# AN ELUCIDATION OF SELECTED PRE-HARVEST PRACTICES AND POSTHARVEST TREATMENT INFLUENCING 'HASS' AVOCADO FRUIT EXOCARP COLOUR DEVELOPMENT DURING RIPENING

by

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THESIS

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## DECLARATION

I declare that this thesis hereby submitted to the University of Limpopo, for the degree of Doctor of Philosophy in Plant production 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 duly acknowledged.

_____ _____

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## DEDICATION

To my beloved daughter Akelo Kylie Shikwambana

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## LIST OF PUBLICATIONS AND CONFERENCE PROCEEDINGS

Publications

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2021. Effect of postharvest glucose infusion treatment on exocarp colour change of 'Hass' avocado (*Persea americana* Mill) during ripening. Journal of Horticulture and Postharvest Research, **DOI**:10.22077/JHPR.2021.4254.1202

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2021. Effect of hand fruit thinning and harvest time on colour development of 'Hass' avocado fruit during ripening. Acta Horticulturae (in press)

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2021. The relationship between peel colour development and pigments, antioxidants and sugars of different size 'Hass' avocado fruit during ripening. Acta Horticulturae (in press)

Conference presentation

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2018. Crop load, harvest time and storage period affect 'Hass' skin colour change during ripening. Combine congress (CC 2018, Poster 25), Ratanga Junction, Cape Town 14 – 18 January 2018

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2019. Effect of glucose pulsing of early-season 'Hass' skin colour development during ripening. Combine congress (CC 2019, Oral), Bloemfontein 21-25 January 2019.

- Shikwambana, K., Mafeo, T.P., Mathaba, N. 2019. The relationship between fruit weight and skin colour of ripe ‘Hass’ avocado fruit. Combine congress (CC 2019, Poster 24), Bloemfontein 21-25 January 2019.

## THESIS STRUCTURE

This thesis is organised in 7 chapters:

**CHAPTER 1:** This chapter discusses the background of exocarp colour development of 'Hass' avocado fruit and elucidate the responsible pigments. Furthermore, it presents the problem statement, rationale, as well as the aims, objectives and hypotheses of the study.

**CHAPTER 2:** This chapter discusses the current knowledge regarding avocado exocarp colour development during ripening. The biochemical changes of anthocyanin and chlorophyll pigments was also reviewed. The role of carbohydrates on anthocyanin biosynthesis and accumulation was discussed. Moreover, preharvest and postharvest factors that affect the synthesis and accumulation of anthocyanins were also reviewed.

**CHAPTER 3:** The effect of crop load adjustments on exocarp colour development at three maturity stages (early, mid- and late) was investigated. A manual hand-fruit thinning method was used to adjust normal (100%) crop load to moderate (50%) and low (25%) load.

**CHAPTER 4:** This chapter examined the effect of girdling and harvest maturity on 'Hass' avocado exocarp colour development during ripening.

**CHAPTER 5:** The effect of $C_6$ sugars on 'Hass' colour development during ripening using exogenously infused glucose was explored in this chapter.

**CHAPTER 6:** The effect of fruit size on the exocarp colouration of ‘Hass’ avocado fruit was studied in this chapter by investigating the relationship between fruit size, exocarp pigments, antioxidants and sugar concentration during ripening.

**CHAPTER 7:** Provides a summary of the study, limitations and proposed future studies.

## GENERAL ABSTRACT

In 'Hass' avocado fruit, pre-harvest and postharvest factors affecting exocarp colour change during ripening are vital to maintain the industry's credibility, competitiveness and profitability. Currently, the South African 'Hass' avocado fruit exocarp colour development is affected by pre- and postharvest factors, ultimately, fruit does not develop the required purple colour during ripening. These pre- and postharvest factors must be understood in order to implement strategies that avoid downgrading of South African 'Hass' avocado fruit by lucrative markets due to insufficient purple colour development during ripening. In 'Hass' avocado fruit, exocarp colour development is associated with an increase in anthocyanin synthesis and accumulation during ripening. However, limited information is available regarding factors regulating anthocyanin synthesis and accumulation in 'Hass' avocado fruit during ripening. Therefore, the overall aims of this study were to investigate pre-harvest practices and postharvest treatment that increase exocarp anthocyanin synthesis during ripening. In addition, determine whether exocarp glucose and other antioxidants contribute to 'Hass' avocado fruit exocarp colour development during ripening.

In chapter 3, the study looked at how crop load adjustment affects 'Hass' avocado fruit exocarp colour development during ripening at three different harvest maturities. The crop load adjustment treatments were applied as: high (100%), moderate (50%) and low (25%) at three harvest times (early, mid- and late). After harvest, fruit were stored at 5.5°C for 28 days, thereafter, ripened at 25°C. The experimental design was carried out as 3 x 3 factorial, arranged in a completely randomized design (CRD) with three replications. The results showed that total anthocyanin and cyanidin 3-*O*-glucoside

concentrations of ‘Hass’ avocados increased following crop load adjustment from normal (100%) to moderate (50%) and low (25%) loads, resulting in improved exocarp colour development during ripening. Furthermore, we discovered that fruit harvested from moderate (50%) and low (25%) crop loads accumulated higher exocarp sugars (D-mannoheptulose and perseitol) at three harvest maturities when compared with high crop load (100%). Moreover, total phenolic concentration of fruit harvested from moderate (50%) and low (25%) crop loads was higher than that obtained from high load fruits, irrespective of harvest maturities.

In chapter 4, the study examined the interaction between branch girdling and harvest maturation on the development of 'Hass' avocado fruit exocarp colour during ripening. The experimental design was carried out as 2 x 2 factorial, arranged in a completely randomized design (CRD). The results showed that fruit harvested from girdled trees had poor exocarp colour development as compared to fruit harvested from control trees, regardless of harvest time. Fruit harvested from girdled and ungirdled avocado trees did not show significant differences in visual exocarp colour during early and mid-maturity. Apart from crop load adjustment and girdling as pre-harvest methods to manipulate postharvest exocarp colour, glucose was also infused through the pedicel.

Studies on the effect of glucose infusion through the pedicel on the exocarp colour of the ‘Hass’ avocado fruit during ripening were presented in chapter 5. The study included five treatments; control fruit with pedicel and infused with distilled water and glucose concentrations (0.05, 0.13 and 0.28 mM). The distilled water, glucose infused and control fruit were stored at 5.5°C for up to 28 days. After cold storage, fruit were kept at ambient temperature 25°C for ripening. The experiment was conducted as a completely randomized design (CRD) with three replications per treatment. The results

showed that glucose infusion through the pedicel markedly increased anthocyanin and cyanidin 3-*O*-glucoside concentration during ripening. Interestingly, glucose concentrations (0.05 and 0.13 mM) resulted in purple colour development after 8 days at 25°C when compared with control, distilled water and highest concentration (0.28 mM).

In chapter 6, the relationship between 'Hass' avocado fruit size, exocarp colour and related pigments with antioxidants capacity and sugar concentration during ripening were investigated. The fruit were categorized by their weight; small (< 200 g) and large (> 201 g). Their diameter and length were also measured using a vernier calliper. Fruit ware stored at 5.5°C for 28 days, then ripened at 25°C. The experimental design was carried out as a completely randomised design (CRD), using 25 fruit replications per category. The results showed that small-sized fruit developed the desirable purple to black exocarp colour when compared with large-sized fruit. Additionally, the results showed that small-sized fruit had higher antioxidant capacity as measured by 2,2 diphenyl 1 picrylhydrazyl (DPPH), ascorbic acid and flavonoid content during ripening when compared with large-sized fruit. Furthermore, it was found that small-sized fruit accumulated higher exocarp and seeds (D-mannoheptulose, perseitol, sucrose and glucose) sugar concentration. We demonstrated and concluded that exocarp colour, pigments, antioxidants and sugar concentration are closely related to size in 'Hass' avocado fruit. Knowledge from this thesis contributes toward the understanding of pre- and postharvest factors that may influence colour development of ‘Hass’ avocado fruit during ripening. This study contributes towards bridging the gap in the literature on the biochemical changes associated with colour development of ‘Hass’ avocado fruit during ripening.

# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1. Background

In the past, the unique characteristics of the ‘Hass’ avocado cultivar have attracted growers and consumers. The ‘Hass’ avocado cultivar is often preferred over other avocado cultivars because of its small size, superior taste and excellent storability (Kok, 2011). The most distinctive characteristic of this cultivar is the change in exocarp colour when ripe, which gives it an attractive appearance on the market. The purple colour development is an important characteristic for consumer acceptance. In addition, ‘Hass’ avocados fruit exocarp colour change is equally important to determine the fruit ripeness (Cox et al., 2004). However, the South African's ‘Hass’ avocado fruit has been showing poor exocarp colour development during ripening (Mathaba et al., 2015). Thus, the poor ‘Hass’ avocado fruit exocarp colour development has compromised quality, thereby, limiting industry’s profitability within lucrative high paying markets. To date, little is known about the factors responsible for this conundrum. Studies conducted on this topic revealed that early and mid-harvest ‘Hass’ avocado fruit are most likely to show poor exocarp colour development during ripening (Mathaba et al., 2015, Mathaba et al., 2017). Furthermore, preharvest factors such as orchard topography, canopy position, mineral status and production region have very little impact (Mathaba et al., 2017). Temperatures during postharvest ripening also had minimal influence, especially for early and mid-season fruit (Mathaba et al., 2015).

The pigment responsible for exocarp colour development in ‘Hass’ avocado fruit is cyanidin 3-*O*-glucoside, a form of an anthocyanin (Cox et al., 2004). In many fruit crops, several environmental and management practices influence anthocyanin biosynthesis and colour change during growth, development and maturation (Hara et al., 2003, He et al., 2010, Zhang et al., 2013). Unlike other fruit crops, ‘Hass’ avocado fruit begins ripening and colour development after harvest, with exception of late harvested fruit. In general, anthocyanin pigments occur as glycoside containing one or more glucose or galactose sugar molecules attached to the hydroxyl group in the central ring (Gu et al., 2019). Sugar is perceived as the trigger for chalcone synthase (CHS), a key enzyme in anthocyanin bio-mechanism and biosynthesis (Gu et al., 2019). Therefore, this study hypothesized that exocarp sugars regulate enzymes, proteins, and gene expression that are involved in the biosynthesis of cyanidin 3-*O*-glucoside. Consequently, avocado fruit colour changes from green to purple to black on the exocarp. However, avocado fruit contains complex sugars distributed throughout the fruit parts, namely: seed, mesocarp and exocarp.

Avocado fruit are rich in seven carbons-$C_7$ (D-mannoheptulose and perseitol) as well as six carbons-$C_6$ sugars (sucrose, glucose and fructose) (Tesfay et al., 2010). Several studies have demonstrated that $C_7$ sugars contribute to antioxidants biosynthesis in the mesocarp (Bertling and Tesfay, 2011, Tesfay et al., 2011). However, $C_6$ sugars are largely ignored in all fruit tissues as they are present in small amounts compared to $C_7$ sugars. To date, the role of both the $C_7$ and $C_6$ sugars in ‘Hass’ avocado fruit exocarp colour development during ripening is unknown.

## 1.2. Problem statement

The South African Avocado Industry (SAAI) is focused on exports and contributes significantly to the agricultural sector. According to Blakey (2011), the ‘Hass’ cultivar contributed about 40% to the avocado volume exported by South Africa to the European market. The European market is highly demanding ‘Hass’ avocado fruit due to its nutty taste and good storage ability. Unfortunately, the industry is losing credibility in this lucrative market because the avocado fruit does not attain intensively purple exocarp colour during ripening as expected by consumers (Mathaba et al., 2015). Therefore, avocado traders and consumers in Europe have complained that South African 'Hass' avocado fruit does not darken during ripening (Mathaba et al., 2015). Traditionally, the colour of the ‘Hass’ avocado skin changes from green to dark purple to black as the fruit ripens. Mathaba et al. (2015) have determined that this phenomenon tends to be an early to mid-season phenomenon. Unfortunately, it is not known which factors are responsible, with the exception of fruit maturity. Studies have indicated that cyanidin 3-*O*-glucoside is responsible for changing ‘Hass’ avocado exocarp colour during ripening (Cox et al., 2004, Ashton et al., 2006). Sugar regulates anthocyanin genes and enzymes during anthocyanin biosynthesis, thus inducing cyanidin 3-*O*-glucoside biosynthesis (Mita et al., 1997, Aza-Gonzalez et al., 2013, Gu et al., 2019). Therefore, management practices such as crop load adjustment and branch girdling can be used to manipulate or increase fruit carbohydrates and partitioning to the exocarp.

## 1.3. Rationale of the study

The SAAI is on the verge of losing its reputation as a supplier of high quality ‘Hass’ avocado fruit to lucrative overseas markets; particularly the European fresh produce market (Mathaba et al., 2015). The conundrum is due to complaints about poor exocarp colour development during the ripening of ‘Hass’ avocado fruit. The ‘Hass’ avocado fruit is currently the most prevalent cultivar in South Africa, contributing approximately 40% to the industry's export value. The poor ‘Hass’ exocarp colour development so far has been documented to be a problem associated with early or mid-season (Mathaba et al., 2015). This study would contribute to the knowledge and understanding by identifying factors that contribute to poor 'Hass' avocado exocarp colour development during ripening. This study would investigate how crop load and tree girdling can be used to increase exocarp sugar accumulation; and consequently, colour development during ripening.

## 1.4. Aims and objectives

### *1.4.1. Aims*

To contribute to the knowledge of improving exocarp colour development of ‘Hass’ avocado fruit during ripening through the use of selective preharvest and postharvest treatments.

### *1.4.2. Objectives*

The specific objectives of this study were to:

a. Determine whether crop load adjustment affect exocarp colour and sugar concentration at different maturities.

b. Assess the effects of girdling on exocarp colour development at different maturation stages.
c. Assess the role played by exogenously infused glucose to the development of exocarp colour.
d. Study the relationships between fruit size, exocarp colour and biochemical composition.

*1.4.3. Hypotheses*

a. Adjusting the crop load at different stages of maturity would encourage exocarp sugar accumulation, concurrently, increasing anthocyanin biosynthesis and, as a result, improving the colour of the ‘Hass' avocado fruit during ripening.

b. Tree branch girdling at different maturity stages would improve the exocarp colour development of ‘Hass’ avocado fruits during ripening.

c. Infusion of glucose after harvest would trigger the biosynthesis of anthocyanin and improve exocarp colour development during the ripening of ‘Hass’ avocado fruit.
d. Different sized ‘Hass’ avocado fruit would differ in exocarp colour change, antioxidants and sugar concentration during ripening.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1. Introduction

In 2018, avocado production in the Southern hemisphere totalled approximately 1.2 million tons. The highest producers were Peru (504 517 tons), Brazil (235 788 tons), Venezuela (139 685), South Africa (127 568 tons), Chile (124 506 tons) and Australia (49 397 tons) (DAFF, 2019). Peru exported the most avocado fruit, accounting for approximately 58.5% of the market share (DAFF, 2019). In the Southern hemisphere, Chile was the second largest exporter contributing to approximately 21.6% of the global avocado export. Peru, Chile and South Africa are export-oriented avocado producers, primarily targeting the European market. The SAAI ranked 8$^{th}$ largest producer contributing 10.4% of global avocado in 2018 (DAFF, 2019). The leading export cultivars in South Africa are 'Fuerte' (42%), 'Hass' (33%), 'Ryan' (11%), and 'Pinkerton' (8.5%) (DAFF, 2019).

The avocado cultivar 'Hass' is a major export cultivar popular on the European market due to its excellent eating quality and storage potential. It consists mostly of Guatemalan (85%) genes and some Mexican (15%) genes (Kok et al., 2010). Fruit is green in colour during fruit growth, maturation and harvest. When the fruit ripens, its colour changes from emerald-green to olive green, then to purplish-black and finally to black (Figure 2.1). It has been reported that growers and consumers use the exocarp colour change of the ‘Hass’ avocado as a ripening indicator (Mathaba et al.,

2015). However, the South African ‘Hass’ avocado fruit does not always follow this quality standard, as the fruit shows poor exocarp colour change when ripe. Therefore, the SAAI faces a postharvest challenge of poor exocarp colouration of ‘Hass’ avocado fruit during ripening (Mathaba et al., 2015). This conundrum could impact the revenue and credibility of the avocado sector in South Africa.


Figure 2.1 Different stages of 'Hass' avocado fruit exocarp colour changes during ripening (Mathaba et al., 2015)

A study conducted by Cox et al. (2004) showed that ‘Hass’ avocado fruit exocarp colour changes during ripening were the result of chlorophyll degradation with an increase in anthocyanin accumulation, specifically cyanidin 3-*O*-glucoside. By understanding how pre- and postharvest factors contribute to exocarp colouration during ripening, we would be able to develop postharvest management practices that would improve fruit quality and maintain industry credibility. There is limited published literature on factors affecting 'Hass' avocado fruit exocarp colour during ripening before and after harvest. This chapter unravel the biosynthesis process of anthocyanin, as well as pre-harvest and postharvest factors that may impact and possibly affect the exocarp colour of ‘Hass’ fruit during ripening.

## 2.2. Anthocyanin biosynthesis and its physiological role in plants

Anthocyanin (ATC) plays a vital role in various biological functions in plant metabolism, such as protection against ultraviolet (UV)-radiation (Dixon et al., 2013). Various anthocyanins give fruits, stems, leaves, tubers and flowers a distinctive colour. The colours in flowers attract insects and animals for pollination and seed dispersal (Aza-González et al., 2012). Anthocyanin exhibits additional antioxidant properties and is widely used in nutraceuticals for human health (Aza-Gonzalez et al., 2013). In the plant cell vacuole, anthocyanin is accumulated as glycoside after being synthesized from phenylalanine enzymes regulated by specific genes (Sparvoli et al., 1994). In horticultural plants, structural genes of anthocyanin biosynthesis pathways have been identified.

The first step in anthocyanin biosynthesis is the condensation of three malonyl-CoA molecules and one *p*-coumaroyl-CoA molecule into chalcone with the enzyme chalcone synthase (CHS). Chalcone is converted to naringenin via chalcone isomerase (CHI) (Chaves-Silva et al., 2018). The biosynthesis of ATC is initiated by colourless naringenin, which is subsequently, converted through hydroxylation by flavonoid 3'-hydroxylase (F3'H) or flavonoid 3', 5'-hydroxylase (F3'5'H) to produce the correspondent dihydro flavonols, dihydroquercetin and dihydromyricetin (Figure 2.2). Thereafter, hydroxylation at carbon (C'3) position on these different groups results in the formation of colourless leucoanthocyanidins by dihydroflavonol 4-reductase (DFR) to produce coloured anthocyanidins by anthocyanin synthase (ANS) (Figure 2.2). ANS yields anthocyanins from leucoanthocyanidins (Chaves-Silva et al., 2018).

This is followed by hydroxyl groups on carbon (C'3) being glycosylated by sugar donor molecules, which are attached to produce a stable and coloured anthocyanin. The flavonoid 3-*O*-glycosyltransferase (UFGT) enzyme is responsible for binding UDP-glucose anthocyanidin molecule to glucose (Castellarin and Di Gaspero, 2007). Anthocyanin production varies between horticultural plants. Horbowicz et al. (2008) identified six common anthocyanins, including cyanidin, petunidin, malvidin, delphinidin, pelargonidin and peonidin (Figure 2.3). Anthocyanins differ in their number of hydroxyl groups on the B-ring and the existence of a methyl group, which influences their colour (Figure 2.4) (Tanaka et al., 2008, Xie et al., 2011). Additionally, it has been demonstrated that the colour of anthocyanin can be influenced by the number of sugar molecules attached to the anthocyanidin, sugar hydroxylation position, and aromatic acid attached to the sugar (Figure 2.4) (Tanaka et al., 2008).

