 4 to ripening (Table 4.1 and 4.2).

Hue angle (*h°)*

Interaction between harvest time, ripening temperature and ripening days had a significant effect (P<0.001) on 'Hass' avocado fruit skin *h*° during ripening (Appendix 7 and 8). However, there were no mean significant differences on skin *h*° for early and late harvest fruit ripened at 21°C for up to day 6 to ripening during the 2014 and 2015 harvest season (Table 4.1 and 4.2). Contrary, late harvest fruit ripened at 25°C showed the highest (111.85±1.01) skin *h*° after 4 days to ripening during the 2014 season. The late harvest fruit ripened at 16°C showed the lowest (75.00±0.59) skin *h*° at day 6 during 2015 season.

Fruit firmness

Interaction between harvest time, ripening temperature and ripening days had a significant effect (P<0.001) on 'Hass' avocado fruit firmness during ripening on both 2014 and 2015 season (Appendix 9 and 10). During 2014 harvest season, mid-harvest fruit ripened at 21°C showed the lowest firmness value (14.76±0.13) at day 6 to ripening. The late harvest fruit ripened at 16°C showed the highest firmness value (27.06±1.01) value at day 8 of ripening (Table 4.1 and 4.2). Moreover, during 2015 harvest season, early and late harvest fruit ripened at 25°C showed the highest firmness value of 25.00±0.13 and lowest 18.06±0.49 at day 4 to ripening.

Ripening percentage

Interaction between harvest time, ripening temperature and ripening days had a significant effect (P<0.001) on 'Hass' avocado fruit ripening percentage during ripening (Appendix 11 and 12). During the 2015 season, early and mid-harvest fruit ripened at 21 and 16°C showed 100% ripeness at day 6 and 8 to ripening, respectively. Meanwhile, late harvest fruit ripened at 16°C showed 100% ripeness at

day 8 to ripening (Table 4.1 and 4.2). Moreover, during 2014 season, mid-harvest fruit ripened at 21 and 16°C obtained 100% ripeness at day 6 and 8, respectively. However, during 2014 and 2015, early and late harvest fruit held to ripened at 25°C showed 0% ripeness at day 2 to ripening.

Chilling injury

An interaction of harvest season and ripening temperature had a significant effect ($P<0.001$) on 'Hass' avocado fruit skin chilling injury during ripening (Appendix 13). In 2014, 'Hass' avocado fruit showed higher skin chilling injury when compared with 2015 harvest season (Figure 4.3). furthermore, 'Hass' avocado fruit skin chilling injury was minimal at lower ripening temperature (16°C), highest at 21°C and decreased at high ripening temperature (25°C) during the 2014 harvest season. During the 2015 harvest season, no skin chilling damage observed on 'Hass' avocado fruit ripened at 16 and 21°C, and about 8% chilling injury was observed at high ripening temperature (25°C).

Table 4.1 Effect of harvest time, ripening temperature and days on Hass' avocado fruit subjective and objective skin colour [Lightness (L*), Chroma (C*) and Hue angle (h°)], fruit firmness and ripening percentage during the 2014 season

Harvest season	Ripening temperature (°C)	Ripening days	Eye colour (1-5)	L*	C*	h°	Ripening %	Firmness (N)
Early harvest	25	0	1.00±0.00	35.00±0.15	22.00±0.44	144.24±0.97	0.00±0.00	69.96±0.92
		2	1.78±0.21	31.18±0.29	16.92±0.92	137.80±1.38	0.00±0.00	41.03±0.45
		4	3.05±0.24	27.01±0.14	8.20±0.15	92.01±0.35	67.00±5.13	25.00±0.13
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	35.74±0.21	21.00±0.22	147.09±0.41	0.00±0.00	51.23±0.81
		2	1.78±0.06	34.13±0.47	20.48±0.34	143.04±0.86	7.00 ±3.33	46.23±0.50
		4	2.99±0.21	31.24±0.12	16.36±0.73	122.68±0.79	50.00±1.50	30.60±0.57
		6	4.00±0.16	29.05±0.17	9.92±0.63	79.04±0.42	100.00±0.00	21.36±0.12
		8	*	*	*	*	*	*
	16	0	1.00±0.00	34.17±0.28	20.11±0.39	146.18±0.25	0.00±0.00	57.33±0.52
		2	2.33±0.05	33.19±0.16	18.60±0.21	142.87±1.19	0.00 ±0.00	45.26±0.37
		4	3.00±0.18	32.37±0.65	15.51±0.54	121.23±0.34	3.00 ±0.15	41.26±0.38
		6	3.46±0.21	30.09±0.18	12.35±0.63	112.63±0.29	13.33±3.33	37.10±0.59
		8	4.23±0.17	29.13±0.24	10.01±0.19	75.25±2.62	83.33±6.22	22.06±0.60
Mid-harvest	25	0	1.00±0.00	34.35±0.26	22.60±0.74	143.65±1.16	0.00±0.00	61.33±0.49
		2	1.83±0.36	33.01±0.18	16.84±0.82	124.85±0.71	37.33 ±5.13	30.80±0.50
		4	3.77±0.15	28.00±0.75	7.13±0.26	81.30±0.36	97.00±1.01	20.36±0.18
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	35.41±0.26	21.59±0.21	146.00±0.66	0.00±0.00	49.83±0.28
		2	2.13±0.10	33.19±0.52	20.40±0.44	142.03±0.29	3.33±0.24	41.93±0.77
		4	3.42±0.18	30.23±0.45	16.42±0.63	126.07±0.80	77.00±1.73	34.23±0.61
		6	4.12±0.11	28.10±0.14	9.62±0.36	89.14±0.42	100.00±0.00	24.30±0.35
		8	*	*	*	*	*	*
	16	0	1.00±0.00	35.92±0.67	20.51±0.69	150.68±0.25	0.00±0.00	58.06±0.65
		2	1.37±0.12	34.46±0.46	18.72±0.21	146.66±0.79	0.00 ±0.00	49.70±0.62
		4	2.41±0.14	34.00±0.28	15.51±0.64	130.23±1.34	33.00±2.13	38.90±0.60
		6	3.67±0.25	32.31±0.10	13.19±0.63	125.12±1.28	70.00±1.80	30.10±0.59
		8	4.36±0.19	27.27±0.15	10.06±0.24	86.17±0.44	100.00± 0.00	21.33±0.47
Late harvest	25	0	1.00±0.00	34.12±0.15	20.88±0.40	142.56±1.17	0.00±0.00	48.76±0.77
		2	1.44±0.12	30.28±0.28	19.02±0.46	133.10±0.49	0.00±0.00	30.93±0.87
		4	3.50±0.15	29.30±0.14	10.12±0.88	100.13±0.84	70.00±2.65	18.06±0.49
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	33.90±0.28	23.07±0.54	151.00±1.17	0.00±0.00	59.76±0.15
		2	1.02±0.00	32.51±0.14	22.53±0.35	148.01±0.89	0.00±0.00	34.36±0.34
		4	1.88±0.10	30.33±0.16	12.00±0.41	127.83±0.63	7.00±4.93	27.53±0.57
		6	3.68±0.16	29.18±0.11	8.19±0.40	85.04±0.28	53.00±2.96	20.96±0.43
		8	*	*	*	*	*	*
	16	0	1.00±0.00	34.12±0.21	22.16±0.38	148.09±0.21	0.00±0.00	45.63±0.39
		2	1.00±0.00	33.15±0.10	20.14±0.13	137.17±0.46	0.00±0.00	39.70±0.43
		4	2.43±0.18	30.56±0.11	16.62±1.15	128.39±1.05	33.00±2.15	37.40±0.12
		6	3.72±0.21	29.78±0.18	11.93±0.53	90.83±0.19	70.00±6.11	31.86±0.76
		8	4.43±0.26	29.03±0.17	9.06±0.24	75.00±0.59	100.00±0.00	24.16±0.69

P value for ripening % = <0.001, P value for firmness (N) = <0.001, P value for eye colour = <0.001, P value for L* =0.356, P value for C* = 0.809, P value for h°= <0.001, *Experiment was terminated, **Each value is a mean of 30 fruit ± S.E.