Structural and regulatory enzymes such as chalcone synthase (CHS), dihydroflavonol 4-reductase (DFR) and flavonoid 3-*O*-glycosyltransferase (UFGT) have been identified for their role in anthocyanin biosynthesis for many horticultural plants (Dedaldechamp et al., 1995, Honda et al., 2002, Ahmed et al., 2009, Degu et al., 2014, Gu et al., 2019). These enzymes are directly or indirectly involved in anthocyanin biosynthesis. Salvatierra et al. (2010) found that the expression of DFR, ANS and UFGT enzymes correlated positively with anthocyanin synthesis in Chilean strawberries. The rate of gene transcription such as chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H) and dihydroflavonol 4-reductase (DFR) increased during anthocyanin accumulation in 'Reine des Valless', 'Yellow Wonder' and 'Hawaii' strawberry cultivars (Härtl et al., 2017). Honda et al.

(2002) suggested that DFR can be used as an indicator of anthocyanin biosynthesis since it is active late in the pathway.

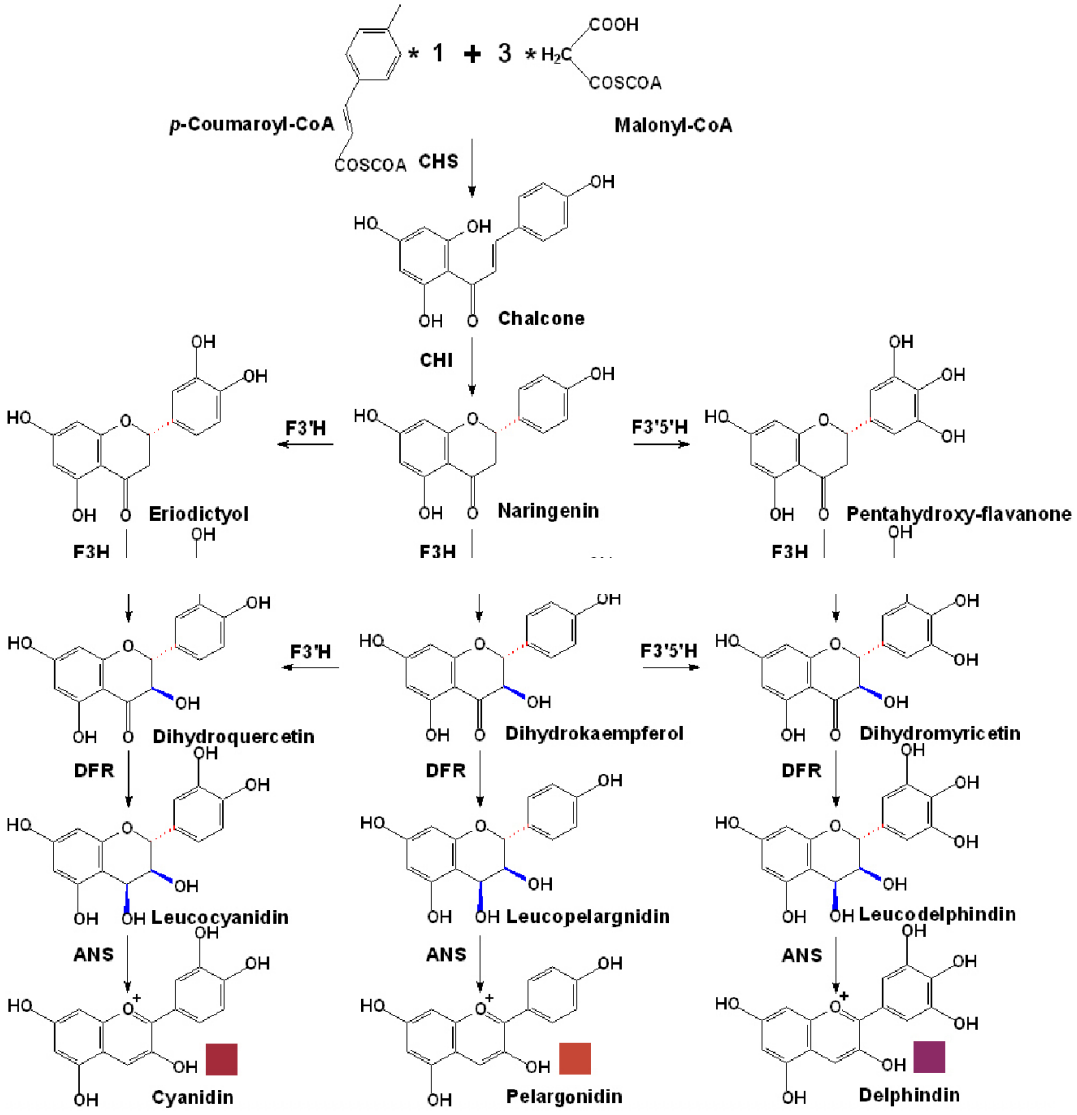



Figure 2.2 Biosynthesis pathway of anthocyanin and main enzymes involved (El Sayed Bashandy, 2016)

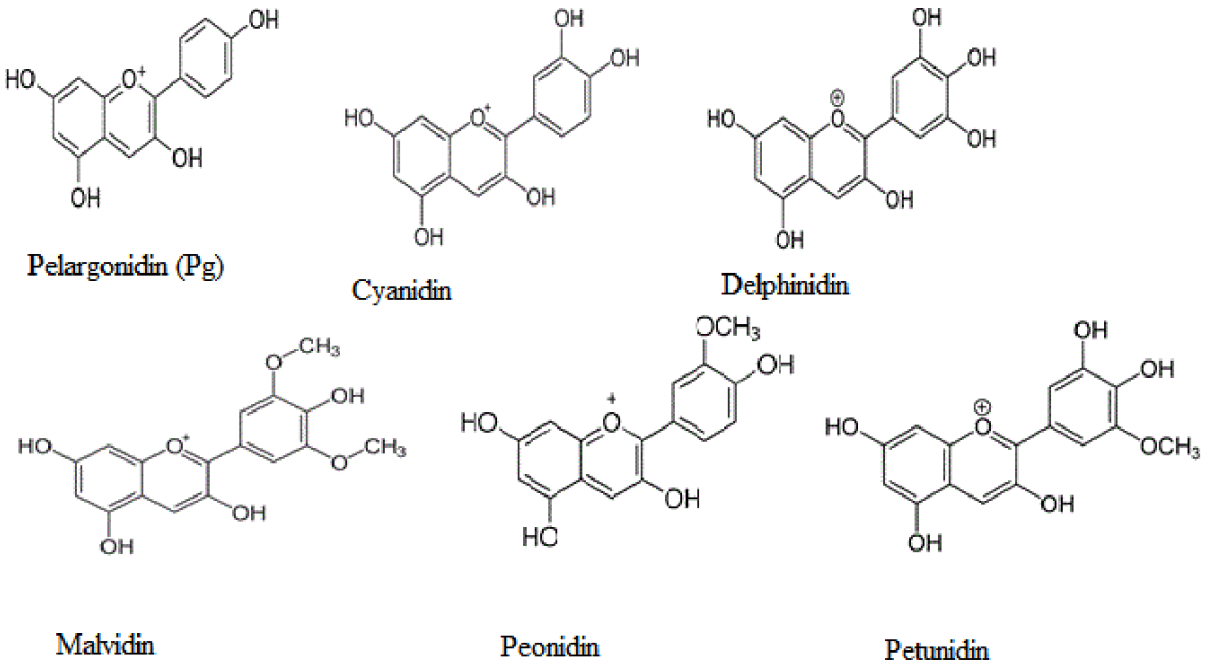



Figure 2.3 Structures of natural occurring anthocyanin in fruit crops (Silva et al., 2016)

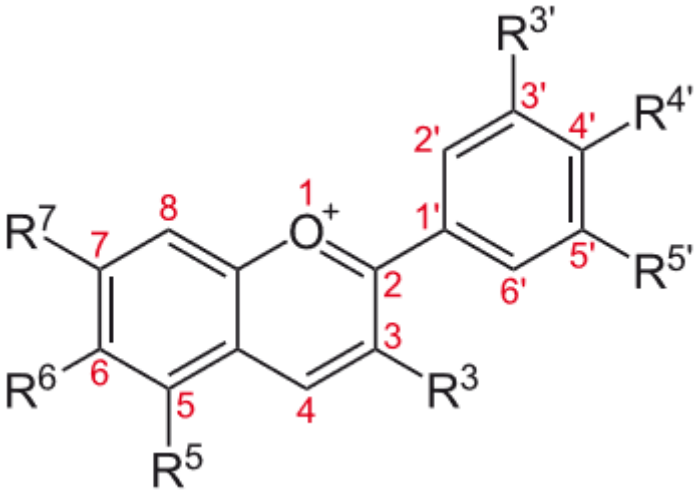



Figure 2.4 Chemical structure of anthocyanin (Tanaka et al., 2008)

The R stands for various functional groups, including hydroxyl (OH), hydrogen (H), methyl (OCH3), and metal ions (Al, mg, Cu and Fe). The arrangement of these functional R group's results in unique anthocyanins. Tanaka et al. (2008) demonstrated that anthocyanin is produced when sugar molecules like glucose bond

to anthocyanin molecules. The bonding occurs at R3 and R6 (Castellarin and Di Gaspero, 2007).

## 2.3. Role of carbohydrates in colour pigment synthesis

Carbohydrates are composed of carbon, hydrogen and oxygen (Bolouri-Moghaddam et al., 2010). In avocado fruit growth and development, carbohydrates are important signalling molecules (Tesfay, 2009). Additionally, carbohydrates are precursors in metabolic processes and the biosynthesis of essential compounds such as amino acids, fatty acids and cellulose (Eckstein et al., 2012). In most parts of the avocado plant at various phenological stages, two types of carbohydrates are present: six-carbon ($C_6$) sugars (glucose, fructose and sucrose) and seven-carbon ($C_7$) sugars (D-mannoheptulose and perseitol) (Liu et al., 1999, Tesfay, 2009).

Tesfay (2009) found that $C_6$ and $C_7$ carbohydrates dominate the seeds, flesh and exocarp of ‘Hass’ avocado fruit. In these tissues, carbohydrates likely affect a variety of physiological processes. Solfanelli et al. (2006) found that carbohydrates in fruit play a major role as both metabolites and signalling molecules. Studies have shown that avocado fruit carbohydrates contribute to seed growth, oil production and fruit development (Blakey et al., 2012, Blakey, 2011). In horticultural crops, glucose and sucrose sugars act as signalling molecules for anthocyanin production (Tsukaya et al., 1991). There is evidence that glucose and sucrose trigger a reaction cascade that induces anthocyanin biosynthesis enzymes. In 'Hass' avocado fruit, the $C_7$ sugars are predominant and occur in greater quantities than the $C_6$ sugars (Tesfay, 2009). According to researchers, $C_7$ sugars are the main carbohydrates determining the shelf-life and quality of ‘Hass’ avocado after harvest (Bertling and Bower, 2005,

Bertling and Bower, 2006, Bertling and Tesfay, 2011, Blakey, 2011, Blakey et al., 2012). Unfortunately, a cascade event of $C_7$ sugars signalling anthocyanin biosynthesis in avocado fruit, and thus, exocarp colour development, has not yet been identified. It is possible that $C_6$ signalling pathways can also contribute to 'Hass' avocado colour changes during ripening. Several studies have established that the 'Hass' avocado fruit exocarp has significantly low sucrose and glucose sugar concentrations (Bertling and Bower, 2005, Tesfay et al., 2010, Bertling and Tesfay, 2011, Blakey, 2011).

Xu et al. (2014) have reported that sucrose can be converted to hexose, such as glucose sugars, via a reversal conversion by sucrose synthase enzyme (SuSy). The SuSy enzyme catalyses the reversal conversion of sucrose to uridine diphosphate glucose (UDP-gluc) and fructose. McKibbin et al. (2006) found that expression of sucrose non-fermenting 1-related protein kinase (SnRK1) reduces the expression of SuSy gene and decreases the sucrose-inducible SuSy transcripts in potato tubers. There is a possibility that avocado fruit uses the same mechanism during colour development. During ripening of 'Hass' avocado fruit, it is possible that glucose and sucrose are the main substrates to be used when it comes to developing the exocarp colour. In addition, an in-vivo model can be used to uncover the mechanism underlying the $C_6$ and $C_7$ carbohydrates influence on colour pigments. The proposed sugar signalling pathway involved in cyanidin 3-*O*-glucoside (Cy3Glu) anthocyanin biosynthesis and exocarp colour in 'Hass' avocado fruits is shown in Figure 2.5.

The signalling system that promotes anthocyanin biosynthesis have sugars inputs includes, the glucose-hexokinase (HXK1), sucrose non-fermenting 1-related protein

kinase (SnRK1) and MYB-bHLH-WD40 (MBW) transcriptional factors signalling network (Figure 2.5) (Gu et al., 2019). It appears that cytosolic glucose and sucrose sugars promote systemically the rate of protein regulation. Recent research found that during early growth and development of 'Hass' avocado fruit, SnRK1 was sensitive to sucrose, while at a later stage, HXK1 is said to take over sugar regulation (Campbell et al., 2000). Chen et al. (2013) found that protein kinase (HXK1 and SnRK1) regulates anthocyanin biosynthesis in part through their phosphorylation of uridine diphosphate (UDP)-glucose. As reported by Xu et al. (2014), HXK1 can phosphorylate glucose sugar molecules at carbon 1 or 6 positions, producing a UDP-sugar nucleotide and cytosine triphosphate (CTP). Studies have shown that UDP-glucose serves as a precursor for glycosylation of secondary metabolites such as flavonoids, phenylpropanoids and batalain (Xu et al., 2014). A role for UDP-glucose in the regulation of anthocyanin biosynthesis has been reported for Arabidopsis (*Arabidopsis thaliana*) (Xu et al., 2014, Chen et al., 2013) and mealies (*Zea mays*) (Pant et al., 2015).

Moreover, it has been reported that the anthocyanin biosynthesis pathway from CHS downward is controlled by transcriptional factors called MYB (Matus et al., 2010). The MYB transcriptional factor belongs to members of MYB-basic-loop-helix (bHLH)-WD-repeat (WD40) (MBW) complex, which is modified at post-translational level in response to various stimuli (Puga et al., 2017). The MYB proteins contain two DNA-binding motifs that are deficient, whereas bHLH proteins contain a basic helix-loop-helix domain that is responsible for specific DNA-binding (Gu et al., 2019). Studies found that MYB forms multiple MYB-bHLH-WD40 (MBW) transcriptional activators to regulate anthocyanin-related genes such as leucoanthocyanidin dioxygenase (LDOX),

DFR and flavonoid 3-O-glucosyltransferase (UDP-Glu: UF3GT) (Jaakola, 2013). Studies in horticultural plants have found that structural genes such as CHS, DFR and UFGT in the flavonoid pathway are regulated by sugars (Teng et al., 2005, Liu et al., 2017). The transmission of the glycosyl molecule from UDP-glucose to the 3-hydroxyl group of anthocyanin stabilizes anthocyanin (Müller et al., 2007).

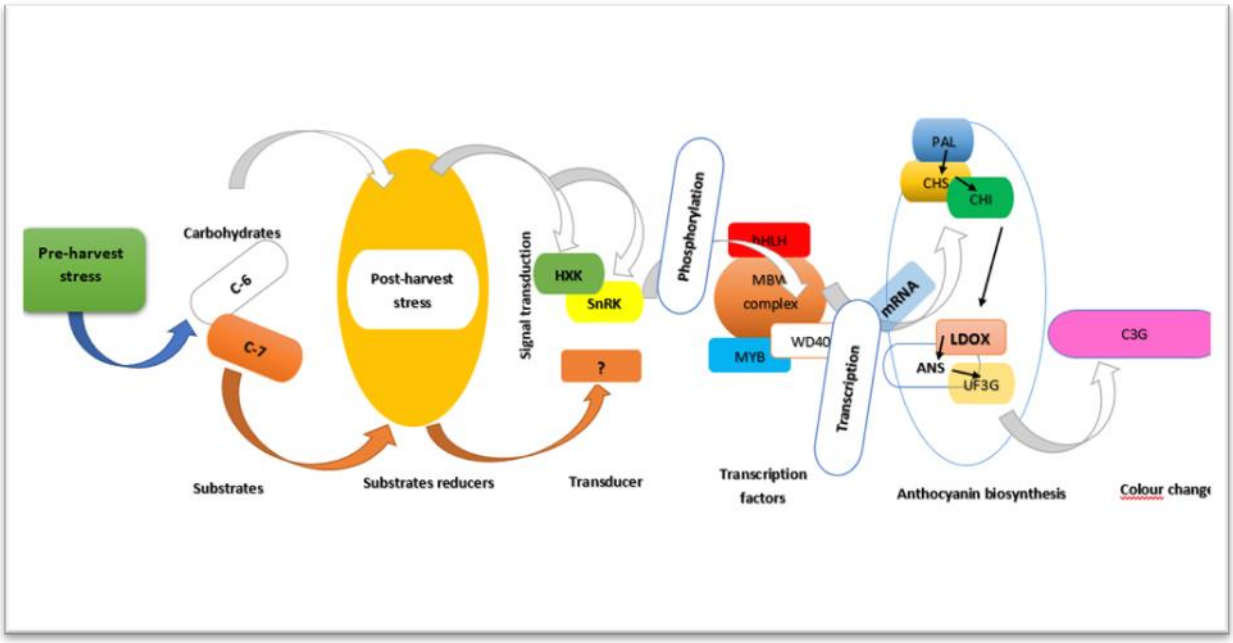



Figure 2.5 Anthocyanin biosynthesis resulting in exocarp colour change in 'Hass' avocado fruit

In the case of preharvest stress, two sets of sugars (substrates) are affected; carbon-six ($C_6$) and carbon-seven ($C_7$). Sugar accumulation could be affected by preharvest and postharvest stresses (Figure 2.5). By using kinase receptors, sugar-mediated signalling will be reduced for anthocyanin biosynthesis. In cells, the hexokinase receptor (HXK1) and the sucrose-non-fermenting-related kinase receptor (SnRK1) are activated in response to the availability of glucose and sucrose in the cytoplasm. Activating these sensors is necessary in order to activate the signalling pathway needed for phosphorylation. In this regard, phosphorylated proteins directly interact

with (MYB-bHLH-WD40) MBW proteins and induce transcription, resulting in anthocyanin synthesis (Figure 2.5). Anthocyanin biosynthesis is evident by early gene expression such as chalcone synthase (CHS), chalcone isomerase (CHI), phenylalanine amino lyase (PAL) and late gene expression (LGE) including leucoanthocyanidin dioxygenase (LDOX), anthocyanin synthase (ANS), dihydroflavonol 4-reductase, flavonoid 3-*O*-glucosyltransferase (Gu et al., 2019).

## 2.4. 'Hass' avocado pigmentation physiology

During ripening, the 'Hass' avocado fruit exocarp colour changes due to the accumulation and degradation of pigments. Anthocyanin, chlorophyll and carotenoid pigments are primarily responsible for the colour development of the exocarp and mesocarp (Cox et al., 2004, Ashton et al., 2006).

### *2.4.1. Chlorophyll*

The 'Hass' avocado fruit is green when unripe and the green colour is due to the chlorophyll pigments in the exocarp tissues. The two forms of chlorophyll are chlorophyll-a and chlorophyll-b (Figure 2.6). Chlorophyll-a and -b content varies with the growing environment and sun exposure (Donetti and Terry, 2011). As the fruit ripens cyanidin 3-*O*-glucoside accumulates in the exocarp, resulting in a colour change from green to dark purple (Ashton et al., 2006). A dark exocarp colour in 'Hass' avocado during ripening is related to chlorophyll degradation according to previous research by Cox et al. (2004).