Table 4.2 Effect of harvest time, ripening temperature and days on Hass' avocado fruit subjective and objective skin colour [Lightness (L*), Chroma (C*) and Hue angle (h°)], fruit firmness and ripening percentage during the 2015 season

Harvest season	Ripening temperature (°C)	Ripening days	Eye colour (1-5)	L*	C*	h°	Ripening %	Firmness (N)
Early harvest	25	0	1.00±0.00	35.00±0.15	22.00±0.44	144.24±0.97	0.00±0.00	69.96±0.92
		2	1.78±0.21	31.18±0.29	16.92±0.92	137.80±1.38	0.00±0.00	41.03±0.45
		4	3.05±0.24	27.01±0.14	8.20±0.15	92.01±0.35	67.00±5.13	25.00±0.13
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	35.74±0.21	21.00±0.22	147.09±0.41	0.00±0.00	51.23±0.81
		2	1.78±0.06	34.13±0.47	20.48±0.34	143.04±0.86	7.00 ±3.33	46.23±0.50
		4	2.99±0.21	31.24±0.12	16.36±0.73	122.68±0.79	50.00±1.50	30.60±0.57
		6	4.00±0.16	29.05±0.17	9.92±0.63	79.04±0.42	100.00±0.00	21.36±0.12
		8	*	*	*	*	*	*
	16	0	1.00±0.00	34.17±0.28	20.11±0.39	146.18±0.25	0.00±0.00	57.33±0.52
		2	2.33±0.05	33.19±0.16	18.60±0.21	142.87±1.19	0.00 ±0.00	45.26±0.37
		4	3.00±0.18	32.37±0.65	15.51±0.54	121.23±0.34	3.00 ±0.15	41.26±0.38
		6	3.46±0.21	30.09±0.18	12.35±0.63	112.63±0.29	13.33±3.33	37.10±0.59
		8	4.23±0.17	29.13±0.24	10.01±0.19	75.25±2.62	83.33±6.22	22.06±0.60
Mid-harvest	25	0	1.00±0.00	34.35±0.26	22.60±0.74	143.65±1.16	0.00±0.00	61.33±0.49
		2	1.83±0.36	33.01±0.18	16.84±0.82	124.85±0.71	37.33 ±5.13	30.80±0.50
		4	3.77±0.15	28.00±0.75	7.13±0.26	81.30±0.36	97.00±1.01	20.36±0.18
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	35.41±0.26	21.59±0.21	146.00±0.66	0.00±0.00	49.83±0.28
		2	2.13±0.10	33.19±0.52	20.40±0.44	142.03±0.29	3.33±0.24	41.93±0.77
		4	3.42±0.18	30.23±0.45	16.42±0.63	126.07±0.80	77.00±1.73	34.23±0.61
		6	4.12±0.11	28.10±0.14	9.62±0.36	89.14±0.42	100.00±0.00	24.30±0.35
		8	*	*	*	*	*	*
	16	0	1.00±0.00	35.92±0.67	20.51±0.69	150.68±0.25	0.00±0.00	58.06±0.65
		2	1.37±0.12	34.46±0.46	18.72±0.21	146.66±0.79	0.00 ±0.00	49.70±0.62
		4	2.41±0.14	34.00±0.28	15.51±0.64	130.23±1.34	33.00±2.13	38.90±0.60
		6	3.67±0.25	32.31±0.10	13.19±0.63	125.12±1.28	70.00±1.80	30.10±0.59
		8	4.36±0.19	27.27±0.15	10.06±0.24	86.17±0.44	100.00± 0.00	21.33±0.47
Late harvest	25	0	1.00±0.00	34.12±0.15	20.88±0.40	142.56±1.17	0.00±0.00	48.76±0.77
		2	1.44±0.12	30.28±0.28	19.02±0.46	133.10±0.49	0.00±0.00	30.93±0.87
		4	3.50±0.15	29.30±0.14	10.12±0.88	100.13±0.84	70.00±2.65	18.06±0.49
		6	*	*	*	*	*	*
		8	*	*	*	*	*	*
	21	0	1.00±0.00	33.90±0.28	23.07±0.54	151.00±1.17	0.00±0.00	59.76±0.15
		2	1.02±0.00	32.51±0.14	22.53±0.35	148.01±0.89	0.00±0.00	34.36±0.34
		4	1.88±0.10	30.33±0.16	12.00±0.41	127.83±0.63	7.00±4.93	27.53±0.57
		6	3.68±0.16	29.18±0.11	8.19±0.40	85.04±0.28	53.00±2.96	20.96±0.43
		8	*	*	*	*	*	*
	16	0	1.00±0.00	34.12±0.21	22.16±0.38	148.09±0.21	0.00±0.00	45.63±0.39
		2	1.00±0.00	33.15±0.10	20.14±0.13	137.17±0.46	0.00±0.00	39.70±0.43
		4	2.43±0.18	30.56±0.11	16.62±1.15	128.39±1.05	33.00±2.15	37.40±0.12
		6	3.72±0.21	29.78±0.18	11.93±0.53	90.83±0.19	70.00±6.11	31.86±0.76
		8	4.43±0.26	29.03±0.17	9.06±0.24	75.00±0.59	100.00±0.00	24.16±0.69

P value for ripening % = <0.001, P value for firmness (N) = <0.001, P value for eye colour = <0.001, P value for L* =0.356, P value for C* = 0.815, P value for h°= <0.001, *Experiment was terminated, **Each value is a mean of 30 fruit ± S.E.

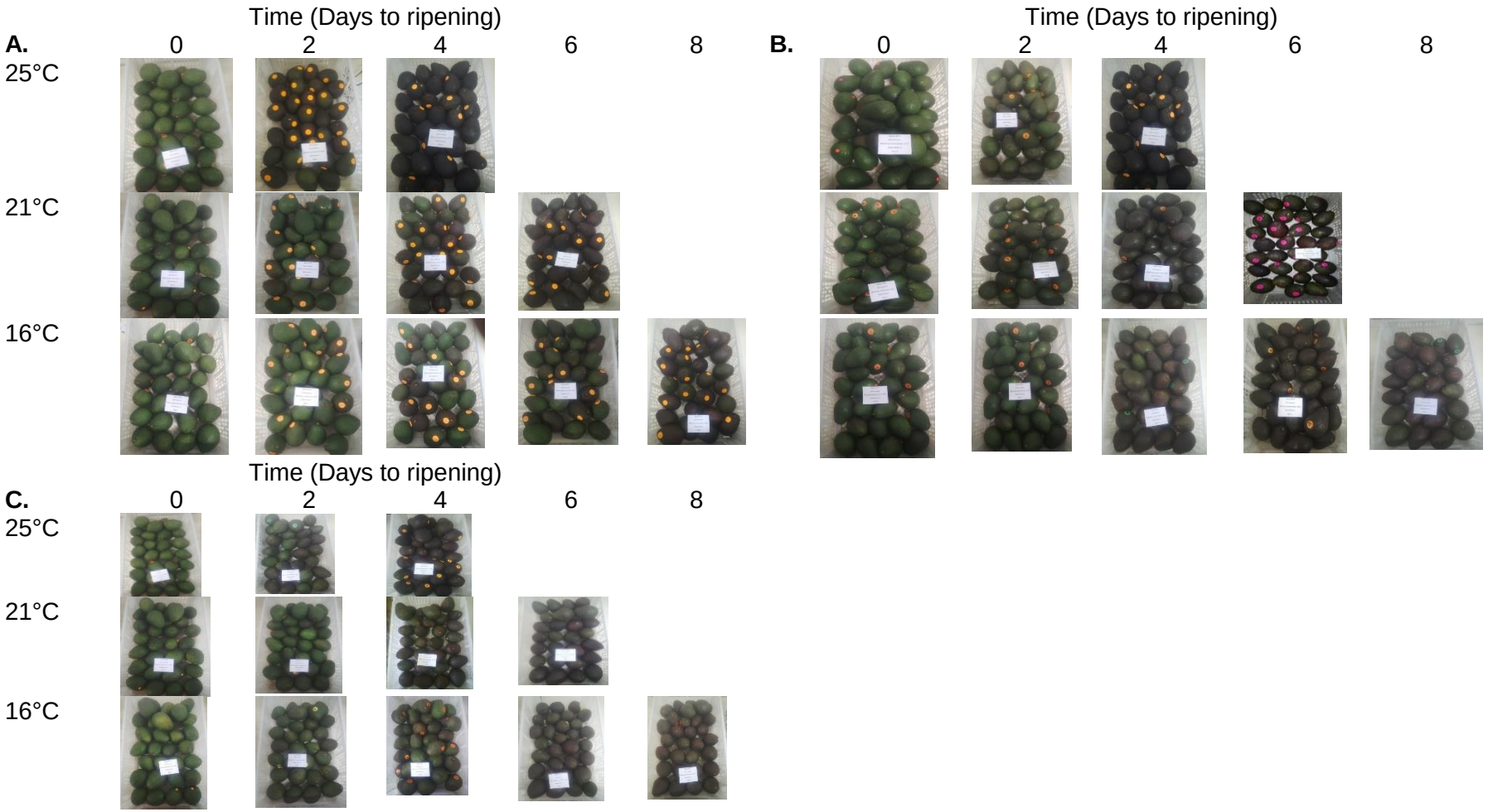

Figure 4.1 Skin colour pictures of **A**. Early **B**. Mid- and **C**. Late season 'Hass' avocado fruit ripened at 16, 21 and 25°C over 0-8 days during the 2014 harvest season

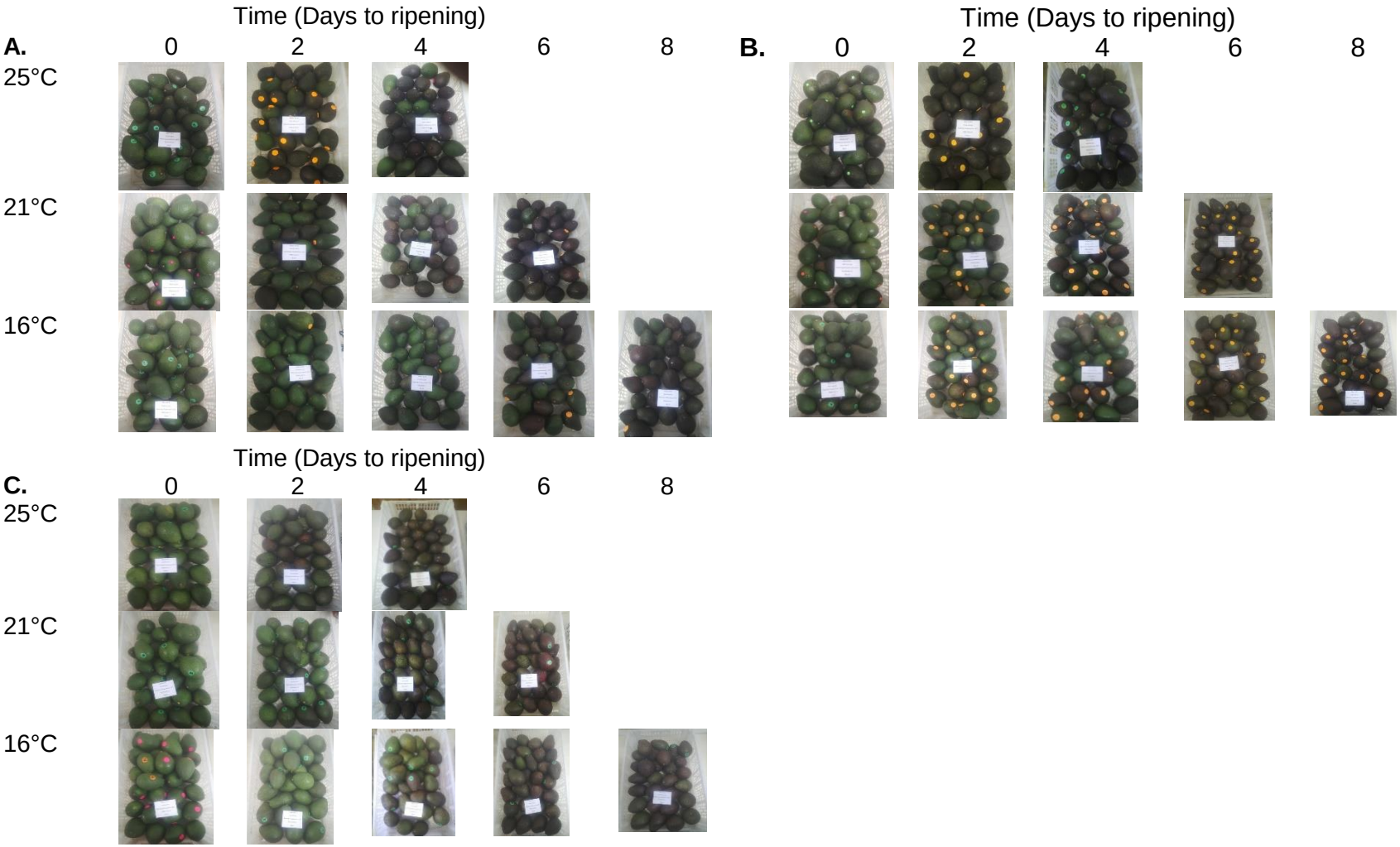

Figure 4.2 Skin colour pictures of **A**. Early **B**. Mid- and **C**. Late season 'Hass' avocado fruit ripened at 16, 21 and 25°C over 0-8 days during the 2015 harvest season