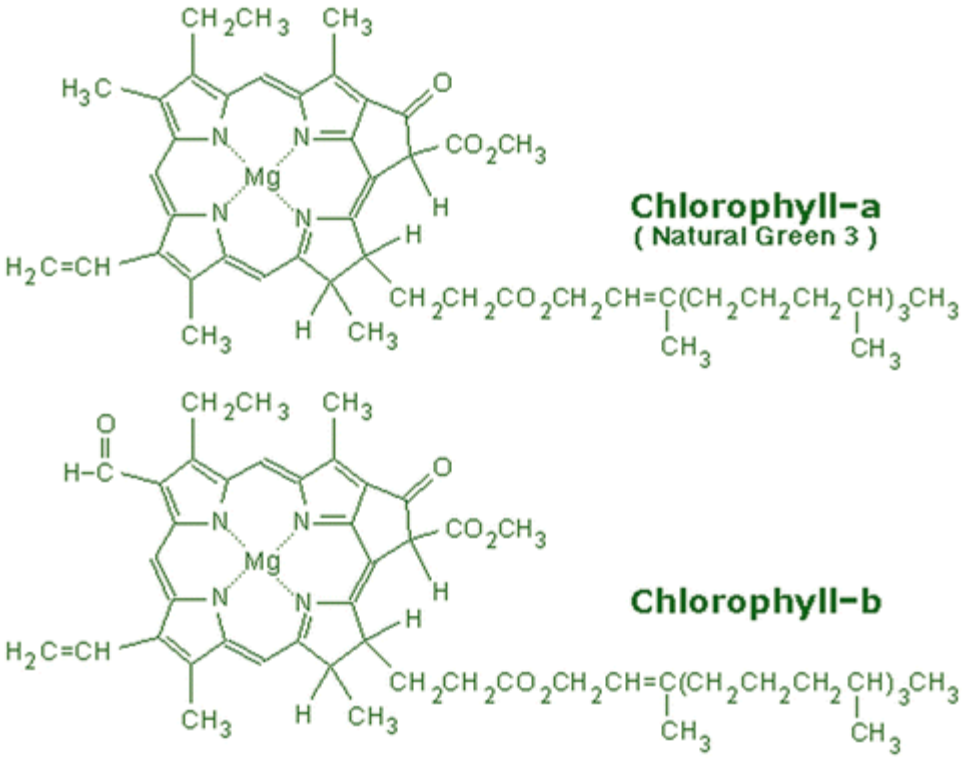



Figure 2.6 Chemical structure of chlorophyll-a and -b (Ramanarayanan et al., 2017)

*2.4.1. Carotenoids*

Some of the major pigments found in 'Hass' avocado fruit are carotenoids. These pigments are responsible for the yellow-green colour of the mesocarp (Lu et al., 2005). Intensive studies have been conducted on carotenoids in 'Hass' avocado fruit (Ashton et al., 2006, Donetti and Terry, 2011). These carotenoids have been determined in various fruit tissues (mesocarp and exocarp) (Ashton et al., 2006). The amounts of carotenoids in mesocarp were higher than in exocarp (Donetti and Terry, 2011). Lu et al. (2009) determined the forms of carotenoids in 'Hass' fruit mesocarp and found the following: lutein (2.93 µg $g^{-1}$), zeaxanthin (0.11 µg $g^{-1}$), β-cryptoxanthin (0.25 µg $g^{-1}$), β-carotene (0.60 µg $g^{-1}$) and α-carotene (0.25 µg $g^{-1}$). The carotenoids concentration in avocado fruit may differ depending on the tissue and stage of ripening (Ashton et al., 2006). It has been observed that lutein carotenoids concentrations decreased with

ripening, both in the mesocarp (1.8-0.3 µg $g^{-1}$) and exocarp (20.5-8 µg $g^{-1}$). Lu et al. (2009) also observed a slower degradation of carotenoids in the mesocarp of 'Hass' avocado fruit when compared with the exocarp. Thus, the change in mesocarp colour during ripening could be a result of a reduction in carotenoids pigment concentration (Ashton et al., 2006).

### *2.4.3. Cyanidin 3-O-glucoside (Cy3Glu)*

Cyanidin 3-*O*-glucoside (Cy3Glu) is one of the primary pigments responsible for the purple colour of the 'Hass' avocado fruit. Many crops have been studied for their biosynthesis of Cy3Glu. The Cy3Glu is first synthesized from naringenin through F3H to form dihydroflavonol, which is then converted through DFR to form leucoanthocyanidin by reducing the carbonyl group (Figure 2.2). Leucoanthocyanidin reductase (LAR) works in the next step, creating flavanol catechin, which then becomes cyanidin through ANS (Figure 2.2) (Ahmed et al., 2014). Moreover, the formed cyanidin is converted into anthocyanin by UDP-D-glucose flavonoid 3-*O*-glucosyltransferase (3GT) to produce Cy3Glu (Wang et al., 2013). The same mechanism occurs in 'Hass' avocado fruit during ripening when the exocarp colour changes from emerald-green to purple then black.

## 2.5. Pre-harvest factors that influence fruit exocarp colour

### *2.5.1. Crop load and thinning*

Many studies have revealed that fruit quality properties are greatly impacted by tree crop load (Drogoudi et al., 2009, Meland, 2009, Cotrut and Stanica, 2015). It has been

found that high crop loads decrease fruit size, chemical properties, make fruit more susceptible to frost damage and increase competition for carbohydrates (Smitha and Samac, 2013). Thinning or adjusting crop load is a horticultural practice that reduces the number of fruits per tree in order to balance tree growth and fruit load (Castro et al., 2015). Adjusting crop load can improve the quality and size of the remaining fruit (Meitei et al., 2013). No studies have been conducted on avocado production. However, there are publications on crop load adjustment by fruit thinning for other fruit crops.

Meitei et al. (2013) examined the effects of chemical thinning on 'Flordasun' peach fruit quality and yield. The authors observed an increase in ascorbic acid, mineral content, phenols, anthocyanin and carotenoids in chemically thinned fruit chemically thinning with Ethrel at a concentration of 150 mg $L^{-1}$. Abdel Hamid (1999) reported similar findings with 'Flordaprince' peaches, by utilizing Ethrel at concentrations of 100 or 200 mg $L^{-1}$ to increase anthocyanin content. In the study, the increased contents of carotenoids and anthocyanin are attributed to decreased competition for minerals, metabolites, and soluble sugars between the fruits (Meitei et al., 2013, Patel et al., 2014).

Drogoudi et al. (2009) found that the total antioxidant capacities and concentration of phenolics in 'Andross' peach fruit from heavily thinned trees increased significantly compared with fruit from light and moderately thinned trees. Reduced crop load during fruit development and growth increased soluble solids (13.16 Brix$^{o}$), soluble sugars (1.81%) and total sugars (6.21%) in ‘Flordasun' peach fruit, according to Patel et al. (2014). Moreover, ascorbic acid, total anthocyanin, total carotenoids, total minerals

and total phenolic contents increased. Consequently, a reduction in crop load resulted in greater carbohydrate synthesis, transport and accumulation in the remaining fruit. In ‘Flordasun’ peach fruit, the levels of skin colour-related pigments, such as anthocyanin and carotenoids, increased (Patel et al., 2014).

Anthocyanin content was found to increase from 15.53 to 18.22 mg 100 $g^{-1}$ and from 14.88 to 17.62 mg 100 $g^{-1}$ in ‘Kala Amritsari’ plum (Rajput and Bhatia, 2017). Additionally, early anthocyanin accumulation improved the colour of ‘Braeburn'/M26 apple fruit harvested from trees with a low fruit load when compared to those from trees with a heavy fruit load. Based on the literature, it appears that different horticultural plants respond differently to fruit thinning (Drogoudi et al., 2009, Meland, 2009, Meitei et al., 2013, Patel et al., 2014, Rajput and Bhatia, 2017). In many horticultural fruit crops, however, crop load adjustment by thinning increased anthocyanin concentration, which resulted in improved colour change.

*2.5.2. Tree/Branch girdling*

In various studies, branch girdling was found to influence fruit colour (Khandeker et al., 2011, Keskin et al., 2013, Castro et al., 2015). Girdling involves the removal of a ring of bark around the full circumference of the tree trunk through the phloem, thus interrupting phloem transport (Goren et al., 2004). According to Patel et al. (2014), girdling improved carbohydrate supply to an apple fruit during maturation, resulting in more available substrate for anthocyanin synthesis. Generally, low levels of anthocyanins in many fruit crops, as well as higher chlorophyll concentrations in the fruit skin, correlate with poor colouration (Wargo et al., 2004).

Girdling prevents the transport of sugars to the roots, resulting in sugar accumulation in the fruit and other above-ground plant organs, thereby enhancing anthocyanin synthesis (Murakami et al., 2008). For example, Khandeker et al. (2011) found that ‘Wax Jambu’ Java apple fruit from girdled branches had the highest concentration of carbohydrates due to carbohydrate and starch accumulation at the upper part of the girdled branches. In 'Hass' avocado, Davie et al. (1995a) found that branch girdling enhanced carbohydrate accumulation and fruit size. According to several studies, branch girdling influences anthocyanin concentrations; and consequently, colour change.

A study by Carreño et al. (1998) found that girdling 'Italia' grapevines at the beginning of berry ripening significantly increased total soluble solids (TSS) and improved skin colour after 5 days. A significant increase in berry weight was also observed. In addition, branch girdling increased skin colouring and anthocyanin concentration in ‘Pione’ berry fruit (Fujishima et al., 2005). Studies in horticultural plants have found that accumulation of anthocyanin is related to sugar accumulation caused by branch girdling. According to Zhang et al. (2013), sugar accumulation activated the expression of a range of anthocyanin genes in ‘Begonia’ semperflorens plants, including CHI, F3H, DFR, ANS, and UFGT. Therefore, the effect of branch girdling on the anthocyanin concentration of 'Hass' avocado fruit could be investigated.

*2.5.3. Harvest time*

Generally, harvest time determines the quality of avocado fruit after harvest (Bower and Cutting, 1988). Several studies have shown that avocado fruits harvested early in the season are vulnerable to mechanical damage, highly sensitive to chilling injury, shrivelling and of poor quality (Kok et al., 2010, Hofman et al., 2013). Donetti and Terry (2014) investigated exocarp colour change as a non-destructive maturity parameter for imported avocado fruit. According to the results, the values of chroma (C*) and hue angle ($h^o$) of ripe 'Hass' avocado decreased significantly across production regions and harvest times. It was found that the development of dark exocarp colour on avocado fruit from South Africa harvested in July (late-harvest) was more pronounced compared with avocado fruit from Spain that was harvested in July (late-harvest).

In the research of Mathaba et al. (2015), poor exocarp colour development of 'Hass' avocado fruit was more prevalent in fruit harvested early (May) and mid-season (June), as opposed to fruit harvested late (July) in the season. Most early harvested fruits are green to olive green in colour (Figure 2.1). Fruit harvested in the middle of the season usually turns purple during ripening, while late season fruit reaches the desired black colour (Figure 2.1) (Mathaba et al., 2015). Generally, poor exocarp colouration of 'Hass' avocado fruit can be attributed to fruit maturity. There could be a possible link between fruit maturity, fruit sugar and cyanidin 3-*O*-glucoside concentrations of 'Hass' avocado fruit, since the dark exocarp colour is due to cyanidin 3-*O*-glucoside accumulation.

*2.5.4. Fruit size*

The ‘Hass’ avocado tree produces two distinct fruit size populations which are categorized as small and large size (Cutting and Wolstenholme, 1992). Various factors can affect the size of the ‘Hass’ avocado fruit. According to Richings et al. (2000), different aspects of fruit growth and responses to the environment are coordinated by the expression of genetic material. Thus, fruit size and duration of growth are also controlled at the genetic level. The chemical form of a physical stimulus must be converted into a form that can elicit effective signalling. In response, the second messengers trigger a cascade of events that alter gene expression. Further, poor climatic conditions, hormonal changes and the availability of water and nutrients can affect the growth of 'Hass' fruit (Richings et al., 2000).

Several studies have shown that fruit size has a significant impact on anthocyanin content (Mes et al., 2008). Thus, it appears that fruit size affects the distribution of anthocyanin in skin cells. The study by Mes (2005) found that tomato fruit size directly affected anthocyanin accumulation. Studies established that the intensity of colour of a particular fruit is a function of several genetically controlled factors which are energy requirements for pigment synthesis, vacuole size and pigment distribution (Xie et al., 2006, Kayesh et al., 2016). It has been reported that the inverse relationship between fruit size and total anthocyanin content is due to the regulation of anthocyanin expression (Mes et al., 2008). The size of the fruit and the concentration of anthocyanin are therefore closely related (Mes et al., 2008).

Stevenson and Scalzo (2012) found that blueberries contain high concentrations of anthocyanin in their skin because of their large surface area. Mes et al. (2008) found that fruit size significantly affected measurable monomeric anthocyanin concentrations

in tomato fruit. Islam et al. (2019) found that small tomato fruit produced more ethylene and respiration than large tomatoes and thus developed colours more rapidly. However, large tomato fruit change colour slowly and produce less ethylene and respiration when compared with medium and small size fruit. Thus, there is need to document the effect of avocado fruit size on concentration of anthocyanin as the proven factor for ‘Hass’ avocado exocarp pigment determinants.

## 2.6. Postharvest factors that affect ‘Hass’ avocado pigmentation and colour changes during ripening

After harvest, avocado fruit undergo continuous physiological changes since they are climacteric. Physiological changes include increased respiration rate and ethylene production, weight loss and exocarp colour changes. Although these physiological events cannot entirely be prevented, they can be delayed by a range of postharvest treatments. Application of postharvest treatments, such as cold-storage (Arendse et al., 2015) and ripening temperature storage (Cox et al., 2004, Mathaba et al., 2015) and infusion (Mathe, 2018) have been reported to affect fruit quality and delayed ripening. Postharvest cold storage, however, may cause cell damage with subsequent physiological problems such as chilling injury symptoms (Mathaba et al., 2015) and insufficient pigment concentrations, resulting in poor exocarp colouration. The following sections will discuss how different postharvest treatments affect the ‘Hass’ avocado fruit pigments and colour.

*2.6.1. Storage temperature*

Storage temperature has been reported to be effective in prolonging storage-life of fruits, thereby extending the shelf-life of fruits. However, it also affects the fruits' exocarp colour during ripening. Therefore, the storage period is crucial to determining post-storage fruit quality and final fruit colour. Effects of storage temperature and storage duration on exocarp colour development have been reported for various horticultural fruit crops such as 'Chandler' strawberry (Ayala-Zavala et al., 2004), 'Kent' mango (Dea et al., 2010) and 'Wonderful' pomegranate (Arendse et al., 2015). The effect of temperature, storage time and packaging on postharvest quality of 'Brighton' and 'Selva' strawberries was studied by Paraskevopoulou-Paroussi et al. (1993). A progressive loss of brightness (decrease in L* value) and darkening of the red exocarp colour (ratio a*/b*) was observed after 8 days of storage at 3°C, as compared to 6 and 20°C.

In contrast, Allong et al. (2000) reported that mango fruit held at 10°C developed colour more quickly than mango fruit held at 5°C. Intense colouring was evident by a reduction in lightness (L* values) of the fruit, which indicates darker yellow colouring. Consequently, lower storage temperatures delay the development of mango colour. Arendse et al. (2015) investigated the effects of storage temperature and duration on postharvest physicochemical and mechanical properties of pomegranate fruit and arils. A study found that the exocarp colour was enhanced in fruit stored at 5°C over a period of 3 months when compared to fruit stored at 21, 10 and 7.5°C. Fawole and Opara (2013) observed an increase in red exocarp colour (a* values) when storing the 'Bhagwa' pomegranate fruit between 5 and 10°C for up to 16 weeks of cold storage

compared with 22 and 7°C. In the experiment by Fawole and Opara (2013), it was observed that fruit colour intensity (C*) decreased significantly as storage temperature and duration increased.

Nanda et al. (2001) found no significant difference in colour change between ‘Ganesh’ pomegranate fruits stored at 8, 15 and 25°C over a 12-week period. Ramin (2007) investigated the effects of cultivars, storage temperature and 1-methylcyclopropene (1-MCP) treatment on postharvest quality of ‘Blady’, ‘Conservolea mission’ and ‘Shengeh’ green olive fruit. In comparison to the lower storage temperature at 5°C, colour development was enhanced at the higher storage temperatures of 10 and 20°C. Red colouring was less intense on fruit storing at 5°C compared to fruit stored at 10 and 20°C for a period of 60 days.

*2.6.2. Ripening temperature*

Ripening temperature is a major factor influencing ‘Hass’ avocado fruit exocarp colour change during ripening. The colouration of avocado fruit of the ‘Hass’ avocado increased with increasing ripening temperature (Cox et al., 2004, Hofman et al., 2013, Mathaba et al., 2017). Cox et al. (2004) investigated exocarp colour and pigment changes in ‘Hass’ avocado fruit during ripening and found similar trends during ripening at 20 and 25°C. In contrast, when ripening at 15°C, the exocarp colouration was much slower, and the fruit colouration did not reach the desired black colour. Mathaba et al. (2015) observed the same trend, in which avocado fruit ripened at 21 and 25°C had better exocarp colour development than fruit ripened at 16°C.

Donetti and Terry (2011) investigated exocarp colour change as a non-destructive parameter of fruit ripeness on imported avocado fruit. According to this study, ripening C* as well as $h^{o}$ values for fruits held at higher temperatures (23°C) were lower than for fruits held at lower temperatures (18°C). The darkening of the exocarp was more apparent on fruit held at 23°C compared with fruit held at 18°C in this instance. Evidence suggests that cyanidin 3-*O*-glucoside's relationship with exocarp colour change is mainly determined by ripening temperature. For instance, Cox et al. (2004) found that the concentration of cyanidin 3-*O*-glucoside was higher at the highest ripening temperature. To ensure better colour development in ‘Hass’ avocado fruit, higher ripening temperatures, preferably above 18°C, are more feasible.

### *2.6.3. Postharvest infusion treatment*

The term ‘infusion’ refers to the continuous supply of sugar solution and water to harvested avocado fruit through the pedicel for a duration of time (during cold storage or postharvest life) to increase sugar concentration, improve quality and extend shelf life. According to the available literature this treatment had been administered to harvested avocado fruit with D-mannoheptulose, perseitol, sucrose sugar solutions and water (Blakey et al., 2009; Bertling and Tesfay, 2011; Mathe, 2018). Mathe (2018) reported that the infusion of a solution containing 9.5 and 4.74 mM of D-mannoheptulose, perseitol and sucrose sugar resulted in firmer ‘Hass’ and ‘Fuerte’ fruit which was followed by water infused fruit. Blakey et al. (2009) found that infusion of water through the pedicel decreased ripening heterogeneity in mid- and late-harvested ‘Hass’ fruit. It has been reported that respiration of 'Hass' and 'Fuerte' fruit infused with D-mannoheptulose and perseitol decreased during the mid-harvest season (Mathe, 2018). A study done on the infusion of sugar in avocado fruit through

the pedicel revealed that infusion of D-mannoheptulose and perseitol increased the concentration of these sugars (D-mannoheptulose and perseitol) in the mesocarp (Bertling and Tesfay, 2011). Whereas infusion of water could only maintain the initial concentration of these sugars (D-mannoheptulose and perseitol). Thus, infusion treatments are useful for improving ‘Hass’ avocado postharvest fruit quality by administering solutions containing compounds through the pedicel.

## 2.7. Conclusion

In spite of numerous studies on exocarp colour changes, the causes and the possible promoting factors for poor colouration of ‘Hass’ avocado fruit during ripening are unknown. This literature review highlighted several factors that contribute to the accumulation of anthocyanin in fruits, therefore, final colour. Further, application of treatments, such as fruit thinning, girdling, harvesting at optimal maturity and fruit size, application of correct storage and ripening temperatures, pre-treatment infusion may also affect exocarp colour. While a huge amount of information is available on the factors influencing and controlling exocarp colour development, little is known about the factors that control the biosynthesis of cyanidin 3-*O*-glucoside in ‘Hass’ avocado fruit during ripening.

Experiments on the biosynthesis of cyanidin 3-*O*-glucoside and factors regulating its biosynthesis are necessary for developing pre- and postharvest management practices to improve 'Hass' exocarp colour development. There was evidence in the literature that soluble sugars are involved in the production of exocarp colour pigments, such as anthocyanin. According to Bertling and Tesfay (2011), the reduction in sugar content of 'Hass' avocado fruit during ripening could be associated with

postharvest quality degradation. There is, however, no understanding of the relationship between sugars and colour pigments in ‘Hass’ avocados. A collaborative research effort is needed to determine which sugar molecules are involved in anthocyanin biosynthesis and how they interact with one another.

There are various pre- and postharvest practices for manipulating sugar accumulation in fruit. This review discussed the effects of these factors on anthocyanin biosynthesis and accumulation, as well as their effects on exocarp colour change. In order to manipulate sugar accumulation during the avocado fruit development and growth, there is a need for future research to focus more intensively on pre- and postharvest practices. This will provide insight into how to improve the appearance of the avocado exocarp during ripening. Additionally, efforts should be made to develop appropriate prediction methods for fruit maturity and exocarp colour change in 'Hass' avocado fruit.