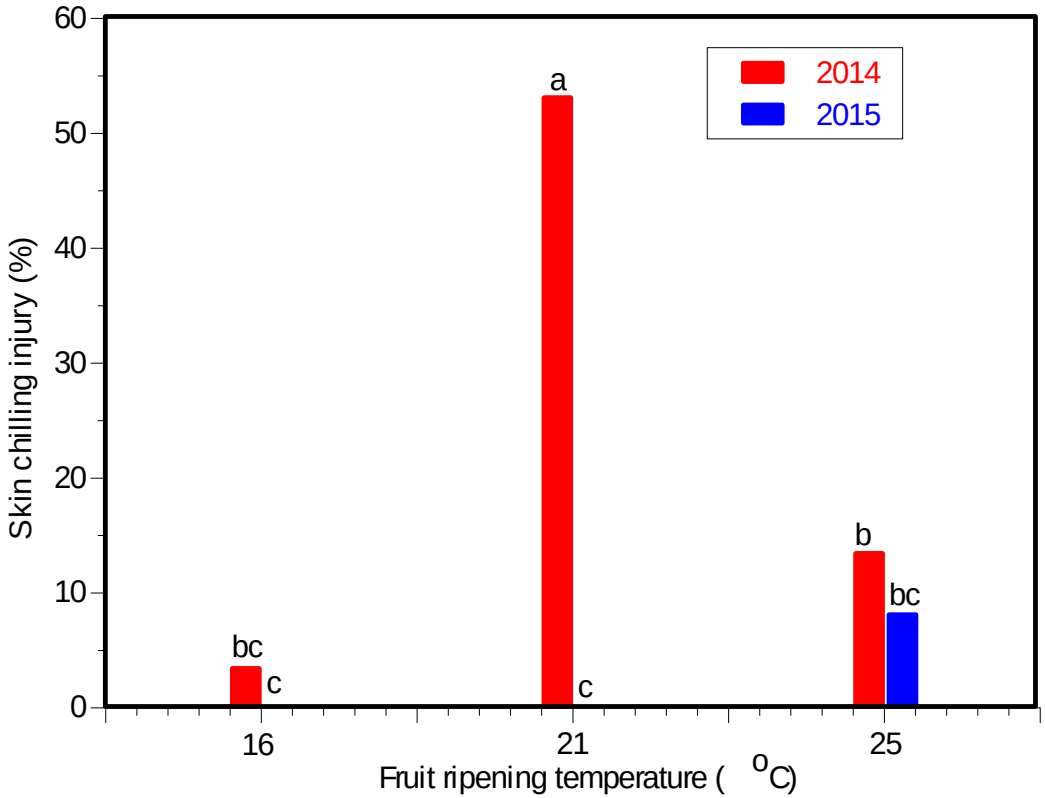

Figure 4.3 Effect of harvest season and ripening temperature on ‘Hass’ avocado fruit skin chilling injury

CHAPTER 5
DISCUSSIONS

5.1 Effect of harvest season and time, ripening temperature and days on skin colour development during ripening

In this study, ‘Hass’ avocado fruit skin colour development was minimal and not affected by harvest season (2014 and 2015) (Table 4.1 and 4.2). This was in agreement with results of Osuna-garcia *et al*. (2010), who showed that harvest season had no significant effect on ‘Hass’ avocado fruit skin colour development during the 2007 and 2008 harvest season. This was attributed to the decrease in chlorophyll, concomitantly, an increase in anthocyanin synthesis, specifically, cyanidin-3-*O*-glucoside (Cox *et al*., 2004). According to Ashton *et al*. (2006), anthocyanins are not only responsible for colour development but also have antioxidant properties. In ‘Kent’ strawberries, high temperature conditions significantly increased flavonoid levels and antioxidant capacity, consequently, influencing colour development. However, ‘Hass’ avocado fruit antioxidants were not affected by the growing temperatures, as anthocyanin accumulate at postharvest during ripening when compared with strawberries (Wang and Zheng 2001), apple (Iglesias *et al*., 2002) and persimmon (ITOO, 1986).

In general, harvest time play a major role in fruit maturation and quality (Léchaudel and Joas 2006) and fruit harvested at immature stage do not ripen proper (Özdemir *et al*., 2009). In this study, poor colour development was observed mainly with the early season ‘Hass’ avocado fruit during ripening, irrespective of harvest season (Table 4.1 and 4.2). The effect of maturity stage has previously been report to be the main factor contributing to poor avocado quality (Johnson and Hofman, 2009). Moreover, early season ‘Bacon’ and ‘Hass’ avocado fruit showed undesired quality such as, irregular ripening and poor skin colour development during ripening (Özdemir *et al*., 2009). Therefore, poor and minimal skin colour development (subjective and objective) for ‘Hass’ avocado fruit may be due to lower sugar accumulation in the early harvest fruit.

Scientific evidence has demonstrated that cyanidin-3-*O*-glucoside synthesis, therefore, colour change is dependent on adequate soluble sugars accumulated

during growing season. According to Mita *et al*. (1997), sugars regulate the expression of an early colour related protein biosynthesis such as, chalcone synthase (CHS) and anthocyanidin reductase (ANR). Chalcone synthase and ANR are metabolic enzymes and proteins encoding anthocyanin pigment production (PAP1 and 2). Therefore, an improved colour development was observed with late season fruit when compared with early season fruit in this study.

Apart from the effect of harvest time and sugar accumulated during pre-harvest growth stage, 'Hass' avocado fruit skin colour change may be affected by ripening temperature. According to Donetti and Terry (2012), ripening temperature affected 'Hass' avocado fruit skin colour change by increasing anthocyanin levels, specifically, cyanidin-3-*O*-glucoside. Moreover, higher ripening temperatures (20 and 25°C) were found to result in improved 'Hass' avocado skin colour change when compared with lower ripening temperature (15°C) (Cox *et al*., 2004). Thus, in this study, higher ripening temperatures (21 and 25°C) showed relatively improved 'Hass' avocado fruit skin colour development when compared with lower temperature (16°C), irrespective of harvest season (Figure 4.1-4.2). However, the effect higher ripening temperatures (21 and 25°C) on 'Hass' avocado fruit skin colour development could not override the effect of harvest time. Therefore, temperature did not play a significant or clear role on early season fruit skin colour development, assumable, due to low skin sugar accumulation.

Furthermore, 'Hass' avocado fruit skin colour change was affected by an interaction between ripening temperature and days to ripening, with longer ripening days associated with improved colour development, depending on harvest time (Table 4.1 and 4.2). In tomato fruit, high temperatures (25 and 30°C) increased skin pigment levels, mainly lycopene after 4 days; and therefore, skin colour change when compared with lower temperature (18°C) (Khairia *et al*., 2015). In addition, 'Hass' avocado fruit skin cyanidin-3-*O*-glucoside significantly increased after 3-6 days during ripening at higher temperatures when compared with lower temperature (Cox *et al*., 2014). Therefore, the role of an interaction between ripening temperature and days to ripening may significant contribute to 'Hass' avocado fruit skin colour development, harvest time has a greater effect.

5.2 Effect of harvest season and time, ripening temperature and days on fruit firmness and ripening percentage during ‘Hass’ avocado fruit ripening

In this study, ‘Hass’ avocado fruit firmness decreased and ripening percentages increased, irrespective of harvest season and time, ripening temperature and days to ripening (Table 4.1 and 4.2). According to Bower and Cutting (1988), early season avocado fruit take longer (about 10 days) to ripening, whereas, late season fruit might ripen within 5-6 days at ambient temperature. In this study, ‘Hass’ avocado fruit were fully ripen within 4, 6 and 8 days when held at 25, 21 and 16°C, irrespective of harvest season and time. Therefore, the effect of harvest time may be overridden by ripening temperature, irrespective of harvest season.

According to Donetti (2011), ‘Hass’ avocado fruit showed an accelerated ripening at higher temperature (23°C) when compared with lower ripening temperature (18°C). In this study, ‘Hass’ avocado fruit stored at higher temperature (25°C) were ripened within 4 days, with higher ripening percentages when compared with lower ripening temperatures, irrespective of harvest season. In general, previous research findings have proved postharvest fruit ripening process to correlate with firmness decline, maturation, storage time and ripening temperature (Mizrach *et al*., 2000).

In ‘Fuerte’ and ‘Hass’ avocado fruit, firmness decreased by 4-6 N during ripening at 20°C (Zamorano *et al*., 1994). While higher ripening temperature (20°C) accelerated firmness decrease of ‘Cavendish’ banana fruit when compared with lower ripening temperatures (14 and 16°C) (Ahmad, 2001). Similarly, in this study, fruit held to ripen at 25 and 21°C became softer at an accelerated rate; whilst, fruit at 16°C became softer at a gradual rate.

5.3 Effect of harvest season and time and ripening temperature on ‘Hass’ avocado fruit external chilling injury

In this study, ‘Hass’ avocado fruit harvested during 2014 season showed significantly higher chilling injury incidence when compared with 2015 season (Figure 4.3). These results were in agreement with Campbell and Hatton (1960) who found severe chilling injury incidence on ‘Pollock’ avocado fruit in 1959 when compared with 1958 harvest season. Moreover, Campbell and Hatton (1960) suggested that chilling injury of ‘Pollock’ avocado fruit during 1958 and 1959 was attributed by maturity stage,

cultural practice and climatic conditions. In the current study, chilling damage during the 2014 and 2015 season was assumed to be attributed to climatic variations due to climate change. According to Wang (2010), chilling injury for tropical and subtropical fruits could be influenced by several factors such as; climatic condition, environmental and cultural practice.

According to Faubion *et al*. (1992), 'Hass' avocado fruit chilling injury susceptibility decreased with physiological maturity increase. Previously, Wu *et al*. (2011) found severe chilling injury on early and mid-harvest 'Chanan' avocado fruit when compared with late harvest fruit. In this study, mid-harvest fruit showed higher chilling damage when compared with early late harvest during 2014 harvest season (Figure 4.3). Bower and Magwaza (2004) found early harvest fruit with higher moisture content to be highly susceptible to chilling injury. Whereas, late harvest fruit with lower moisture content showed low chilling susceptibility when stored at low temperature (Kok *et al*., 2010). Furthermore, Wang (2010) suggested that physiological maturity is one factor determining chilling injury susceptibility of tropical and subtropical fruits. In this study, higher chilling damage for mid-harvest fruit might be due to higher moisture content.

Avocado fruit chilling injury involves mesocarp discoularation, hardening of vascular trends (Van Rooyen and Bower, 2007). However, avocado fruit does not exhibit chilling symptoms, while under cold storage but manifest once kept to ripen at higher temperature (Donkin, 1995). In this study, fruit kept to ripen at higher temperature (25°C) showed higher chilling symptoms when compared with lower temperature (16°C). Hopkirk *et al*. (1994) found 'Hass' avocado fruit ripening at lower temperature (15 to 20°C) to reduce external damage (chilling injury) when compared with ripening at higher temperature (20 to 30°C). Presumably, chilling damage increased with increase in ripening temperature. Similarly; in this study, fruit held to ripen at higher temperature showed higher chilling damage when compared with lower temperature.

CHAPTER 6
SUMMARY, RECOMMENDED FUTURE RESEARCH AND CONCLUSION

6.1 Summary

In this study, the effect of harvest season, harvest time, ripening temperature and ripening days on skin subjective colour change was investigated. During ripening, skin colour, fruit firmness, ripening percentage and chilling injury were affected by harvest season, harvest time, ripening temperature and ripening days. However, harvest time, ripening temperatures and days to ripening were the main factors contributing to 'Hass' avocado skin colour change, fruit firmness and ripening percentage. Moreover, 'Hass' avocado fruit skin colour development slightly changed during the 2014 and 2015 harvest season. Furthermore, early and mid-harvest fruit showed poor skin colour change during ripening when compared with late harvest. Mid-season 'Hass' avocado fruit showed significantly higher chilling damage when compared early and late season fruit.

6.2 Recommendation and future research

The present study examined only a few pre-harvest factors, however, several pre-harvest factors such as orchard design, elevation, production location, planting design, girdling, pruning, plant growth, plant nutrition, irrigation and fertilizer application are responsible for the determination of avocado fruit post-harvest quality. Moreover, advanced technologies such as; magnetic resonance imaging (MRI) and near-infrared spectroscope (NIR) can be used to further understand and develop non-destruction of 'Hass' skin colour problem. Future research should focus in quantifying sugar accumulation and skin pigments during harvest time to predict post-harvest fruit colour change during ripening.

6.3 Conclusion

The present study showed that harvest season, harvest time, ripening temperatures and ripening days influenced 'Hass' skin colour development, firmness and chilling injury occurrence during 2014 and 2015 harvest season. Moreover, poor skin colour development and occurrence of chilling injury symptoms were more apparent on early and mid- harvest fruit in comparison with late harvest during ripening at higher temperature (25^{o}C).

REFERENCES

AHMAD, S., THOMPSON, A.K, AHMED, I.H. and A.A. ASGHAR. 2001. Effect of temperature on the ripening behavior and quality of banana fruit. *International Journal of Agriculture and Biology* 3: 1560-8530.

ANDERSEN, O. M. 2001. Anthocyanins. Encyclopedia of Life Sciences. *John Wiley and Sons, Ltd. doi:10.1038/npg.els.0001909. ISBN 0470016175.* [online]. http://www.hortnet.co.nz/publications/science/pers5.htm. [Accessed on 07 October 2016].

ARZATE-VAZQUEZ, I., CHANONA-PEREZ, J.J., de JESUS PEREA-FLORES, M., CALDERÓN-DOMÍNGUEZ G., MORENO-ARMENDÁRIZ.M.A., CALVO, H., GODOY-CALDERÓN, S., QUEVEDO, R. and GUTIÉRREZ-LÓPEZ, G. 2011. fgImage processing applied to classification of avocado variety 'Hass' (*Persea americana* Mill.) during the ripening process. *Food and Bioprocess Technology* 4: 1307-1313.

ASHTON, O.B.O., WONG, M., MCGHIE, T.K., VATHER, R., WANG, Y., JACKMAN, C.R., RAMANKUTTY, P. AND WOOLF, A. B. 2006. Pigments in avocado tissue and oil. *Journal of Agricultural and Food Chemistry* 54: 10151-10158.