# CHAPTER 3

# EFFECT OF CROP LOAD ADJUSTMENT AND HARVEST TIME ON 'HASS' AVOCADO EXOCARP COLOUR DEVELOPMENT DURING RIPENING


## Abstract

Crop load adjustment is an important horticultural practice that influences the source-sink relationship and fruit quality. However, little is known about its impact on 'Hass' avocado fruit during ripening. The purpose of this study was to examine the effect of crop load adjustment on exocarp colour development of 'Hass' avocado fruit during ripening at three harvest times (early, mid- and late harvest). Three crop load adjustment treatments were applied as the high (100%), moderate (50%) and low (25%). After each harvest time, fruit were stored at 5.5°C for 28 days; thereafter, ripened at 25°C for 4 days and evaluated for fruit firmness, visual colour and objective colour parameters (lightness-L*, chroma-C* and hue angle-*h*°), total chlorophyll, and carotenoids, total anthocyanin, cyanidin 3-*O*-glucoside, D-mannoheptulose, perseitol and total phenolics. The fruit firmness was greater in the low load followed by moderate after 4 days at 25°C during middle and late harvest. Firmness loss was positively ($p < 0.001$) correlated with L* (r= 0.838), C* (r= 0.822), *h*° (r= 0.581), and negatively ($p < 0.001$) correlated with visual colour (r= -0.631). In this study, lower and moderate crop load resulted in improved visual colour after 4 days at 25°C during early and mid-harvest. There was no significant effect of crop loads on visual colour for late harvest fruit. During early and mid-harvest, the total chlorophyll and carotenoids content of fruit from lower crop loads declined rapidly. During all harvest times, higher anthocyanin and cyanidin 3-*O*-glucoside concentrations were observed in fruit harvested from low load followed by moderate load. In conclusion, 'Hass' avocado fruit exocarp colour

development during early and middle harvest was improved by low (25%) and moderate (50%) crop load.

**Keywords*:*** Anthocyanin, chlorophyll, cyanidin 3-*O*-glucoside, fruit quality, source-sink relationship

## 3.1. Introduction

In South Africa, ‘Hass’ avocado (*Persea americana* Mill) is grown mainly for lucrative export markets. However, the South African ‘Hass’ avocado fruit have been displaying inconstant exocarp colouration when ripe, consequently, affecting acceptability by consumers and profitability of the industry (Mathaba et al., 2015). Exocarp colour of 'Hass' avocado fruit remains a major commercial trait, since it measures fruit ripeness, which provides the basis for consumers to purchase the fruit (Mathaba et al., 2017). Commercially, it is frequently desirable to have uniform exocarp colour development rather than variable colouration. In general, ‘Hass’ avocado fruit exocarp colouration is attributed to their anthocyanin biosynthesis and accumulation. The development of colour and anthocyanin accumulation should coincide with softening (Cox et al., 2004). Avocado 'Hass' fruit differ from other fruit crops by their inherent ability not to ripen on the tree (Bower and Cutting, 1988, Wolstenholme, 1989). In avocado fruit, 'Hass' colour development and anthocyanin accumulation occur shortly after harvest; consequently, the exocarp colour changes from green to black (Cox et al., 2004). Therefore, ‘Hass’ avocado fruit poor exocarp colour development might be ascribed to insufficient anthocyanin accumulation during ripening.

Inadequate anthocyanin accumulation is attributed to numerous pre-harvest factors; harvest maturity is the most important factor. In avocado fruit quality, harvest maturity is fundamental and has a considerable effect on exocarp colour development during ripening (Mathaba et al., 2015, Mathaba et al., 2017). Mathaba et al. (2015) found that the ‘Hass’ avocado fruit harvested relatively early in the growing season developed poor exocarp colouration during ripening compared with the late harvest. Donetti and Terry (2014) reported that 'Hass' avocado exocarp colour development depends on

the production region and harvest time. According to their study, dark exocarp colour of ‘Hass’ avocado fruit was most prominent in South African fruit harvested in July (late harvest) when compared to Spanish fruit harvested in July (late harvest). However, the practice of late harvest with excess fruit production beyond the tree’s carbohydrate storage capacity leads to competition; and consequently, poor fruit quality (Wolstenholme, 1989).

Therefore, crop management strategies such as crop load adjustment is necessary. Several studies evaluated the physiological response of fruit to crop load adjustment on a wide range of fruit crops. The results showed that crop load adjustment influenced the source/sink relationship; and ultimately, enhance carbohydrate accumulation and partitioning to improve fruit quality. A study conducted by Steyn et al. (2002) found that horticultural practices that increase carbohydrates status of tissues are often linked to higher anthocyanin levels. In general, crop load adjustment by fruit thinning reduces competition between developing fruits for resources and improves the accumulation of carbohydrates in the fruit. It was shown that crop load adjustment improved total soluble sugars, ascorbic acid, anthocyanin, carotenoids and phenolic concentration on the 'Flerdasun' peach tree (Patel et al., 2014). When crop loads were adjusted for ‘Honeycrisp’ apples, dry matter, fruit size, firmness, red blushed over colour percentage and soluble solid content improved (Kalcsits et al., 2019). According to Rajput and Bhatia (2017), crop load adjustments on 'Kala amritsari' plums increased anthocyanin concentration. Meland (2009) found that crop adjustment of the 'Braeburn/M26' tree resulted in early anthocyanin accumulation. Based on the relationship between carbohydrate metabolism and anthocyanin biosynthesis, we hypothesized that crop load adjustment would enhance colour development during

ripening. This study was conducted to examine the effects of crop load adjustment on the colour development of 'Hass' avocado fruit during three harvest times.

## 3.2. Materials and Methods

### *3.2.1. Plant materials*

In this study, avocados (cv. Hass) fruit were sourced from the commercial farm Nico Swart Estate in the Kiepersol area, Mpumalanga province of South Africa (25° 04' 12.7" S 31° 00' 35.8" E). Four months before early harvest (March 2018), twenty-four 'Hass' avocado trees of uniform size and vigour were selected for crop load adjustment treatments. After harvest, fruit were transported to the postharvest laboratory at Agricultural Research Council – Tropical and Subtropical Crops (ARC-TSC) (25° 27' 04.6" S 30° 58' 09.1" E), Nelspruit, Mpumalanga, South Africa for the postharvest experiment, storage and analysis.

### *3.2.2. Crop load adjustment treatments in the field*

In this experiment, the total number of fruit on each tree wasn't considered. The treatments were applied as; (a) high crop load (100%) (control), where no fruit was removed per panicle. (b) Moderate crop load adjustment (50%) was achieved by thinning the initial fruit set to half the number of fruit per panicle. (c) Low crop load adjustment (25%) was achieved by thinning the initial fruit set to quarter number per panicle (Figure 3.1). To study the subsequent effect of harvest time, the thinned (50% and 25%) and control (100%) trees were divided into nine trees per treatment and three trees per treatment were harvested in three commercial maturity stages *viz:* early, mid- and late harvest as summarised in Table 3.1.


Figure 3.1 Crop load adjustment treatments; high crop load (**A**), moderate crop load (**B**) and low crop load (**C**)

Table 3.1 Details of crop load adjustment treatments applied, number of trees used and trees harvested at each harvest maturity

| Treatments | Trees used | Harvest times | | |
|---|---|---|---|---|
| | | Early | Mid- | Late |
| 1. High crop load (100%) | Nine trees, no fruit were removed per panicle | Three trees were harvested and six continued | Three trees were harvested and three continued | Three trees were harvested |
| 2. Moderate crop load (50%) | Nine trees, thinning fruit to half number per panicle | Three trees were harvested and six continued | Three trees were harvested and three continued | Three trees were harvested |
| 3. Low crop load (25%) | Nine trees, thinning fruit to quarter number per panicle | Three trees were harvested and six continued | Three trees were harvested and three continued | Three trees were harvested |

*3.2.3. Postharvest experimental design and treatments*

The experimental design was carried out as 3 x 3 factorial, factor A (crop load adjustments) and B (harvest times) arranged in a completely randomized design (CRD) with three replications. Uniform-sized fruit were packed into the crates; each contained 30 fruit, replicated three times per treatment. The fruit was then cold stored at 5.5°C for 28 days and ripened at 25°C for 4 days. Fruit quality was evaluated every second day (0, 2 and 4 days) until they reached ‘eat ripe’ firmness. During ripening three fruit per replicate were sampled and frozen in liquid nitrogen; and subsequently, cold-stored at -81°C for further analysis.

*3.2.4. Chemicals and standards*

The following chemicals and standards were used methanol, sodium nitrite, ethanol, aluminium chloride, sodium hydroxide, quercetin, gallic acid monohydrate, sodium

carbonate, folin-ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetic acid, phosphoric acid, acetonitrile, cyanidin 3-*O*-glucoside stock solution, acetone, 1% metaphosphoric acid, 2,6-dichlorophenolindophenol dye and L-ascorbic acid.

### *3.2.5. Determination of dry matter content*

The dry matter content at harvest was determined using the method of Sugiyama and Tsuta (2010) with little modification. Avocado fruit samples were peeled and mesocarp grated to about 10 grams samples. The samples were weighed then oven dried (270 Labtech Eco Therm Economy, South Africa) at 30°C for 3 days until they were completely dry. The dried samples were reweighed and DM content was estimated by dry mass divided by fresh mass multiplied by 100.

Dry matter (%) = (Dry mass/Fresh mass) x 100

### *3.2.6. Determination of firmness*

The fruit firmness was measured using a non-destructive Sinclair IQ$^{TM}$ automated desktop machine (51DFTB, Jarrold Way, Bowthorpe, Norwich, Norfolk, NR5 9JD, England). The firmness of each fruit was determined at three points along the equatorial region and expressed in Newton (N).

### *3.2.7. Determination of colour parameters*

Avocado ‘Hass’ fruit exocarp colour change was measured subjectively using the visual colour rating scale as shown in Figure 3.2. The same samples were measured objectively using CIELAB colour coordinates L*, a* and b* with a calibrated Minolta

Chromameter (CR-400, Minolta Corp. Osaka, Japan). The chroma (C*) and hue angle (*h*°) were calculated according to McGuire (1992) using the following formulas:

Chroma (C*) = ((a*) ^2 + (b*)^2)½

Hue angle (*h*º) = θ + $\tan^{-1}$ (b*/a*)

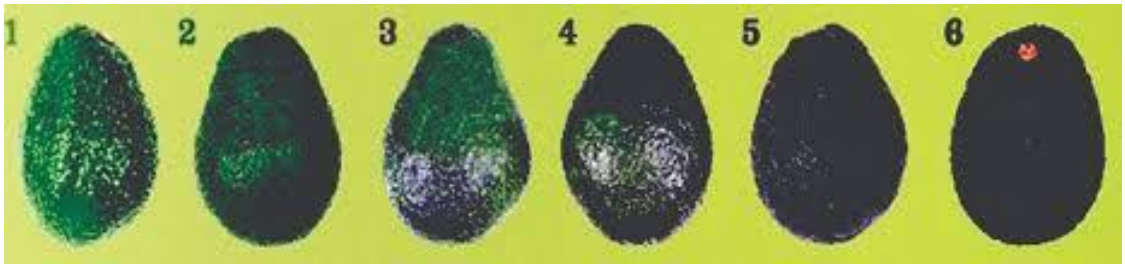



Figure 3.2 Visual colour rating scale (1= emerald-green, 2 = forest green, 3 = olive green, 4 = violet, 5 = purple, and 6 = black) of 'Hass' avocado fruit during ripening

*3.2.8. Sample preparation*

Fruit exocarp were separated manually and vacuum dried using a freeze dryer (BTP-9ES0VX, Benchtop Pro with Omnitronics™, Genevac ipswich, England). Thereafter, the dried exocarp samples were ground in liquid nitrogen to a fine powder using a pistol and mortar. The resultant fine powder was put in clean vials and stored at -80°C until needed.

*3.2.9. Determination of total chlorophyll and total carotenoids*

The concentration of chlorophyll and carotenoids were determined using the method of Lichtenthaler (1987) with modifications. A sample of 0.5 grams of fine powder avocado fruit exocarp was mixed with 10 mL of 80% (v/v) acetone kept on ice for 30

minutes. Thereafter, centrifuged at 6000 × g for 5 minutes, the supernatant was read in a spectrophotometer (UV-1800, Shimadzu, Corp. Japan) at 663 nm, 646 nm and 470 nm. Total chlorophyll and carotenoids concentration were calculated using the following equations.

$Ca = 12.25 \times A_{663} - 2.79 \times A_{646}$

$Cb = 12.50 \times A_{646} - 5.10 \times A_{663}$

$\text{Total chlorophyll} = 20.2 \times A_{646} + 8.02 \times A_{663}$

$\text{Total carotenoids} = 4.37 \times A_{470} + 2.11 \times A_{663} - 9.10 \times A_{646}$

Where C*a* referred to chlorophyll-*a* and C*b* referred to chlorophyll-*b*

*3.2.10. Determination of total anthocyanin and cyanidin 3-O-glucoside concentration*

The extraction was conducted according to Cox et al. (2004) with minor modification. A sample of 0.5 grams of fine avocado fruit exocarp powder was mixed with 5 mL of 10% acetic acid/methanol (v/v) at room temperature, followed by centrifugation at 3000 × g for 10 minutes, the supernatant was diluted 1:1 with methanol: water: acetic acid (50:50:10, v/v/v). Total anthocyanin concentration was determined using the pH differential method previously described by Giusti and Wrolstad (2001). The diluted 1:1 supernatant was filtered through 0.45 mm nylon filters into clean vials and mixed with 1 µL potassium chloride buffer ($pH_{1.0}$) and sodium acetate buffer ($pH_{4.5}$). The mixture was incubated in the dark for 10 minutes thereafter read in a spectrophotometer (UV-1800, Shimadzu, Corp. Japan) at 530 nm and 700 nm. Total anthocyanin was calculated using equations below.

$A = (A_{530} - A_{700})pH_{1.0} - (A_{530} - A_{700})pH_{4.5}$

$\text{Total anthocyanin} = (A \times MW \times Df)/ (\varepsilon \times L)$

Where A= Absorbance, Ɛ = cyanidin 3-glucoside molar absorbance (26900), MW = anthocyanin 164 molecular weight (449.2), Df = dilution factor, L = cell path length (1 cm)

Cyanidin 3-*O*-glucoside concentration was analysed using an HPLC equipped with JASCO units (LC-4000 Series, Madrid, Spain). The chromatography column was a Phenomenex AQUA 5u C18 125A 5 um PR-18e 4.6 × 150 mm (California, USA), maintained at 35°C. Mobile phases were: (A) 1.5 % $H_3PO_4$, and (B) acetic acid: acetonitrile: $H_3PO_4$: water (20: 24: 1.5: 54.5, v/v/v/v). The solvent program started with solvent (B) at 20%, increasing to 70% after 25 minutes then 90% at 30 minutes. After 35 minutes the solvent composition was returned to the initial 20% solvent (B) and ready for the next injection. The sample injection volume was 2 µL and detection was at 530 nm.

*3.2.11. Determination of sugar concentration*

Sugar concentration in the exocarp and seed were determined according to Tesfay et al. (2016) with slight modification. A sample of 1 gram of fine powder avocado fruit exocarp and seed was mixed with 10 mL of 80% (v/v) ethanol and vortexed for 60 seconds. Thereafter, the mixture was incubated in an 80°C water bath for 60 minutes; subsequently kept at 4°C overnight. After centrifugation at 10 000 × g for 5 minutes at 4°C, the supernatant was filtered through glass wool and taken to dryness in Genevac personal evaporator (EZ-2-3, SP, scientific, Genevac Ltd, Ipswhich, England). Afterwards, dried samples were re-suspended in 2.5 mL of ultra-pure water, vortexed for 60 seconds, then filtered through 0.45 mm nylon filters and sugar were analysed

using an HPLC (LC-20 AT, Shimadzu Corp. Kyoto, Japan) equipped with a refractive index detector and a Rezex RCM-monosaccharide column (8 mm pore size, Phenomenex, Torrance, CA, USA).

### *3.2.12. Determination of total phenolic concentration*

A method described by Hertog et al. (1992) with minimum modification was used to determine total phenolic. A sample of 2 grams of fine powder avocado fruit exocarp was mixed with 10 mL of 99.8% methanol and vortexed for 60 seconds thereafter, kept at room temperature for 6 hours. Subsequently, 1 mL of extract was mixed with 5 mL distilled water + 1 mL Folin-Ciocalteu reagent + 10 mL of 7% $Na_2CO_3$ + 8 mL distilled water and incubated in 90°C for 120 minutes. The absorbance was read at 750 nm using a spectrophotometer (UV-1800, Shimadzu, Corp. Japan) and expressed as mg $g^{-1}$ Gallic acid equivalent (GAE) DW.

### *3.2.13. Statistical analysis*

The statistical analysis was conducted using statistical software (GenStat, version 16$^{th}$, VSN International, UK) with the data subjected to two-factor analysis. Means were separated using Duncan multiple range tests (DMRT) at 5% level of significance. Data were further subjected to a Pearson correlation test in Statistix software version 10.1 to determine the relationship between postharvest fruit quality parameters.

## 3.3. Results and Discussion

### *3.3.1. Dry matter (DM) content at harvest*

In this study, DM at harvest was significantly influenced by harvest time ($p < 0.001$) and crop load adjustment ($p < 0.001$) (Figure 3.3). However, the interaction effect between harvest time and crop load was not evident on DM content at harvest ($p = 0.552$). A significant increase in DM content occurred with advancing harvest time for all studied crop loads. Dry matter (DM) content in avocado fruit is one of the most important indicators of fruit harvest maturity (Olarewaju, 2014). Early harvest fruit was found to have a lower DM content, while mid- and late harvest fruit had a higher DM content (Figure 3.3). These results are in agreement with those reported previously by several researchers on 'Hass' avocado fruit (Kruger et al., 1995, Osuna-García et al., 2010, Olarewaju, 2014). Bowen et al. (2018) reported an increasing trend in DM content in 'Hass' avocado fruit during early (28.9%), mid- (30.6%) and late harvest (36.9%). The significant increase in DM content was attributed to the cumulative effect of increasing oil content in avocado fruit during growth and development as previously reported by Magwaza and Tesfay (2015).

In this study, fruit maturity was significantly influenced by crop load adjustment ($p < 0.001$). Dry matter content of fruit from high crop load increased from 25 to 25.3 and 31% at early, mid- and late harvest, respectively (Figure 3.3). The DM content of fruit from moderate load increased from 27 to 29.6 and 32.3% at early, mid- and late harvest, respectively. There are currently no published studies reporting on the effect of crop load on the DM content of avocado fruit at harvest. Our results are within the

range of 20 - 33.7% DM content previously reported by Osuna-García et al. (2010) and Olarewaju (2014). In this study, the DM content of fruit from high load and moderate crop load did not differ significantly during early and late harvest times. However, significant differences were observed with lower DM content in fruit from low crop load at early and late harvest.

As a result, the highest DM content was found in fruit harvested from low crop load during early (31%), mid- (31.6%) and late harvest (36.6%). Our study found higher DM contents in avocado fruit than those reported in the literature. Therefore, the finding of the current study suggested that harvest maturity of 'Hass' avocado fruit by DM content was advanced with crop load adjustment, particularly for moderate and low crop loads, which could be in relation to reduced carbohydrates competition between developing fruit within each panicle as reported by Castro et al. (2015) in 'Caricia' and 'Eva' apple fruit. The results of this study are still in agreement with Gamble et al. (2010) who indicated that avocado fruit with a dry matter of 20 and 40% should be considered minimal and very mature, respectively.

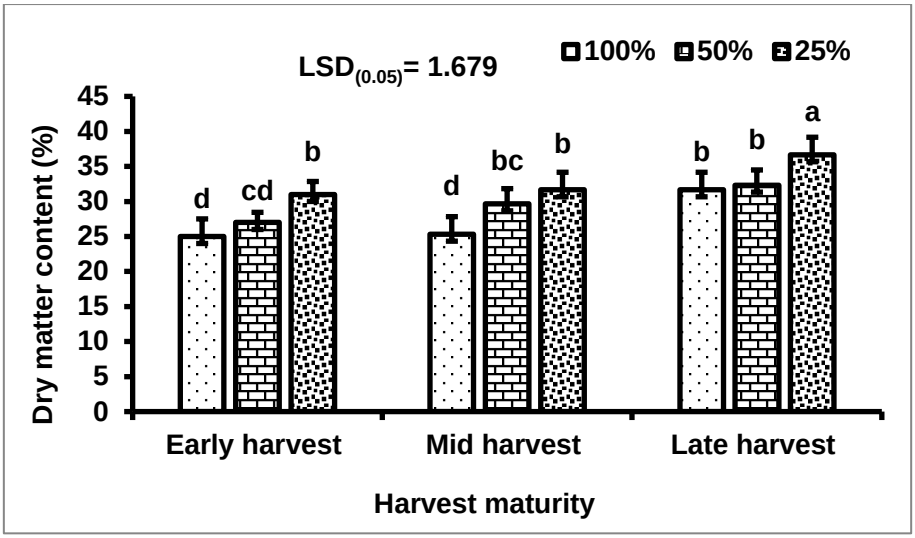



Figure 3.3 Dry matter content at harvest of 'Hass' avocado fruit from high (100%), moderate (50%) and low crop load (25%) during early, mid- and late harvests. Means followed by different letters are significant different. Error bars indicate ±SE of means at p≤ 0.05

*3.3.2. Fruit firmness*

In this study, 'Hass' avocado fruit firmness at three harvest times decreased continuously with ripening days at 25°C, irrespective of crop load adjustment (Figure 3.4). The decrease in firmness during ripening was consistent with the results by other authors on 'Hass' avocado fruit harvested at different maturity stages (Blakey, 2011, Mathaba et al., 2015). Furthermore, firmness declined with ripening days at 25°C and significantly (p= 0.051) interactive effect between harvest time and crop load was observed (Figure 3.4). During the initial ripening days at 25°C (0 and 2 days) of early harvest, the firmness of fruit from high crop load was higher compared to those from moderate and low crop loads (Figure 3.4a). However, firmness did not differ between all crop load adjustments after 4 ripening days at 25°C. During the three harvest stages, firmness was higher in fruit from the low load than in those from moderate and high crop loads after day 4 at 25°C. In this study, fruit from low crop load were firmer

compared to those from high and moderate during ripening at 25°C during the mid- and late harvest stages (Figure 3.4b). These results were in agreement with previous findings of Alcobendas et al. (2012); whereby, ‘Flordaster’ peach fruit from the low crop load was greater and firmer than those from the commercially loaded tree.

However, fruit harvested from high crop load were firmer at early harvest during the initial (0 and 2 days) at 25°C and softer at late harvest after 2 and 4 days at 25°C (Figure 3.4c). In addition, no significant differences in firmness were observed between fruit from high and moderate crop loads during mid-harvest across all days at 25°C. According to Olarewaju (2014), higher firmness is attributed to lower dry weight content; thus, in this study, fruit from high load showed lower dry matter content of 25% during early harvest. Furthermore, rapid softening during mid- and late harvest in fruit from high and moderate crop load could be associated with an interactive effect between carbohydrates, water stress, abscisic acid (ABA) and ethylene biosynthesis as previously explained by Blakey et al. (2009). This reason was supported by Yeshitela (2006), who reported that crop load adjustment by fruit thinning for ‘Sensation’ mango reduced carbohydrates competition amongst developing fruits subsequently, improving fruit quality in terms of firmness, soluble solids content and anthocyanin production.

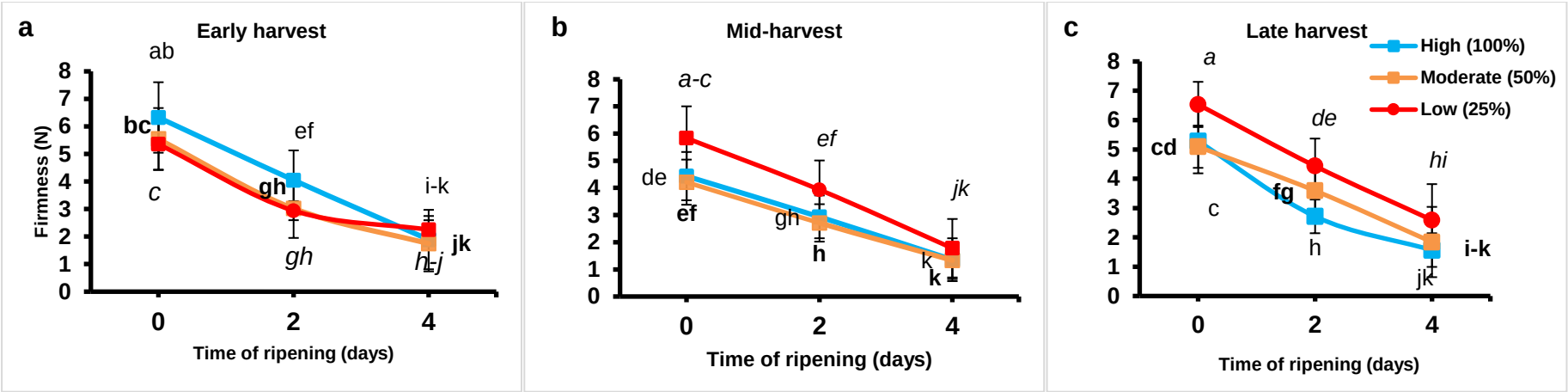



Figure 3.4 Changes in firmness of 'Hass' avocado fruit from high, moderate and low crop load during early, mid- and late harvests. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

*3.3.3. Exocarp colour change*

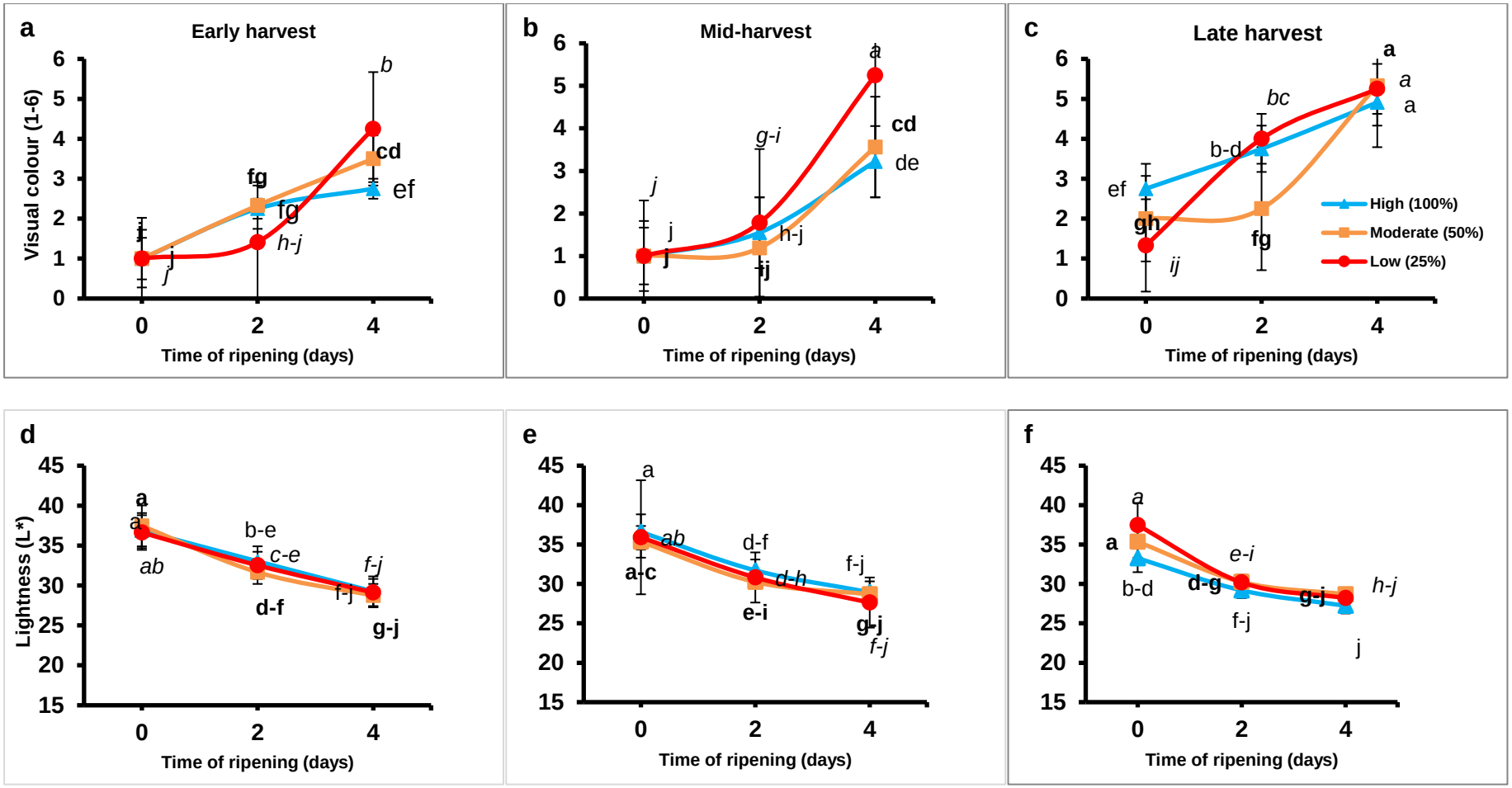
The colour development of ‘Hass’ avocado fruit is very important for the industry, it influences marketability and consumers preference (Mathaba et al., 2015). However, exocarp colour development should correlate with fruit softening (Cox et al., 2004). In this study, a strong negative correlation (r= -0.630**) was observed between visual colour and firmness during ripening at 25°C (Table 3.2). Changes in colour parameters during ripening of ‘Hass’ avocado fruit harvested from trees of different crop loads at three maturity stages are presented in Figure 3.5. In general, exocarp colour change as measured by visual colour increased with ripening days at 25°C. This trend was significantly ($p< 0.001$) influenced by both harvest time and crop load adjustment and their interaction ($p< 0.001$) during ripening (Figure 3.5a-c). The visual colour index showed an increase with days at 25°C as the fruit changed exocarp colour from green (≈ 1 visual colour rating) to purple (≈ 5 visual colour rating) or black (≈ 6 visual colour rating), as the result of anthocyanin accumulation (Cox et al., 2004). In this study, the increase in exocarp visual rating colour at 25°C was in agreement with the previous reports on ‘Hass’ avocado fruit (Ashton et al., 2006, Donetti and Terry, 2014, Mathaba et al., 2015).

Moreover, early harvest fruit showed poor exocarp colour development during ripening when compared with those at advanced maturity stages (mid- and late harvest). The observed insufficient exocarp colour development after 4 ripening days at 25°C indicated that early harvest fruit were still actively undergoing the maturation process compared to mid- and late harvest. This was in agreement with Mathaba et al. (2015), whereby, poor colour development was predominantly prevalent in early and mid-

harvested than late harvest. According to these authors, maturity status may be the key factor influencing postharvest exocarp colour development in ‘Hass’ avocado fruit exocarp. In this study, exocarp colour of fruit from moderate load and low load showed improved colour development compared to those from high crop load as evident on day 4 at 25°C across all harvest times (Figure 3.5 and 3.6). During early harvest, fruit from low crop load changed significantly from green (≈ 1 visual colour rating) to violet colour (≈ 4 visual colour rating) after 4 ripening days at 25°C while those from moderate and high crop load changed to olive green (≈ 3 visual colour) and forest green (≈ 2 visual colour), respectively (Figure 3.5a and 3.2). In this study, early harvest showed reduced colour development particularly for fruit from high and moderate crop loads. This could be attributed to lower anthocyanin concentrations in fruit exocarp as presented in Figure 3.8a. However, fruit from low load reached the desirable purple colour (≈ 5 visual colour) on day 4 at 25°C while those from moderate and high crop load did not differ significantly in visual colour and had changed to violet (≈ 4 visual colour) during mid-harvest (Figure 3.5b). Moreover, there were significant differences between all crop load treatments on day 0 during late harvest; however, fruit from all crop load adjustments reached the desirable purple colour (≈ 5 visual colour rating) after 4 days at 25°C (Figure 3.5c). The observed differences on day 0 during late harvest corroborated the general phenomenon of ‘Hass’ avocado, whereby late hanging fruit change colour while still attached to the mother plant. The late hanging of avocado commonly coincides with the flower bud development period. Therefore, we suppose that continuous carbohydrate supply during this period could contribute to anthocyanin biosynthesis and accumulation and possibly colour development.

Furthermore, fruit exocarp lightness (L*) was significantly affected by harvest time (p= 0 .020) but not by crop load adjustment (p= 0.857) and their interaction (p= 0.696) during ripening (Figure 3.5d-f). However, 'Hass' avocado fruit exocarp colour intensity (C*) differed significantly with harvest time (p< 0.001) and fruit load (p< 0.001) and their interaction (p= 0.014) (Figure 3.5g-i). Also, fruit exocarp hue ($h^o$) was significantly influenced by harvest time (p< 0.001) and crop load adjustment (p= 0.042) and the interaction between the two factors (p< 0.001) (Figure 3.5j-l). In the three harvest times, all exocarp colour chromaticity properties as measured by L*, C* and $h^o$ showed a decline with ripening period regardless of fruit load, probably resulting from chlorophyll degradation featured by anthocyanin accumulation in the exocarp of the fruit (Ashton et al., 2006). The observed decline in colour chromaticity properties values during ripening in this study confirmed findings reported by Cox et al. (2004) and Donetti and Terry (2014); whereby, colour properties L*, C* and $h^o$ decreased as fruit change exocarp colour from green to purple and black as the fruit ripens. Interestingly, during early and mid-harvest, the $h^o$ values decreased gradually with advancing ripening, with the highest decline occurring on day 4 at 25°C for fruit harvested from low crop load followed by moderate load (Figure 3.5j-k). This could be as a result of greenness loss due to chlorophyll degradation (Figure 3.7g-h), concurrently, anthocyanin and cyanidin 3-*O*-glucoside accumulation in fruit exocarp; which result in colour development for fruit from low and moderate compared to high crop load during early and mid-harvest as presented in Figure 3.8. This was also supported by strong negative correlation between $h^o$ and visual colour (r= -0.912**), total anthocyanin (r= -0.715**) and cyanidin 3-*O*-glucoside (r= -0.712**) (Table 3.2). Furthermore, the lower $h^o$ values in fruit from all crop load adjustment after 4 ripening days at 25°C did not differ significantly during late harvest. However, all late harvest

fruit attained the desirable purple colour after 4 ripening days at 25°C, regardless of crop load treatments (Figure 3.5). Based on the results of this study, it can be deduced that the overall exocarp colour development of ‘Hass’ avocado fruit during ripening was influenced by harvest time. However, adjusting crop load to moderate and low crop load also played a significant role in improving exocarp colour during ripening in early and mid-harvested as observed on day 4 at 25°C.


a
Early harvest
Visual colour (1-6)
Time of ripening (days)
b
Mid-harvest
Time of ripening (days)
c
Late harvest
Time of ripening (days)
High (100%)
Moderate (50%)
Low (25%)
d
Lightness (L*)
Time of ripening (days)
e
Time of ripening (days)
f
Time of ripening (days)

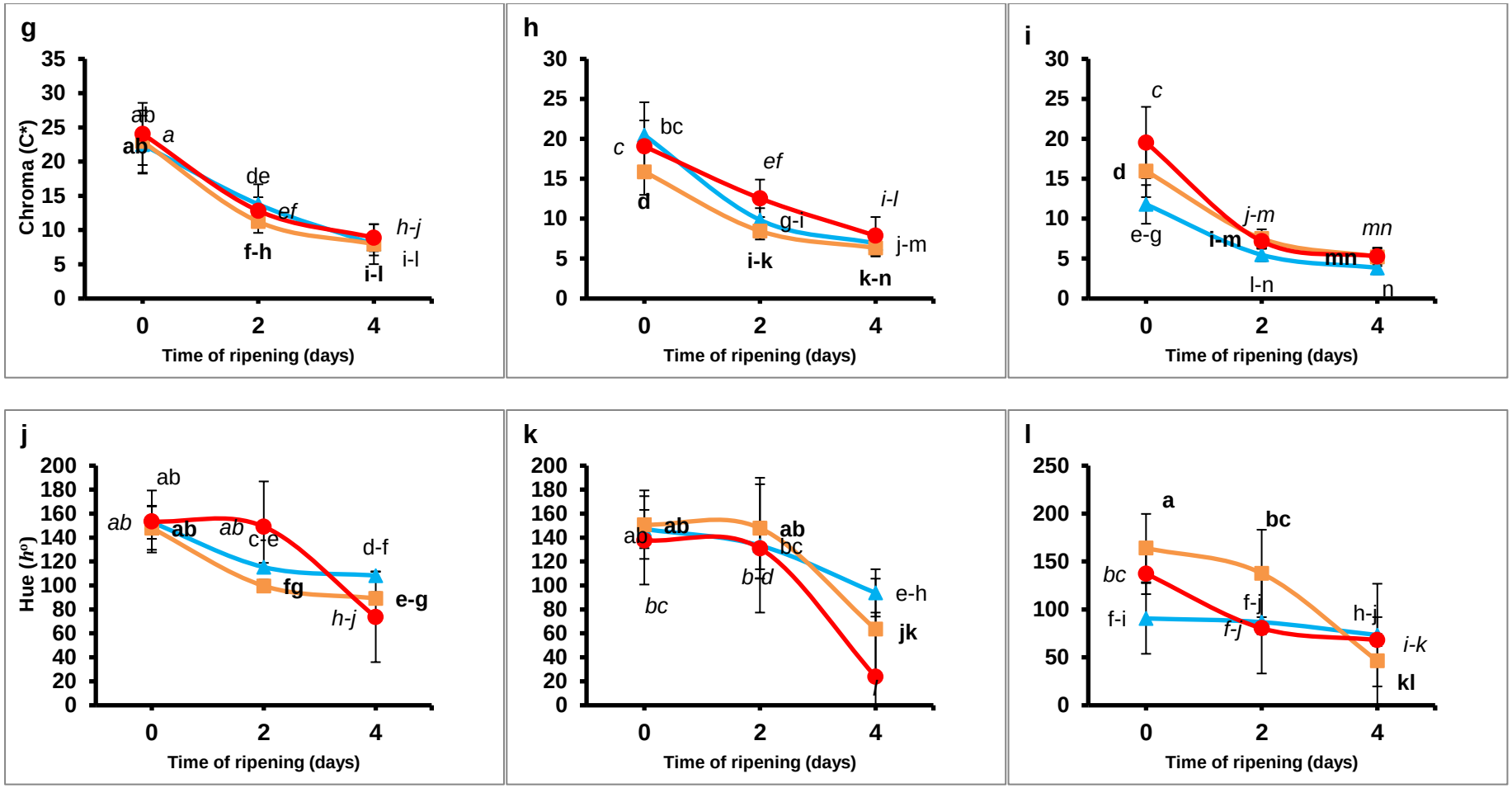



Figure 3.5 Changes in colour parameters in visual colour (**a-c**), lightness (**d-f**), chroma (**g-i**) and hue (**j-l**) of 'Hass' avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