BARRETT, D.M., BEAULIEU, J.C. AND SHEWFELT, R., 2010. Critical, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: Desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition* 50: 369-389.

BLAKEY, R.J. 2011. Management of avocado postharvest physiology. Discipline of Horticultural Science School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. PhD thesis.

BOSSE, R. 2012. Effect of systemic resistance inducers applied pre- and postharvest for the development of a potential control of *Colletotrichum gloeosporioides* on *Persea americana* Mill. cv 'Fuerte'. Discipline of Horticultural Science School of Agricultural, Earth and Environmental

Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. MSc. dissertation.

BOWER, J.P. and J.G.M. CUTTING. 1988. Avocado fruit development and ripening physiology. *Horticultural Reviews* 10: 229-271.

BOWER, J.P. and L.S. MAGWAZA. 2004. Effect of coatings and packaging on external and internal quality with emphasis on “cold injury”. *South African Avocado Growers' Association Yearbook* 27: 35-39.

CAMPBELL, C. W. and T. T. HATTON. 1960. Chilling injury in Pollock avocados during cold storage. *Proceedings of Florida State Horticultural Society.* 72: 337-338.

CANTÍN, C.M., CRISOSTO, CH., OGUNDIWIN, E.A., GRADZIEL, T., TORRENTS, J. and M.A. MORENO. 2010. Chilling injury susceptibility in an intra-specific peach [*Prunus persica* (L.) Batsch] progeny. *Postharvest Biology and Technology* 58: 79-87.

CHEN, N.J., WALL, M.M., PAULL, R.E. and P.A. FOLLETT. 2009. Variation in ‘Sharwil’ avocado maturity during the harvest season and resistance to fruit fly infestation. *Horticultural science* 44: 1655-1661.

CORELLI-GRAPPADELLI, L. and A.N. LAKSO. 2002. Fruit development in deciduous tree crops as affected by physiological factors and environmental conditions. *Acta Horticulturae* 636: 820-823.

COX, K.A., MCGHIE, T.K., WHITE, A. and A.B. WOOLF. 2004. Skin colour and pigment changes during ripening of ‘Hass’ avocado fruit. *Postharvest Biology and Technology* 31: 287-294.

CRISOSTO, C.H., JOHNSON R.S., DeJONG, T. and K.R. DAY. 1997. Orchard factors affecting postharvest stone fruit quality. *Horticultural science* 32: 820-823.

DONETTI, M. 2011. Evaluation of factors affecting shelf-life and quality biomarkers of imported avocado fruit. Cranfield University, Cranfield Health, Plant Science Laboratory, PhD thesis.

DONETTI, M. and L.A. TERRY. 2012. Investigation of skin colour changes as non-destructive parameter of fruit ripeness of imported 'Hass' avocado fruit, *Acta Horticulturae* 945: 189-192.

DONKIN, D.J. 1995. Some aspects of low storage temperature of 'Fuerte' avocados (*Persea americana* Mill.) grown in the Natal Midlands. Discipline of Horticultural Science School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. MSc. dissertation.

FAUBION, D.F., MITCHELL, F.G. and G. MAYER. 1992. Response on 'Hass' avocado to post-harvest storage in Controlled Atmosphere Conditions. *Proceedings of Second World Avocado Congress* 40: 94-100.

FLITSANOV U., MIZRACH A., LIBERZON A., AKERMAN M. and G. ZAUBERMAN. 2000. Measurement of avocado softening at various temperatures using ultrasound. *Postharvest Biology Technology* 20: 279-286.

HEWETT, W. 2006. An overview of pre-harvest factors influencing post-harvest quality of horticultural products. *International Journal of Postharvest Technology and Innovation* 1: 4-15.

HOFMAN P.J., FUCHS, Y. and D.L. MILNE. 2002. Harvesting, packing, postharvest technology, transport and processing. *In*: Whiley, A.W., Schaffer, B.A. and Wolstenholme, B.N. (eds.). 2002. *The Avocado: Botany, Production and uses*. CABI Publishing, Wallingford, Oxon, UK. pp. 363-391.

HOPKIRK G., WHITE, A., BEEVER, D.J. and S.K. FORBES. 1994. Influence of postharvest temperatures and the rate of fruit ripening on internal postharvest rots and disorders of New Zealand 'Hass' avocado fruit. *New Zealand Journal of Crop Horticulture Science* 22: 305-311.

IGLESIAS I., SALVIA J., TORGUET L. and C. CABUS. 2002. Orchard cooling with over tree micro sprinkler irrigation to improve fruit colour and quality of 'Topred Delicious' apples. *Scientia Horticulturae* 93: 39-51.

ITOO, S. 1986. Persimmons. *In*: *MONSELISE, S. P. and J. ROTHSCHILD (eds.). Handbook of Fruit set and Development*. CRC Press. Inc. pp. 355 - 370.

JOHNSON G.I. and P.J., HOFMAN. 2009. Postharvest technology and quality treatment. *In*: Litz R.E (*ed.*), *The mango: Botany, production and uses* (2nd ed.), CAB International, pp 537-571.

KADER, A. A. 1992. Postharvest biology and technology. *An overview. In: Kader, A.A. (ed). Postharvest Technology of Horticultural Crops*, University of California, California, *Division of Agriculture and Natural Resources Publication* 3311, pp 535.

KANE, O., BOULET, M. and F. CASTAIGNE. 1982. Effect of chilling injury on texture and fungal rot of mangoes (*Mangifera indica* L.). *Journal of Food Science* 47: 992-995.

KHAIRIA, A. N., FALAHA, M. A. F., SUYANTOHADIA, A., TAKAHASHIB, N. and H. NISHINAB. 2015. Effect of storage temperatures on color of tomato fruit (*Solanum lycopersicum* Mill.) cultivated under moderate water stress treatment. *Agriculture and Agricultural Science Procedia* 3: 178-183.

KOK R.D., BOWER, J.P. and I. BERTLING. 2010. Low temperature shipping and cold chain management of 'Hass' avocados: An opportunity to reduce shipping costs. *South African Avocado Growers' Association Yearbook* 33: 33-37.

KOK R.D. 2011. Enhancement of 'Hass' avocado shelf life using ultra- low temperature shipping or 1-MCP treatment and cold chain management. Discipline of Horticultural Science School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. MSc Dissertation.

LÉCHAUDEL, M. and J. JOAS. 2006. Quality and maturation of mango fruits of cv. 'Cogshall' in relation to harvest date and carbon supply. *Australian Journal of Agricultural Research* 57: 419-426.

LLOYD, J. and G. D. FARQUHAR. 2008. Effects of rising temperatures and [CO_2] on the physiology of tropical forest trees. *Philosophical Transactions of the Royal Society of Biological Sciences* 363: 1811-1817.

LÜTGE, A. 2011. Ultra-Low Temperature Shipping and Cold Chain Management of 'Fuerte' Avocado (*Persea americana* Mill.) Grown in the KwaZulu-Natal Midlands. Discipline of Horticultural Science School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. MSc Dissertation.

MATHABA, N., MAFEO, T.P. and F.J. KRUGER. 2015. The skin colouring problem of 'Hass' avocado fruit during ripening. *South African Avocado Growers Association Yearbook* 38: 51-58.

McGUIRE, R.G. 1992. Reporting of objective color measurements. *HortScience* 27: 1254-1255.