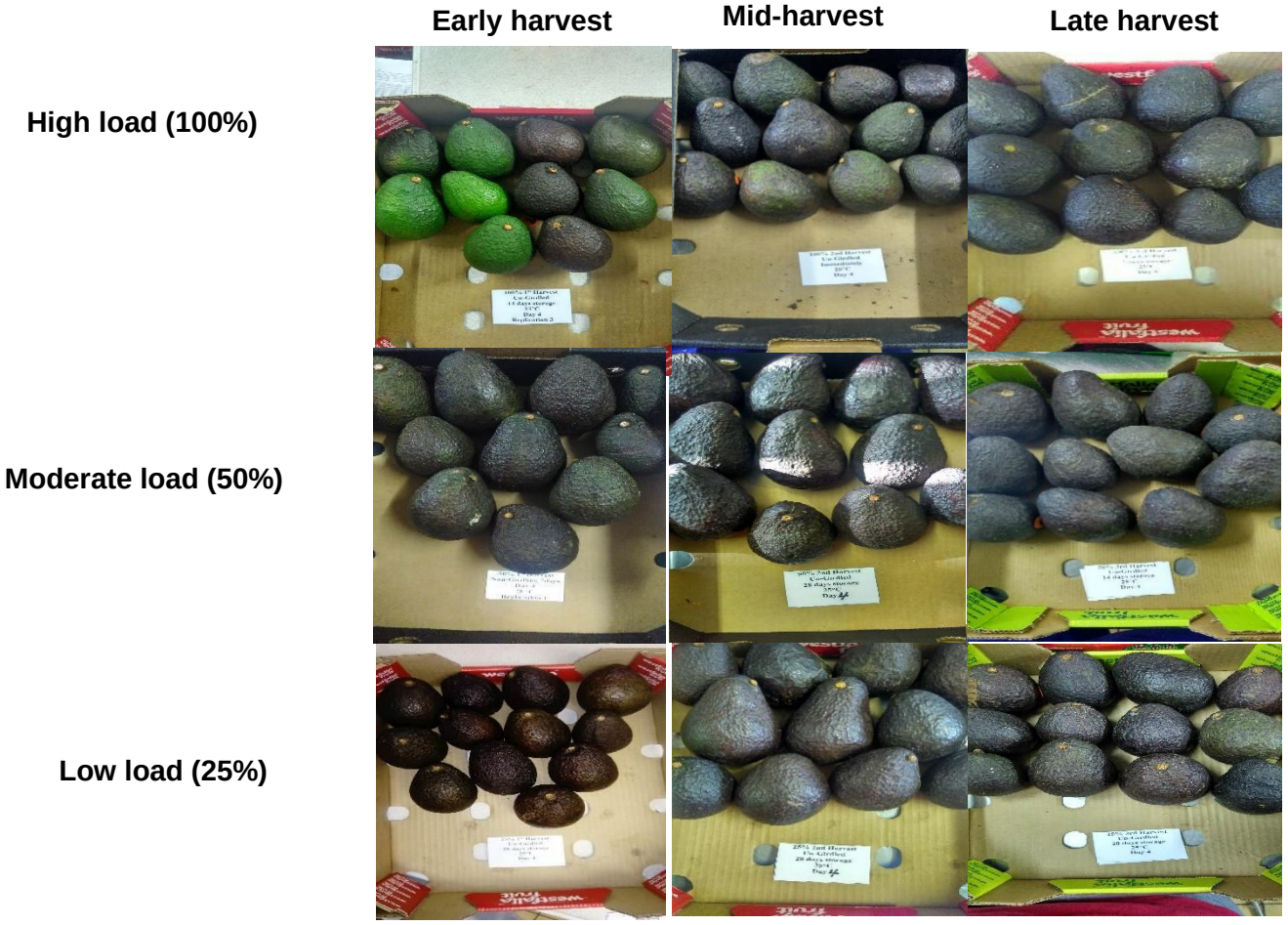



Figure 3.6 The difference in colour development of ‘Hass’ avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons after 4 days at 25°C

### *3.3.4. Exocarp pigments changes*

#### *3.3.4.1 Chlorophyll content*

Chlorophyll degradation during ripening is directly related to anthocyanin synthesis and accumulation in 'Hass' avocado fruit (Ashton et al., 2006, Cox et al., 2004). In this study, an interaction effect between harvest time and crop load adjustment on chlorophyll-a and chlorophyll-b significantly ($p< 0.001$) affected fruit exocarp colour development during ripening (Figure 3.7a-f). The changes in chlorophyll-a and chlorophyll-b showed similar decreasing trends regardless of crop load adjustment and harvest time. Our results are consistent with Donetti and Terry (2011) and Ashton et al. (2006) who reported a significant decrease in chlorophyll-a and chlorophyll-b in 'Hass' avocado fruit harvested at different harvest times. The observed decrease in chlorophyll-a and chlorophyll-b concentration during ripening at 25°C is an indication of chlorophyll degradation. In this study, chlorophyll-a and chlorophyll-b were strongly correlated with total chlorophyll ($r= 0.908^{**}$ and $0.832^{**}$, respectively) (Table 3.2).

During early harvest, fruit from high and low crop loads showed no significant differences in chlorophyll-a content on day 4 at 25°C while fruit from moderate crop load showed significantly higher content. Fruit from low crop load showed lower chlorophyll-a content with all ripening days at 25°C during mid harvest compared to high and moderate crop loads. During late harvest, fruit from low and moderate crop loads showed significantly higher chlorophyll-a content compared to those from high crop load on day 0 at 25°C. In terms of chlorophyll-b, fruit from low crop load showed a significantly rapid decline with ripening across all harvest times (Figure 3.7d-f). The

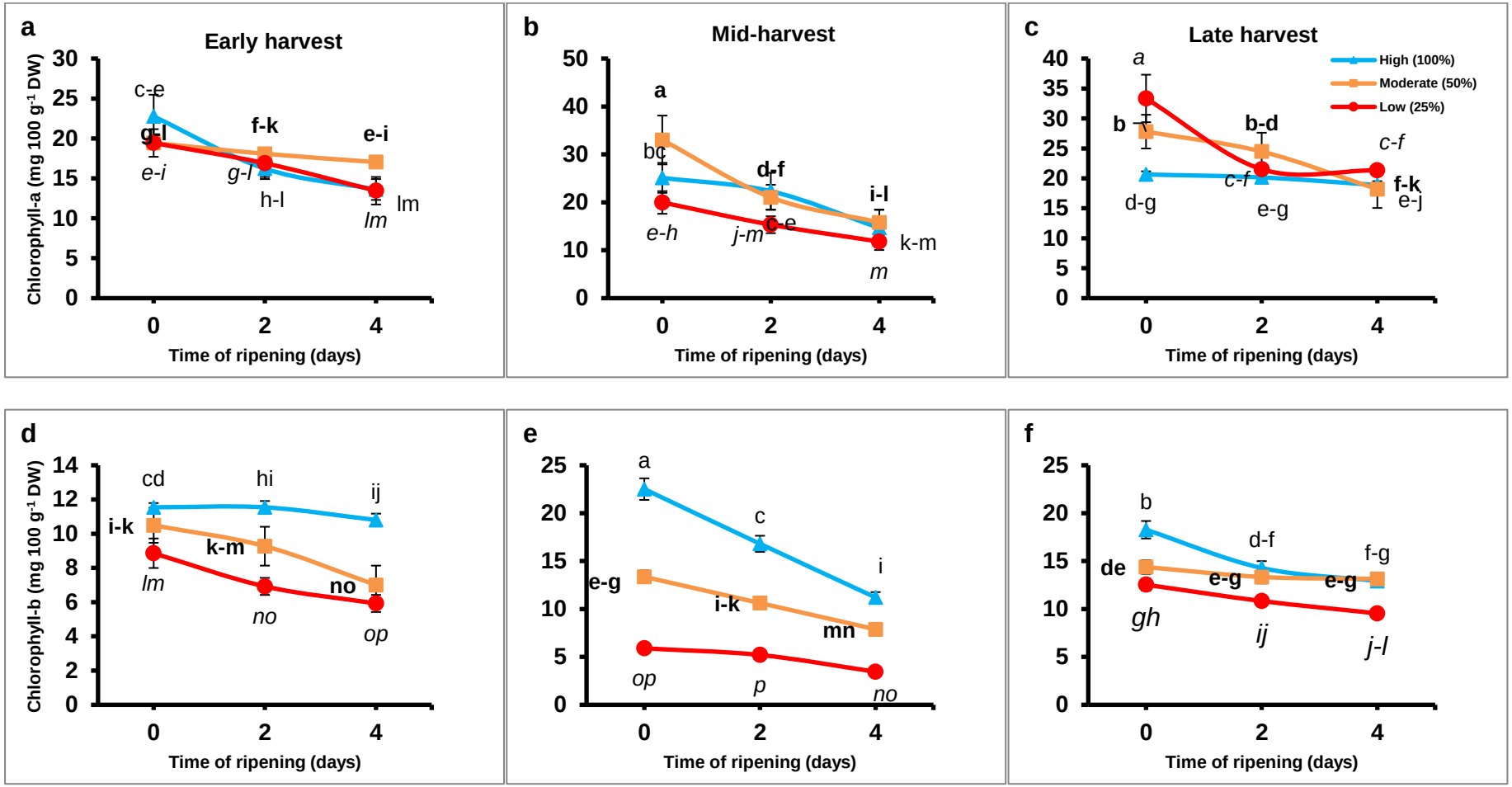
observed rapid decline in chlorophyll-b for fruit from low crop load could be attributed to the cumulative effects of total chlorophyll degradation as the fruit ripens.

Consequently, fruit from low crop load showed rapid decline in total chlorophyll content with ripening days at 25°C than high and moderate crop loads during early and mid-harvest. In this study, total chlorophyll content in 'Hass' avocado exocarp was significantly influenced by both harvest time ($p< 0.001$) and crop load adjustment ($p< 0.001$) and their interaction ($p< 0.001$) during ripening. In general, chlorophyll degradation occurred in 'Hass' avocado fruit exocarp during ripening. However, total chlorophyll degradation rate was higher in fruit from low crop load than those from moderate and high loads during early and mid-harvest (Figure 3.7g-i). These results are in agreement with Wünsche et al. (2005), whereby increased crop load resulted in higher chlorophyll concentration for 'Braeburn' apples. However, the decline in chlorophyll content in 'Hass' avocado fruit during postharvest ripening has been ascribed to an increase in anthocyanin pigment accumulation (Cox et al., 2004, Ashton et al., 2006, Donetti and Terry, 2011). This further supports the higher total anthocyanin and cyanidin 3-*O*-glucoside concentration found in the exocarp of fruit harvested from low crop load during early and mid- harvest (Figure 3.8a-f). Our results also suggest that different harvest time and crop load might influence the postharvest change of chlorophyll content in 'Hass' avocado exocarp.

The exocarp of 'Hass' avocado fruit contains a considerable amount of carotenoids and contributes to colour development (Ashton et al., 2006). In this study, total carotenoids content decreased during ripening for all fruit load treatments across all harvest times (Figure 3.7j-l). These findings agree with Donetti and Terry (2011),

whereby carotenoids content in ‘Hass’ avocado declines during ripening, irrespective of harvest time. The influence of harvest time and crop load on decreasing tendency of total carotenoids was not significantly evident. In terms of harvest time, total carotenoids content increased from early to mid-harvest then decreased from mid- to late harvest for crop load treatments. Consequently, late harvest fruit showed lower total carotenoids than early and mid-harvest. These observations were in agreement with Donetti and Terry (2011) who also found lower carotenoids content in late season ‘Hass’ avocado fruit on the initial days of ripening. This could be related to the phenomenon; whereby, late season ‘Hass’ avocado fruit develops colour while still hanging. We suppose that carotenoids degradation in late harvest occurred while fruit was still hanging.

Moreover, total carotenoids content showed no significant difference between fruit from high and low crop loads on day 4 at 25°C during early and mid-harvest (Figure 3.7j-k). However, fruit from low crop load showed significantly higher carotenoids content followed by those harvested from moderate while fruit from high load recorded the lowest content on day 0 and 2 at 25°C during mid harvest. Similarly, fruit from low load showed significantly higher total carotenoids content at late harvest when compared with fruit from moderate and high crop load throughout the ripening period.


a
Early harvest
Chlorophyll-a (mg 100 g-1 DW)
Time of ripening (days)
b
Mid-harvest
Time of ripening (days)
c
Late harvest
High (100%)
Moderate (50%)
Low (25%)
Time of ripening (days)
d
Chlorophyll-b (mg 100 g-1 DW)
Time of ripening (days)
e
Time of ripening (days)
f
Time of ripening (days)

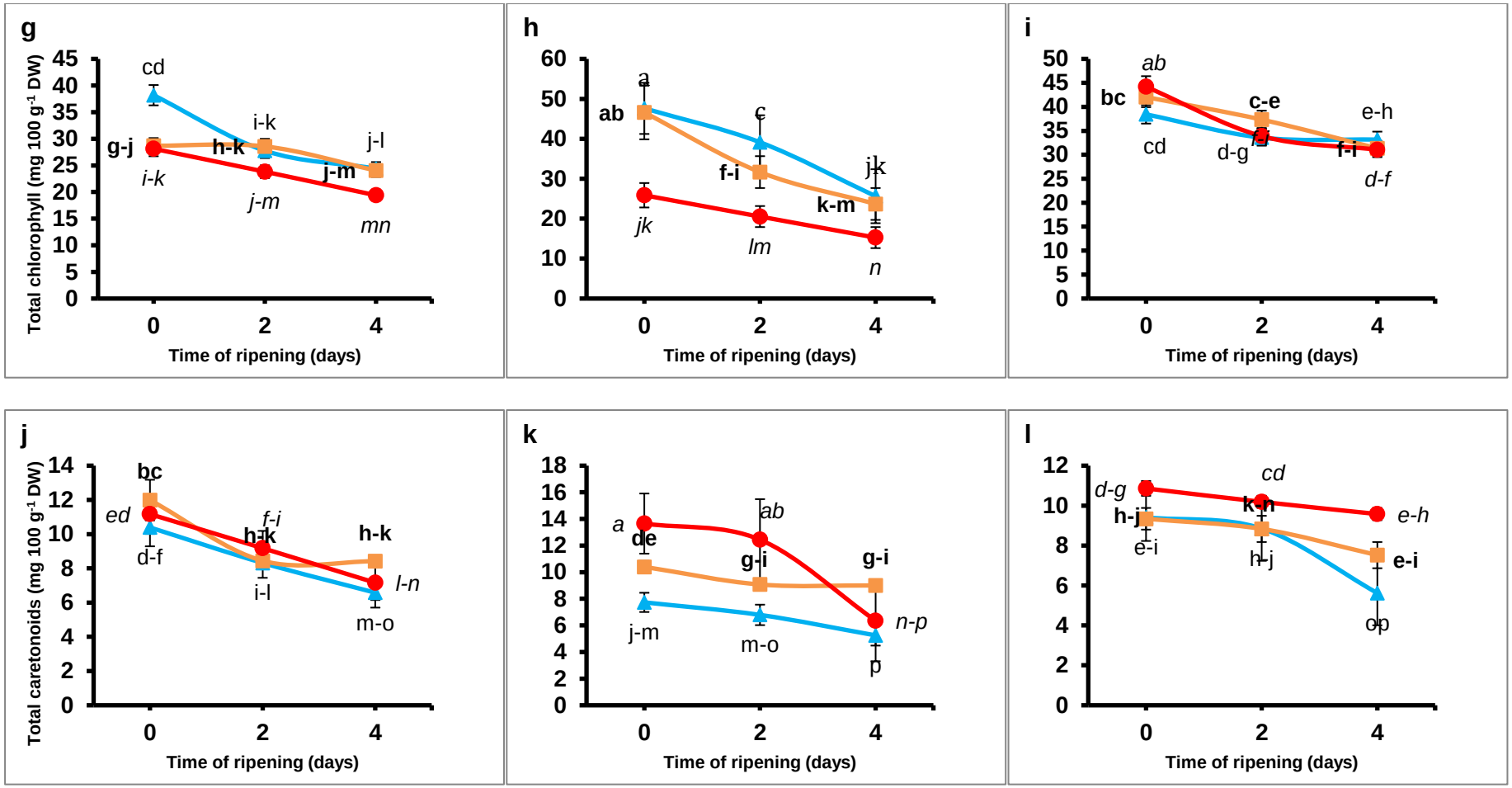



Figure 3.7 Changes in chlorophyll a (**a-c**), chlorophyll b (**d-f**), total chlorophyll (**g-i**) and total carotenoids (**j-l**) of 'Hass' avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

*3.3.4.2. Total anthocyanin and cyanidin 3-O-glucoside concentrations*

There was a significant ($p < 0.001$) interaction between harvest time and crop load adjustment on total anthocyanin during ripening (Figure 3.8a-c). Across all harvest times, anthocyanin concentration increased significantly ($p < 0.001$) with ripening days at 25°C. In this study, late harvest measured high total anthocyanin concentration followed by mid-harvest, while early harvest showed lower concentration during ripening. Our results are similar to those reported by Bertling et al. (2007), who found that anthocyanin concentration in the exocarp of 'Hass' avocado harvested later was higher when compared with those harvested earlier and middle in the season. In this study, during early harvest total anthocyanin concentration was significantly higher in fruit from moderate and low crop loads than high crop load on day 4 at 25°C. The influence of crop load adjustment on anthocyanin concentration was evident during mid-harvest, whereby higher total anthocyanin concentration was found in fruit from low load throughout ripening days at 25°C, followed by moderate load while the lowest concentration was observed in fruit from high crop load (Figure 3.8b). The increase in anthocyanin concentration resulted in enhanced colour development in fruit from low and moderate loads as measured by visual colour as presented in Figure 3.6b. This result corroborates the general notion that crop load adjustment increases carbon supply during growth and development which contribute to the formation of flavonoids (Awad et al., 2001).

However, there was no significant difference in total anthocyanin concentration among crop load treatments after 4 ripening days at 25°C during late harvest (Figure 3.8c). This could be attributed to the significant ($p < 0.001$) effect of both harvest time and

crop load adjustment on fruit maturity level as presented in Figure 3.3. It can be deduced that the effect of fruit maturity is critical in predicting the colour development of ‘Hass’ avocado during ripening.

According to Cox et al. (2004), cyanidin 3-*O*-glucoside has been identified as the dominant pigment in ‘Hass’ avocado fruit exocarp and its concentration increases during ripening. In this study, the interaction between harvest time and crop load was significant ($p< 0.001$) for cyanidin 3-*O*-glucoside concentration during ripening. In general, cyanidin 3-*O*-glucoside concentration significantly increased during ripening across all harvest times (Figure 3.8d-f). The observed increasing trend in cyanidin 3-*O*-glucoside concentration in this study was in agreement with those reported by several researchers for ‘Hass’ avocado during ripening (Ashton et al., 2006, Cox et al., 2004, Donetti and Terry, 2011, Donetti and Terry, 2014).

Furthermore, the influence of fruit loads on cyanidin 3-*O*-glucoside concentration was significantly evident during ripening across all harvest times. When all crop load adjustments were compared, it was evident that fruit from low load had significantly higher cyanidin 3-*O*-glucoside after 4 ripening days at 25°C during mid-harvest than moderate and high crop loads (Figure 3.8d-e). This further supports higher total anthocyanin concentration found in fruit from low crop load in Figure 3.8b which resulted in improved colour development (≈ 5 visual colour) as presented in Figure 3.5b. Cyanidin 3-*O*-glucoside showed significantly strong correlation with visual colour ($r= 0.768^{**}$) and total anthocyanin ($r= 0.803^{**}$) (Table 3.2). In this study, crop load adjustment effect is in accordance with the phenomenon that high carbon availability due to reduced competition is favourable to the production of anthocyanin through the

influence of carbohydrate as the main precursor (Yeshitela, 2006). Furthermore, the positive relationship between carbohydrate level and anthocyanin is also well documented.

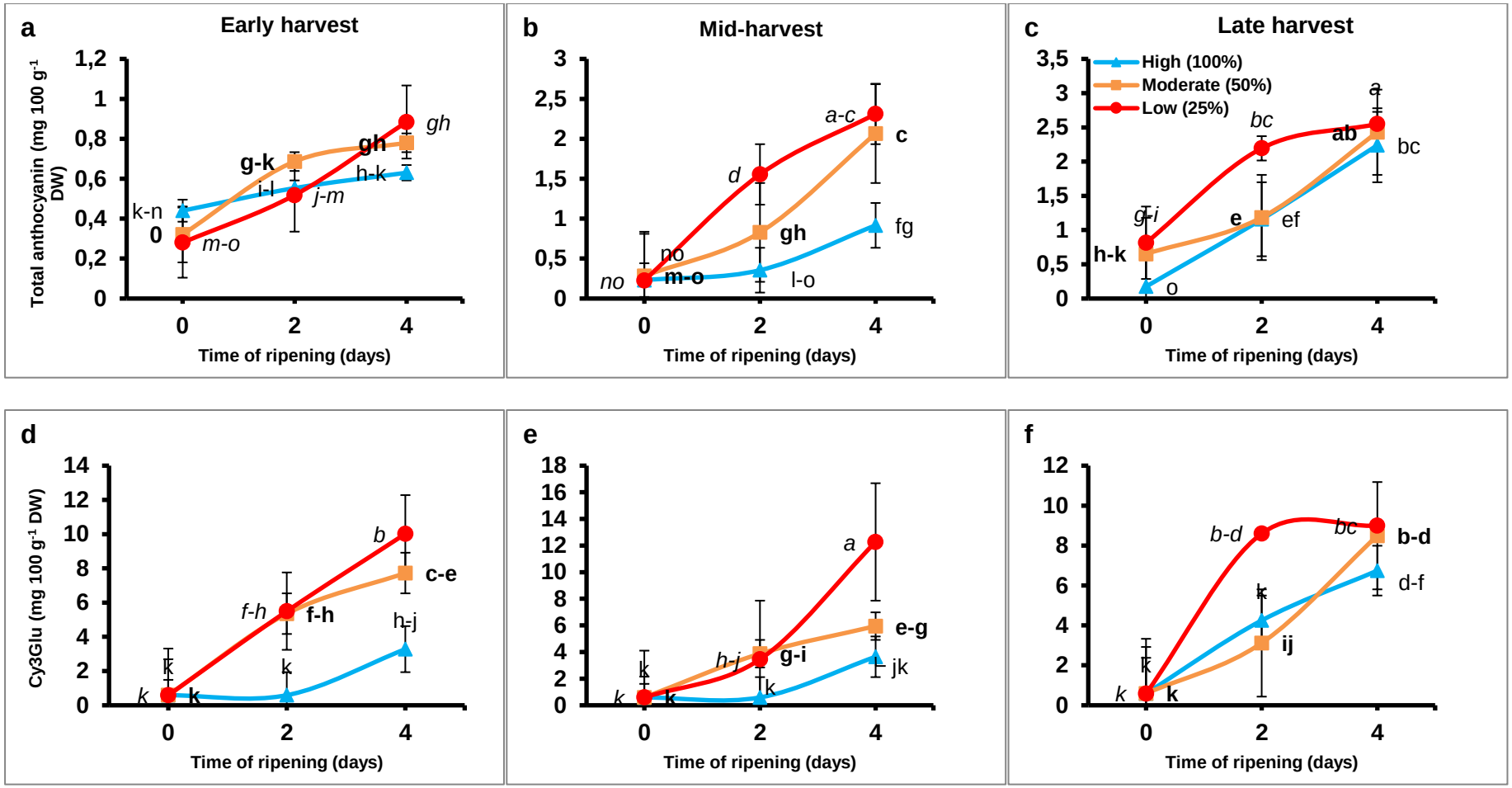



Figure 3.8 Changes in total anthocyanin (**a-c**) and cyanidin 3-*O*-glucoside (Cy3Glu) (**d-f**) concentration of 'Hass' avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

*3.3.5. Change in exocarp sugars concentration*

In this study, the results showed that D-mannoheptulose and perseitol accumulation were significantly influenced by harvest time ($p< 0.001$) and crop load adjustment ($p< 0.001$) but not by their interaction ($p= 0.213$) during ripening. Changes in D-mannoheptulose and perseitol concentrations in 'Hass' avocado exocarp harvested from different crop loads at three maturity stages are presented in Figure 3.9. In this study, the change in D-mannoheptulose and perseitol concentration showed similar decreasing trends with ripening days at 25°C regardless of crop load adjustment and harvest time. Our observations were in agreement with previous studies (Blakey, 2011, Donetti, 2011, Donetti and Terry, 2011), who reported that D-mannoheptulose and perseitol concentration decreases during ripening irrespective of harvest time. At the beginning of ripening (day 0), D-mannoheptulose and perseitol concentration was higher in mid-harvest fruit followed by late harvest while early harvest fruit had lower concentration. These results were in agreement with those previously reported by Donetti (2011). Also, a study conducted by Blakey (2011) showed that D-mannoheptulose and perseitol concentrations in the mesocarp of 'Hass' avocado fruit were significantly affected by harvest date. Furthermore, the seasonal fluctuation of sugars such as fructose, glucose, D-mannoheptulose and perseitol in 'Hass' avocado mesocarp and exocarp were also previously reported by Liu et al. (1999). The same authors also found that 'Hass' avocado fruit at advanced maturity stage varied a little in perseitol concentration and was found to be 3% of the dry weight in the exocarp, this was attributed to the reason that perseitol act as non-structural storage carbohydrates for D-mannoheptulose. In this study, there was a significant and positive correlation ($r= 0.877^{**}$) between exocarp D-mannoheptulose and perseitol concentration during ripening (Table 3.2).

Furthermore, fruit harvested from low and moderate crop loads showed significantly higher D-mannoheptulose and perseitol concentration at day 0 when compared with fruit from high crop load during all three harvest times (Figure 3.9). However, no significant differences were found in D-mannoheptulose concentration between moderate and high crop load during mid- and late harvest (Figure 3.9b-c). During mid- and late harvest, fruit harvested from low crop load showed significantly higher D-mannoheptulose concentration after 2 and 4 ripening days at 25°C than those from moderate and high crop load. In this study, crop load adjustment clearly increased D-mannoheptulose and perseitol accumulation at harvest. The reason for high exocarp D-mannoheptulose and perseitol accumulation might be attributed to the fact that crop load adjustment to moderate or low crop load reduced competition between fruit for minerals, water, nutrients and stem carbohydrate reserved and advanced fruit maturation as indicated by dry weight. Supporting this, fruit harvested from low and moderate loads showed increased dry matter content during early, mid- and late harvest when compared with fruit from high crop load (Figure 3.3). In agreement with previous studies (Wolstenholme and Robert, 1991, Shalom et al., 2012), low crop load tends to partition high amounts of carbohydrates into developing fruit whereas, heavy/high crop load compete for available carbohydrate. These studies have also shown that high crop load has an adverse bearing on flowering, fruit set and fruit development and maturation. Consequently, the tree is forced to enter into the resting phase which results in the production of small-sized and poor-quality fruit the following season. Several studies in ‘Hass’ avocado fruit reported that carbohydrate partition during fruit development is the most important factor that influences postharvest fruit quality. This is further supported in this study by improved fruit quality including

firmness (Figure 3.4), colour development (Figure 3.5) and pigments (Figure 3.7 and 3.8).

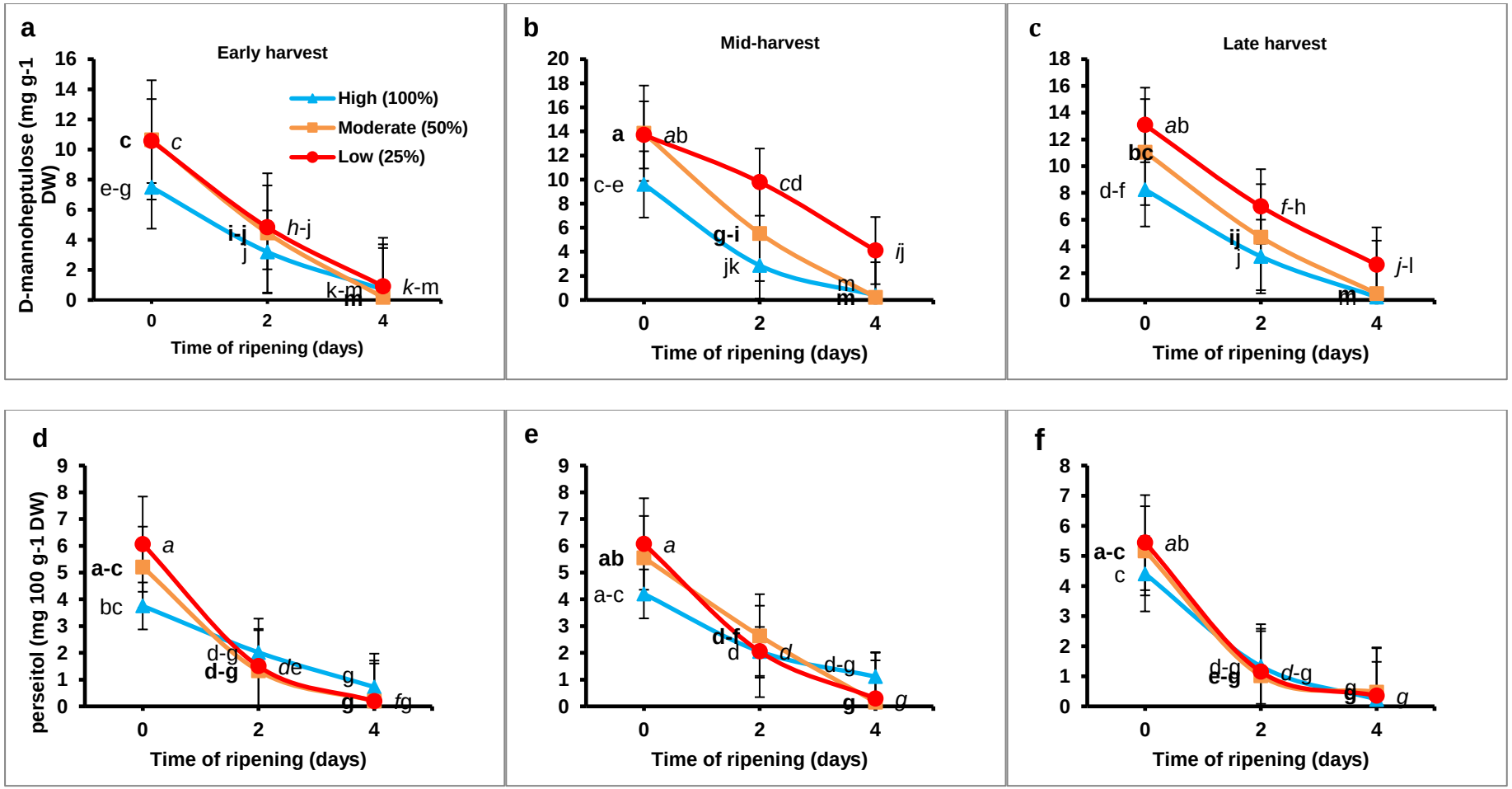



Figure 3.9 Changes in exocarp D-mannoheptulose (**a-c**) and perseitol (**d-f**) concentration of 'Hass' avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

*3.3.6. Change in exocarp total phenolic concentration*

The effect of crop load adjustment and harvest time and their interaction on total phenolic were significant ($p < 0.001$) during ripening (Figure 3.10a-c). In general, total phenolic concentration increased significantly during ripening regardless of crop load adjustment and harvest time until 4 days. In this study, late harvest fruit had higher total phenolic concentration than mid- and early harvest, however, fluctuation in total phenolic concentration was observed with crop load adjustment. The findings in the present study on change in total phenolic concentration were in agreement with Cutting and Wolstenholme (1992) who reported a significant increase in mesocarp phenolic concentration with advancing fruit maturity. During early harvest, fruit from low and moderate crop loads had higher total phenolic concentration from 2 to 4 ripening days at 25°C when compared with those from high crop load (Figure 3.10a). Furthermore, fruit from low and moderate crop loads showed significantly higher total phenolic concentration throughout the ripening period than fruit from high crop load in mid-harvest. The significant increase in total phenolic concentration in fruit from low and moderate crop load could be attributed to high sugar accumulation in the exocarp (Figure 3.9b-c) and advanced fruit maturation (Figure 3.3). Several studies reported that many factors may affect total phenolic concentration including variety, fruit maturity, sugar accumulation, growing condition, and abiotic and biotic stress (Tesfay et al., 2010, Wang et al., 2010).

Furthermore, there was no significant difference observed in total phenolic concentration between fruit harvested from low and moderate crop loads after 4 ripening days during mid- and late harvest (Figure 3.10b-c). Wang et al. (2010) reported that phenolic compounds are ubiquitous in avocado fruit tissues: seed,

exocarp and mesocarp and forms an integral part of antioxidant capacity. The most abundant phenolic compounds in the exocarp were catechin and epicatechin (Tesfay et al., 2010, Donetti, 2011). We did not quantify individual phenolic compounds in this study. However, Donetti (2011) quantified individual phenolic compounds including catechin, epicatechin and procyanidin B2. In the same study, individual phenolic epicatechin and procyanidin B2 increased during early and late harvest while in mid-harvest concentration declined. According to Donetti (2011), changes in the individual phenolic compound was influenced by ripening temperature with fewer changes at 23°C when compared to ample change at 18°C. In the present study, the ripening temperature was kept constant at 25°C, therefore, we supposed that changes in exocarp total phenolic was to a large extent due to the effect of both harvest time and crop load adjustment.

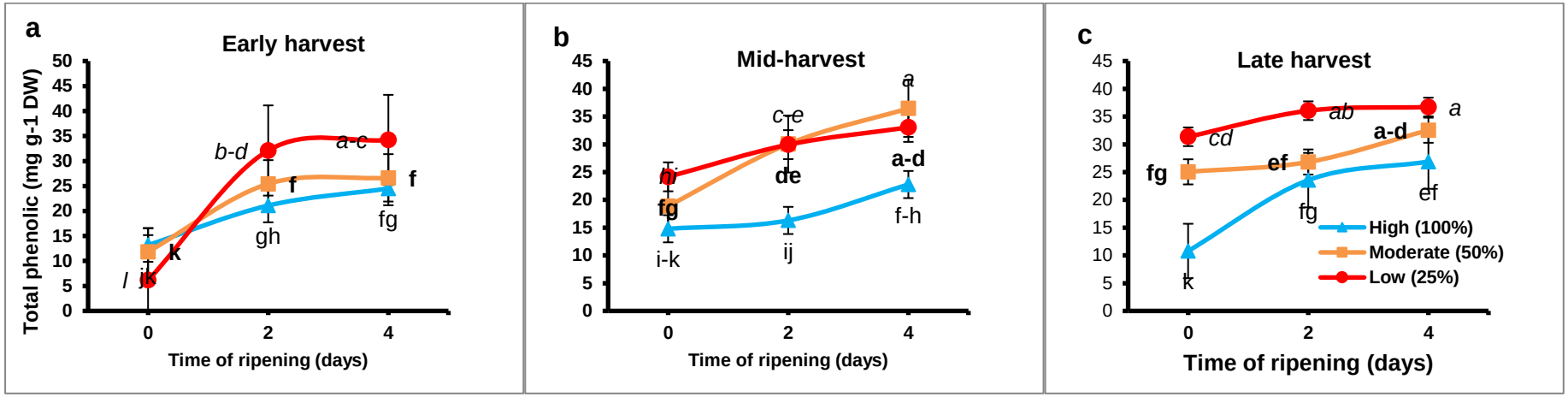



Figure 3.10 Changes in exocarp total phenolic concentration of 'Hass' avocado fruit from high, moderate and low crop load treatments harvested at early, mid- and late seasons. Error bars indicate ±SE of means at p≤ 0.05. Bold letters denote significant differences of means corresponding to moderate crop load while italicized letters correspond to low crop load

*3.3.7. Correlation analysis*

In this study, Pearson correlation was conducted to determine the relationship between fruit quality variables of 'Hass' avocado fruit during ripening (Table 3.2). Significant negative correlation was found between visual colour and L* (r= -0.749**), C* (r= -0.764**) and $h^o$ (r= -0.912**), indicating the relationship between subjective and objective colour measurements. Furthermore, visual colour had positive correlation with total anthocyanin (r= 0.803**) and cyanidin 3-*O*-glucoside (r= 0.768**), highlighting that colour development of 'Hass' avocado fruit during ripening is predominantly dependent on change in anthocyanin pigments. These findings were in agreement with several studies that reported that exocarp colour development of 'Hass' avocado during ripening was attributed to high accumulation of cyanidin 3-*O*-glucoside.

Table 3.2 Pearson correlation coefficient between visual colour and colour parameter (L*, C* and *h*°) of 'Hass' avocado fruit exocarp colour measurement/firmness and total anthocyanin, cyanidin 3-*O*-glucoside, D-mannoheptulose, perseitol and total phenolic concentrations in response to crop load adjustment and harvest time during ripening

| Variables | Firmness | L* | Vis-colour | C* | *h*° | Chl-a | Chl-b | Total chlo | Total carot | Total anth | Cy3Glu | D-Mamm | pers | Total phen |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Firmness** | **1** | | | | | | | | | | | | | |
| **L*** | **0,838**** | **1** | | | | | | | | | | | | |
| **Vis-colour** | **-0,630**** | **-0,749**** | **1** | | | | | | | | | | | |
| **C*** | **0,822**** | **0,904**** | **-0,764**** | **1** | | | | | | | | | | |
| ***h*°** | **0,581**** | **0,696**** | **-0,912**** | **0,673**** | **1** | | | | | | | | | |
| **Chl-a** | **0,579**** | **0,530**** | **-0,409**** | **0,369**** | **0,459**** | **1** | | | | | | | | |
| **Chl-b** | **0,287*** | **0,335**** | **-0,232*** | **0,169$^{ns}$** | **0,276*** | **0,545**** | **1** | | | | | | | |
| **Total chlo** | **0,479**** | **0,471**** | **-0,346*** | **0,289*** | **0,409**** | **0,907**** | **0,832**** | **1** | | | | | | |
| **Total carot** | **0,648**** | **0,486**** | **-0,422**** | **0,538**** | **0,361**** | **0,267*** | **-0,197$^{ns}$** | **0,060$^{ns}$** | **1** | | | | | |
| **Total anth** | **-0,525**** | **-0,658**** | **0,803**** | **-0,677**** | **-0,715**** | **-0,257*** | **-0,300*** | **-0,283*** | **-0,184$^{ns}$** | **1** | | | | |
| **Cy3Glu** | **-0,623**** | **-0,693**** | **0,768**** | **-0,641**** | **-0,712**** | **-0,474**** | **-0,507**** | **-0,527**** | **-0,267*** | **0,739**** | **1** | | | |
| **D-Mamm** | **0,712**** | **0,731**** | **-0,562**** | **0,645**** | **0,556**** | **0,560**** | **0,592**** | **0,620**** | **0,310**** | **-0,611**** | **-0,697**** | **1** | | |
| **pers** | **0,809**** | **0,794**** | **-0,647**** | **0,777**** | **0,594**** | **0,606**** | **0,509**** | **0,610**** | **0,457**** | **-0,622**** | **-0,688**** | **0,877**** | **1** | |
| **Total phen** | **-0,672**** | **-0,686**** | **0,593**** | **-0,764**** | **-0,532**** | **-0,338**** | **0,062$^{ns}$** | **-0,172$^{ns}$** | **-0,496**** | **0,573**** | **0,389**** | **-0,375**** | **-0,544**** | **1** |

L* = Lightness, C* = Chroma, *h*° = hue angle, Vis-colour = visual colour Total anth = Total anthocyanin, Cy3Glu = Cyanidin 3-*O*-glucoside, Chl-a = Chlorophyll-a, Chl-b = Chlorophyll-b, Total Chlo = Total Chlorophyll, Total carot = Total carotenoids, D-Mamm = D-mannoheptulose and pres = perseitol, Total phen = Total phenolic * = $p<0.05$ and ** = $p<0.01$, ns = not significant

## 3.4. Conclusion

This study showed that exocarp colour development during ripening was improved due to crop load adjustment at different harvest maturity. Fruit harvested from low and moderate crop loads accumulated higher total anthocyanin and cyanidin 3-*O*-glucoside concentration during ripening. Ultimately, showed improved visual colour during ripening during early and mid-harvest when compared with high crop load. In this study, crop load adjustment to low and moderate increased pre-harvest D-mannoheptulose and perseitol accumulation in the exocarp of ‘Hass’ avocado at all harvest time. Therefore, it can be further deduced that colour development of ‘Hass’ avocado fruit could be influenced by crop load which entails the accumulation of pre-harvest carbohydrates. Consequently, increased carbohydrates accumulation increases the ability of fruit to synthesize anthocyanin during ripening.