MITA, S., MURANO, N. AKAIKE, M. and K. NAKAMURA. 1997. Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that is inducible by sugars. *Plant Journal* 11: 841-851.

MIZRACH A., FLITSANOV U., AKERMAN, M. and G. ZAUBERMAN. 2000. Monitoring avocado softening in low-temperature storage using ultrasonic measurements. *Computers and Electronics in Agriculture-Journal* 26: 199-207.

MOHAMMED, M, and J. K. BRECHT. 2002. Reduction of chilling injury in 'Tommy Atkins' mangoes during ripening. *Scientia Horticulturae* 95: 297-308.

MOWAT, A. D. 1992. Managing persimmon quality. Persimmon Profile, Autumn (1999), pp. 5-6.

MOWAT, A. D. and A. A. CHEE. 2011. Persimmon quality in New Zealand orchards. [online] htt://www.hortnet.co.nz/publications/science/pers6.htm (Accessed on October 2016).

NEWETT S.D.E., CRANE J.H. and C.F. BALERDI. 2002. Cultivars and rootstocks. *In*: Whiley A.W. (ed.) The Avocado. Botany, Production and Uses, Schaffer B. and Wolstenholme B.N., CABI Publishing, UK, Pp 161-187.

NILSEN, E.T. and D.M. ORCUTT. 1996. The physiology of plants under stress. Abiotic factors. John Wiley and Sons Inc, New York.

OCHOA-ASCENCIO S., HERTOG M.L.A.T.M. and B. NICOLAÏ. 2009. Modelling the transient effect of 1-MCP on 'Hass' avocado softening: A Mexican comparative study. *Postharvest Biology and Technology* 51: 62-72.

OSUNA-GARCÍA, J.A., DOYON G., SALAZAR-GARCÍA S., GOENAGA R. and I.J.L. GONZALZ-DURAN. 2010. Effect of harvest date and ripening degree on quality and shelf-life of Hass avocado in Mexico. *Fruits* 65: 367-375.

ÖZDEMIR, A. E., ÇANDIR, E. E., TOPLU, C., KAPLANKIRAN, M., DEMIRKESER, T. H. and E. YILDIZ. 2009. The effects of physical and chemical changes on the optimum harvest maturity in some avocado cultivars. *African Journal of Biotechnology* 8: 1878-1886.

PRIOR, R.L. and X. WU. 2006. Anthocyanins: Structural characteristics that result in unique metabolic patterns and biological activities. *Free Radical Research* 40: 1014-1028.

TESTONI, A. 2002. Post-harvest and processing of persimmon fruit. *In*: BELLINI, E. and E., GIORDANI (eds.). First Mediterranean Symposium on Persimmon. CIHEAM-IAMZ 1: 53-70.

THOMPSON, A. K. 2003. Pre-harvest factors on post-harvest life. *In*: Fruit and vegetable harvesting, handling and storage. Blackwell. Oxford OX4 2DQ, UK. Chap. 1. pp 1-8.

VAN ROOYEN, Z. 2005. Factors affecting mesocarp discolouration severity in 'Pinkerton avocados. Discipline of Horticultural Science School of Agricultural, Earth and Environmental Sciences University of KwaZulu-Natal Pietermaritzburg, South Africa. PhD thesis.

VAN ROOYEN, Z. and J.P. BOWER. 2007. Postharvest treatments used to reduce external chilling injury in 'Pinkerton' avocado (*Persea americana* Mill.), *Proceedings VI World Avocado Congress*, Vina Del Mar, Chile. pp 96-122.

VSN INTERNATIONAL. 2014. GenStat for Windows 16th Edition. VSN International, Hemel Hempstead, UK.

WANG, C.Y. 2010. Alleviation of chilling injury in tropical and subtropical fruits. *Acta Horticulture* 864: 267-273.

WANG, S.Y. and W. ZHENG. 2001. Effect of plant growth temperature on antioxidant capacity in strawberry. *Journal of Agricultural Food Chemistry* 49: 4977-4982.

WILLS, A.W., LEE, T.H., McGLASSON, W.B., GRAHAM, D. and E.G. HALL. 2008. Post-harvest: An Introduction to the Physiology and Handling of Fruit and Vegetables. 5th ed. CAB International, London, UK.

WOOLF A. B. and W. A. LAING 1996. Avocado fruit skin fluorescence following hot water treatments and pretreatments. *Journal of the American Society for Horticultural Science 121*: 147-151.

WOOLF A.B. and I.B. FERGUSON. 2000. Postharvest responses to high fruit temperatures in the field. *Postharvest Biology and Technology* 21: 7-20.

WU C.T., ROAN S.F., HSIUNG T.C., CHEN I.Z., SHYR J.J. and A. WAKANA. 2011. Effect of harvest maturity and heat pretreatment on the quality of low temperature storage avocados in Taiwan. *Journal of the Faculty of Agriculture for Kyushu University* 56: 255-262.

ZAMORANO, J.P., DOPICO B., LOWE, A.L., WILSON, I.D., GRIERSON, D. and C. MERODIO. 1994. Effect of low temperature storage and ethylene removal on ripening and gene expression changes in avocado fruit. *Postharvest Biology and Technology* 4: 331-342.

ZAUBERMAN, G., FUCHS, Y. and M. AKERMAN. 1986. Peroxides activity in avocado stored at chilling temperatures. *Scientia Horticulture* 26: 261-265.

APPENDICES

Appendix 1: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit skin eye-colour during 2014 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		50.8625	1.7539	3.17	
Harvest time	2		31.5892	15.7946	28.56	<.001
Ripening temperature	2		145.5112	72.7556	131.55	<.001
Ripening days	4		1863.0462	465.7615	842.16	<.001
Harvest time × Ripening temperature	4		27.2442	6.8111	12.32	<.001
Harvest time × Ripening days	8		34.3880	4.2985	7.77	<.001
Ripening temperature × Ripening days	5	(3)	117.6115	23.5223	42.53	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	16.5372	1.6537	2.99	0.001
Residual	1015	(261)	561.3510	0.5531		
Total	1079	(270)	1930.7769			

Appendix 2: ANOVA table for the effect of harvest time, ripening temperature and days of 'Hass' avocado fruit skin eye-colour during 2015 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		47.2012	1.6276	2.85	
Harvest time	2		32.7773	16.3887	28.71	<.001
Ripening temperature	2		145.2480	72.6240	127.23	<.001
Ripening days	4		1880.9753	470.2438	823.85	<.001
Harvest time × Ripening temperature	4		27.1622	6.7905	11.90	<.001
Harvest time × Ripening days	8		34.5774	4.3222	7.57	<.001
Ripening temperature × Ripening days	5	(3)	117.7005	23.5401	41.24	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	16.5449	1.6545	2.90	0.001
Residual	1015	(261)	579.3510	0.5708		
Total	1079	(270)	1952.9546			

Appendix 3: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit skin lightness during the 2014 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		432.705	14.921	3.64	
Harvest time	2		11445.694	5722.847	1397.29	<.001
Ripening temperature	2		1129.379	564.689	137.87	<.001
Ripening days	4		19047.390	4761.847	1162.65	<.001
Harvest time × Ripening temperature	4		109.940	27.485	6.71	<.001
Harvest time × Ripening days	8		8333.325	1041.666	254.33	<.001
Ripening temperature × Ripening days	5	(3)	870.725	174.145	42.52	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	45.177	4.518	1.10	0.356
Residual	1015	(261)	4157.117	4.096		
Total	1079	(270)	36238.642			

Appendix 4: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit skin lightness during the 2015 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		412.888	14.238	3.48	
Harvest time	2		11290.837	5645.418	1381.71	<.001
Ripening temperature	2		1134.381	567.191	138.82	<.001
Ripening days	4		18679.440	4669.860	1142.94	<.001
Harvest time × Ripening temperature	4		110.909	27.727	6.79	<.001
Harvest time × Ripening days	8		8471.123	1058.890	259.16	<.001
Ripening temperature × Ripening days	5	(3)	868.858	173.772	42.53	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	45.088	4.509	1.10	0.356
Residual	1015	(261)	4147.101	4.086		
Total	1079	(270)	36059.159			

Appendix 5: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit skin chroma during the 2014 harvest

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		1090.84	37.62	3.44	
Harvest time	2		3630.13	1815.06	165.93	<.001
Ripening temperature	2		4544.18	2272.09	207.71	<.001
Ripening days	4		42057.92	10514.48	961.22	<.001
Harvest time × Ripening temperature	4		263.59	65.90	6.02	<.001
Harvest time × Ripening days	8		5242.21	655.28	59.90	<.001
Ripening temperature × Ripening days	5	(3)	2479.95	495.99	45.34	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	66.39	6.64	0.61	0.809
Residual	1015	(261)	11102.72	10.94		
Total	1079	(270)	48494.49			

Appendix 6: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit skin chroma during the 2015 harvest

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		1087.10	37.49	3.41	
Harvest time	2		3271.07	1635.53	148.78	<.001
Ripening temperature	2		4583.37	2291.68	208.47	<.001
Ripening days	4		39892.62	9973.16	907.24	<.001
Harvest time × Ripening temperature	4		269.21	67.30	6.12	<.001
Harvest time × Ripening days	8		5483.17	685.40	62.35	<.001
Ripening temperature × Ripening days	5	(3)	2468.65	493.73	44.91	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	65.89	6.59	0.60	0.815
Residual	1015	(261)	11157.77	10.99		
Total	1079	(270)	47714.34			

Appendix 7: ANOVA table for the effect of harvest time, ripening temperature and days of 'Hass' avocado fruit skin hue angle during the 2014 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		31174.9	1075.0	3.26	
Harvest time	2		16802.5	8401.2	25.50	<.001
Ripening temperature	2		46792.8	23396.4	71.02	<.001
Ripening days	4		729635.4	182408.9	553.68	<.001
Harvest time × Ripening temperature	4		29987.6	7496.9	22.76	<.001
Harvest time × Ripening days	8		84371.4	10546.4	32.01	<.001
Ripening temperature × Ripening days	5	(3)	42856.2	8571.2	26.02	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	19877.1	1987.7	6.03	<.001
Residual	1015	(261)	334386.9	329.4		
Total	1079	(270)	962910.5			

Appendix 8: ANOVA table for the effect of harvest time, ripening temperature and days of 'Hass' avocado fruit skin hue angle during the 2015 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		29707.2	1024.4	3.00	
Harvest time	2		18587.8	9293.9	27.22	<.001
Ripening temperature	2		46440.1	23220.1	68.01	<.001
Ripening days	4		757321.1	189330.3	554.52	<.001
Harvest time × Ripening temperature	4		29778.9	7444.7	21.80	<.001
Harvest time × Ripening days	8		85816.2	10727.0	31.42	<.001
Ripening temperature × Ripening days	5	(3)	42990.3	8598.1	25.18	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	19896.9	1989.7	5.83	<.001
Residual	1015	(261)	346549.6	341.4		
Total	1079	(270)	985138.9			

Appendix 9: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit firmness during the 2014 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		2795.21	96.39	2.43	
Ripening temperature	2		18648.98	9324.49	234.63	<.001
Harvest time	2		26558.64	13279.32	334.14	<.001
Ripening days	4		268825.00	67206.25	1691.09	<.001
Ripening temperature × Harvest time	4		1027.35	256.84	6.46	<.001
Ripening temperature × Ripening days	5	(3)	6202.28	1240.46	31.21	<.001
Harvest time × Ripening days	8		14365.76	1795.72	45.19	<.001
Ripening temperature × Harvest time × Ripening days	10	(6)	2556.08	255.61	6.43	<.001
Residual	1015	(261)	40337.42	39.74		
Total	1079	(270)	294293.78			

Appendix 10: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit firmness during the 2015 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		5588.82	192.72	2.13	
Ripening temperature	2		185862.23	9510.49	234.63	<.001
Harvest time	2		16258.64	14258.32	254.14	<.001
Ripening days	4		258365.00	67206.25	1691.09	<.001
Ripening temperature × Harvest time	4		513.68	128.84	3.46	<.001
Ripening temperature × Ripening days	5	(3)	6202.28	1240.46	31.21	<.001
Harvest time × Ripening days	8		14365.76	1795.72	45.19	<.001
Ripening temperature × Harvest time × Ripening days	10	(6)	1556.08	255.61	6.43	<.001
Residual	1015	(261)	32137.42	39.74		
Total	1079	(270)	535215.67			

Appendix 11: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit ripening percentage during the 2014 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		29707.2	1024.4	3.00	
Harvest time	2		15418.0	7709.0	75.96	<.001
Ripening temperature	2		6766.8	3383.4	33.34	<.001
Ripening days	4		124881.4	31220.3	307.65	<.001
Harvest time × Ripening temperature	4		488.9	122.2	1.20	0.316
Harvest time × Ripening days	8		9161.6	1145.2	11.28	<.001
Ripening temperature × Ripening days	5	(3)	24232.6	4846.5	47.76	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	4187.5	418.8	4.13	<.001
Residual	72	(18)	7306.7	101.5		
Total	107	(27)	155628.2			

Appendix 12: ANOVA table for the effect of harvest time, ripening temperature and days of ‘Hass’ avocado fruit ripening percentage during the 2015 season

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum	29		29707.2	1024.4	3.00	
Harvest time	2		15986.9	7993.4	78.77	<.001
Ripening temperature	2		9570.6	4785.3	47.15	<.001
Ripening days	4		141592.8	35398.2	348.81	<.001
Harvest time × Ripening temperature	4		602.5	150.6	1.48	0.216
Harvest time × Ripening days	8		8862.5	1107.8	10.92	<.001
Ripening temperature × Ripening days	5	(3)	19641.1	3928.2	38.71	<.001
Harvest time × Ripening temperature × Ripening days	10	(6)	3950.4	395.0	3.89	<.001
Residual	72	(18)	7306.7	101.5		
Total	107	(27)	155628.2			

Appendix 13: ANOVA table for the effect of harvest season and ripening temperature of ‘Hass’ avocado fruit chilling injury

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication stratum	2	478.11	239.06	6.14	
Harvest season	1	1901.39	1901.39	48.85	<.001
Ripening temperature	2	1896.78	948.39	24.37	<.001
Harvest season × Ripening temperature	2	2371.44	1185.72	30.46	<.001
Residual	10	389.22	38.92		
Total	17	7036.94			