# CHAPTER 4

# EFFECT OF GIRDLING AND HARVEST MATURITY ON 'HASS' AVOCADO FRUIT POSTHARVEST EXOCARP COLOUR DEVELOPMENT


## Abstract

In 'Hass' avocado fruit, poor exocarp colour development from emerald-green to purple then black is regulated by anthocyanin; specifically, cyanidin 3-*O*-glucoside during ripening. The cyanidin 3-*O*-glucoside synthesis is dependent on pre-harvest sugars accumulated by the fruit during growth and development. In horticultural fruit trees, branch girdling and maturity have been shown to increase carbohydrates accumulation and fruit colour development. Therefore, the objective of this study was to determine the interaction between girdling and harvest maturity on 'Hass' avocado fruit exocarp colour development during ripening. In this study, early (25 and 29% dry matter content) and mid-maturity (28 and 32% dry matter content) fruit harvested from girdled and control trees were cold stored at 5.5°C for 28 days. After withdrawal from cold storage, fruit were evaluated for firmness, visual colour and chromaticity (lightness-$L^*$, chroma-$C^*$, hue-angle-$h^o$) during ripening over 8 days. In addition, exocarp total anthocyanin and carotenoids, chlorophyll-a and -b, while cyanidin 3-*O*-glucoside were also quantified during the ripening period. Fruit harvested from girdled trees showed poor exocarp colour development during ripening. Consequently, the interaction between girdling and harvest maturity did not increase total anthocyanin and low cyanidin 3-*O*-glucoside enzymatic synthesis; therefore, exocarp colour change during ripening. In conclusion, girdling did not promote carbohydrate accumulation into 'Hass' avocado fruit exocarp, thereby, resulted in poor colour

change as indicated by low cyanidin 3-*O*-glucoside during ripening for both harvest maturities.

**Keywords:** Anthocyanin, carbohydrates accumulation, chlorophyll-a and -b, cyanidin 3-*O*-glucoside

## 4.1. Introduction

The development of purple exocarp colour is an important quality trait of ‘Hass’ avocado fruit commercially. Avocado fruit differs from other crops as they do not ripen on the tree (Bower and Cutting, 1988). In general, ‘Hass’ avocado fruit exocarp colour development can only be observed after several days of ripening, a distinct phenomenon to other climacteric fruit crops. Avocado fruit requires an adequate pre-harvest supply of carbohydrates during development and growth (Wolstenholme, 1985); equally, the carbohydrates improve yield and postharvest fruit quality parameters including; exocarp colour, firmness and prevention of disorders (Bower and Cutting, 1988, Dixon et al., 2004, Tesfay et al., 2009). Specialized horticultural practices such as girdling, thinning and harvest time can significantly promote carbohydrate allocation and accumulation; ultimately, improving harvest and postharvest fruit quality. Several studies reported that girdling of avocado trees improved carbohydrates and starch accumulation, fruit size, increased yield and fruit retention (Davie et al., 1995a, Davie et al., 1995b, Davie and Stassen, 1997).

Ianaro and McNeil (1992) found that girdling of ‘Hass’ avocado trees increased alternate fruit bearing habits. In South Africa, Davie and Stassen (1997) recommended that girdling of an avocado tree be performed in October or November (early fruit growth stage) in order to counteract alternative bearing effects. Girdling avocado trees during the late fruit growth stage has been reported to have a negative influence on carbohydrate accumulation (Bertling et al., 2008). Both Bertling et al. (2008) and Davie and Stassen (1997) found that the influence of girdling on avocado trees favours the

accumulation of six carbon ($C_6$) over seven carbon ($C_7$) carbohydrates. Köhne (1992) conducted a study to increase yield through girdling of young 'Hass' trees prior to thinning and recommended that healthy young trees should be girdled in the year before thinning to improve yield. In fruit crops such as grape (Koshita et al., 2011), litchi (Shu et al., 2016), sweet cherry (Michailidis et al., 2020), persimmon (Choi et al., 2010) and apple (Fallahi et al., 2018), girdling has been reported to improve pre-harvest fruit skin colour development. Thus, girdling could be used to improve postharvest exocarp colour development in 'Hass' avocado fruit, but the existing literature lacks information on the effect of girdling on 'Hass' avocado fruit colour development during ripening.

Exocarp colour development during ripening is affected by harvest timing and maturity in 'Hass' avocado fruit (Mathaba et al., 2015, Mathaba et al., 2017). Mathaba et al. (2015) reported that 'Hass' avocado fruit harvested at low maturity develops poor exocarp colour during ripening. In contrast, fruit harvested in middle and late harvest maturity develop purple and black exocarp colour during ripening (Cox et al., 2004, Donetti and Terry, 2011). In 'Hass' avocado exocarp, anthocyanin; specifically, cyanidin 3-*O*-glucoside naturally increases during postharvest ripening. Consequently, leading to exocarp colour change with a breakdown of chlorophyll (Cox et al., 2004, Donetti and Terry, 2014). The accumulation of this anthocyanin has been attributed to a high content of six-carbon ($C_6$) sugars (Das et al., 2012, Gu et al., 2019); particularly, sucrose that dominates in 'Hass' avocado fruit exocarp (Bertling and Bower, 2005, Tesfay et al., 2010 Bertling and Tesfay, 2011). Therefore, we hypothesized that (i) individual $C_6$ sugar content at harvest are biochemical markers for determining the potential of 'Hass' avocado fruit to change exocarp colour during postharvest ripening,

and (ii) girdling and an increase in fruit maturity increases $C_6$ sugars concentration pre-harvest, which promotes anthocyanin synthesis and concentration, thereby, resulting in exocarp colour change during postharvest ripening. The objective was to determine the interaction between girdling and harvest maturity to improve 'Hass' avocado exocarp colour development during ripening.

## 4.2. Materials and Methods

### *4.2.1. Plant materials*

This study was conducted at a commercial avocado orchard in 2017 at Nico Swart estate (25° 04' 12.7" S 31° 00' 35.8" E), Kiepersol, Hazyview, Mpumalanga. The area has an average monthly minimum and maximum temperature of 13.4 and 26.0°C, respectively. The area experiences an average annual rainfall of less than 667 mm. Uniform 11-year-old 'Hass' avocado trees were used in this study. Ten trees were randomly selected in the orchard in early February 2018, three months prior to an early harvest and they were divided into two categories [**A** = girdled; **B** = control]. All the trees used received the same commercial production management practices.

### *4.2.2. Branch girdling treatment and harvesting*

Five trees selected at the fruit growth stage for category **A** were prepared for girdling. In brief, cuts of 1.5-2 mm wide were made using a hooked girdling knife by removing the bark (phloem) of all secondary branches containing developing fruit. After three months fruit were harvested from six trees; three of category **A** and three of category **B** during early harvest maturity and dry matter was determined (control ≈ 25% and girdled ≈ 29% dry matter content). In this study, girdling wounds were not reopened

from the trees that were not harvested early. Again, after three months, fruit were harvested from four trees; two of category **A** and two of category **B** to represent mid-harvest (control ≈ 28 % and girdled ≈ 32% dry matter content) fruit samples. During both harvest maturity (early and middle) fruit were harvest from both girdled and control, regardless of canopy position and immediately transported to the Agriculture Research Council-Tropical and Subtropical Crops (ARC-TSC) postharvest laboratory (25° 27' 04.6" S 30° 58' 09.1" E), Nelspruit, Mpumalanga, South Africa for the postharvest experiment, storage and analysis.

*4.2.3. Postharvest experimental design and treatments*

The experimental design was carried out as 2 x 2 factorial, factor A (girdled and non-girdled) and factor B (early and middle harvest maturity), arranged in a completely randomized design (CRD). Fruit of uniform size were packed into avocado crates, each containing 30 fruit, replicated three times per treatment [factor A ($n$ = 90) and B ($n$ = 90)]. Fruit were then cold stored at 5.5°C for 28 days. After removal from cold storage, the fruit ripened at 21°C for 6-8 days. During ripening, fruit were sensory evaluated every second day until they reached ‘eat ripe’ firmness. Fruit quality parameters were firmness, colour (visual and chromaticity colour parameters) and three fruit per replicate were sampled and frozen in liquid nitrogen and subsequently, cold-stored at -80°C for further analysis of total anthocyanin, cyanidin 3-*O*-glucoside, sucrose, total carotenoids and chlorophyll.

The methodology for determining fruit firmness (***cf. section* 3.2.6**), exocarp colour (***cf. section* 3.2.7**), total chlorophyll and carotenoids (***cf. section* 3.2.9**) and total

anthocyanin (***cf. section* 3.2.10**) were done as described in Chapter 3. The statistical analysis was also done as described in ***section* 3.2.13** of Chapter 3.

## 4.3. Results and Discussion

### *4.3.1. Fruit firmness*

The results showed that fruit firmness was significantly (p= 0.044) affected by the interaction between harvest maturity and girdling treatment during ripening at 21°C (Figure 4.1a-b). In this study, 'Hass' avocado fruit harvest from girdled trees ripened earlier when compared with the control fruit, irrespective of harvest maturity (Figure 4.1a-b). These results were in agreement with Sharma (2011) on 'Satluji' purple plums, whereby, girdling treatment significantly decreased firmness when compared with control. Similar to the results of Ilha et al. (1999), trunk girdling decreased firmness of 'Japanese' plums. In this work, early maturated fruit from control trees showed a significantly different firmness trend when compared with fruit harvested from girdled trees in the same ripening days (Figure 4.1a). In mid-harvest, control and fruit harvested from girdled trees showed similar firmness decline and fully ripening after 6 days (Figure 4.1b). These results suggested that the interaction effect of tree girdling and harvest maturity accelerated fruit maturation as indexed by dry matter content. At early maturity, fruit from girdled trees had higher dry matter content of 28% when compared with control fruit (25% DM). Similarly, fruit harvested from girdled trees had higher dry matter (32%) when compared with control fruit (28%) at mid-maturity.

Girdling effects have been linked to increased fruit size, advanced fruit maturity, manipulation of tree flowering, productivity and fruit quality (Annabi et al., 2019). In

avocado, Cutting and Wolstenholme (1992) explained that ripening time is highly dependent on fruit maturity, which increases with dry matter. Results obtained in this study were in accordance with the findings reported in literature for other crops including; sweet cherry (Quentin et al., 2013) and pears fruit (Aly et al., 2012, Teng et al., 1998), whereby, girdling treatment resulted in early fruit maturity. Therefore, from our results, a rapid decrease in firmness in fruit from the girdled tree during early harvest was attributed to accelerated fruit maturity induced by girdling treatment.

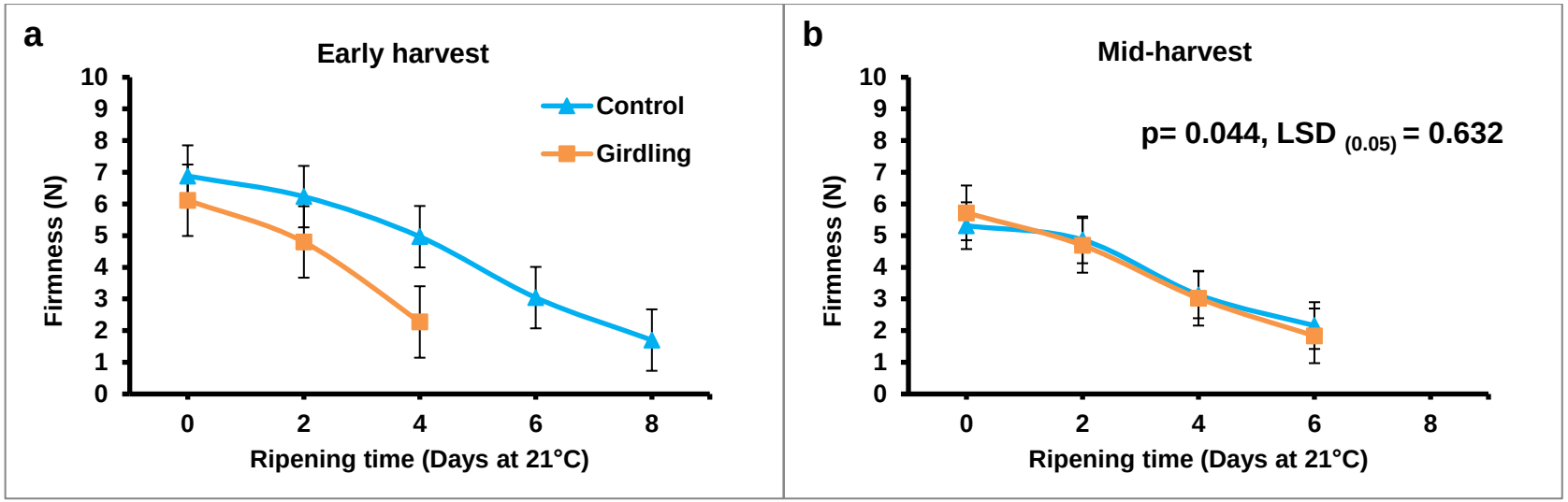


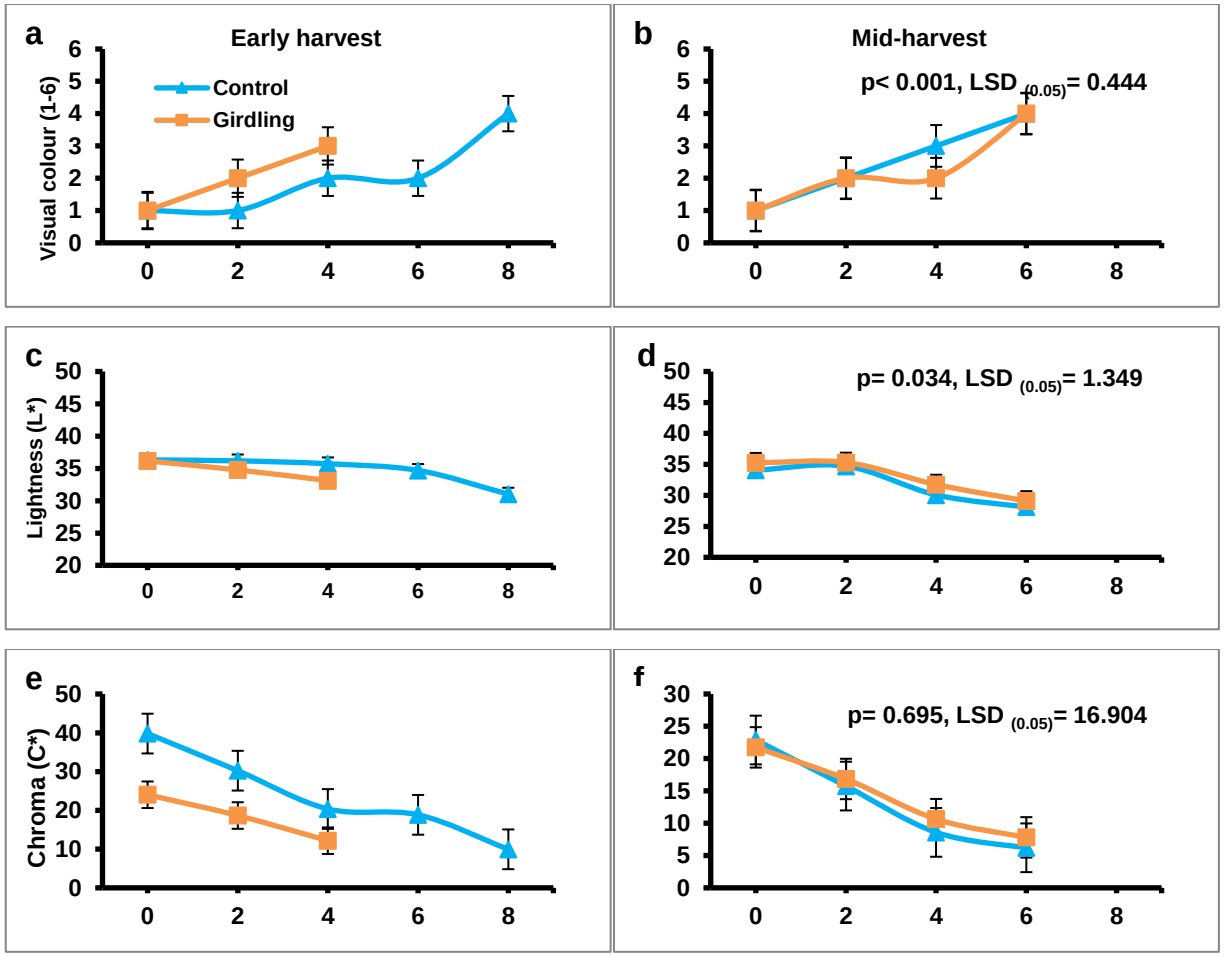

Figure 4.1 Effect of tree girdling and harvest maturity on 'Hass' avocado fruit firmness after 28 days cold storage (5.5°C) and ripening at 21°C. Error bars indicate ±SE of means at p≤ 0.05

*4.3.2. Exocarp colour development*

In the current study, tree girdling and harvest maturity interaction was significant ($p < 0.001$) for visual exocarp colour during ripening (Figure 4.2a-b). Control and fruit harvested from girdled trees showed an increase in visual colour during ripening only at early harvest, but no significant difference was observed at mid-harvest (Figure 4.2a-b). However, early harvested fruit from girdled trees showed a low visual colour (≈ 3 olive green), while control fruit showed higher exocarp visual colour (≈ 4 violet) after 4 ripening days at 21°C (Figure 4.2a). In mid-harvest, there was no significant difference in visual colour of fruit harvested from control and girdled tree, but treatment reached an improved visual colour (≈ 4 violet) after 6 ripening days at 21°C when compared with early harvest (Figure 4.2b). In terms of chromaticity colour parameters (L*, C* and $h^o$), tree girdling and harvest maturity showed significant interaction ($p = 0.034$) on lightness (L*) during ripening (Figure 4.2a-b). However, tree girdling and harvest maturity had no significant interaction on chroma (C*) ($p = 0.694$) and hue angle ($h^o$) ($p = 0.961$) during ripening (Figure 4.2c-h). Furthermore, the chroma (C*) and hue angle ($h^o$) declined at a slower rate in this study (Figure 4.2e-h) for both control and girdle but control fruit took longer to fully ripen as shown in Figure 4.1a. In the mid-harvest, chroma (C*) and hue angle ($h^o$) showed a similar trend during ripening (Figure 4.2f and h).

Our results in this study were unexpected considering that girdling has been shown in previous studies to increase carbohydrates accumulation, which act as precursors of anthocyanin, flavonoids and improved fruit colour develop (Choi et al., 2010, Keskin et al., 2013, Shu et al., 2016, Michailidis et al., 2020). According to the findings of Wilton (2000), girdling increased fruit colour for ’Pacific Rose’ apple. Fujishima et al.

(2005) also studied girdling in ‘Pione’ grapevine and found that colour and anthocyanin concentration were increased through girdling. In this study, girdling treatment resulted in poor colour development (Figure 4.2a-b) and low anthocyanin concentration (Figure 4.4a-b) for ‘Hass’ avocado during ripening. In the present study, the reason for these results may be related to the girdling time. According to Bertling et al. (2008), girdling an avocado tree during late fruit growth has a negative influence on carbohydrate accumulation. These authors found that girdling ‘Hass’ avocado trees in March and April decreased carbohydrate accumulation, particularly seven-carbon ($C_7$). Moreover, the highest $C_6$ sugar concentration was found in matured fruit harvested from girdled trees around May and June (Bertling et al., 2008). In this study, we concluded that girdling ‘Hass’ avocado tree in February possibly resulted in decreased accumulation of $C_6$ sugars, which led to low sucrose for enzymatic synthesis of cyanidin 3-*O*-glucoside and colour development during ripening. Supporting this, cyanidin 3-*O*-glucoside (Figure 4.4c-d) concentration was considerably lower in fruit harvested from girdled trees, therefore, leading to poor exocarp visual colour (Figure 4.2a). According to Wolstenholme (1985), carbohydrates ($C_6$-sugars) derived from photosynthesis are prioritized for oil production and seed development during early maturity. The inability of early maturity fruit harvested from girdled trees to develop purple exocarp colour during ripening could also be linked to oxidative damage during cold storage (Mathaba et al., 2015).


a
Early harvest
Control
Girdling
Visual colour (1-6)
b
Mid-harvest
p< 0.001, LSD (0.05)= 0.444
c
Lightness (L*)
d
p= 0.034, LSD (0.05)= 1.349
e
Chroma (C*)
f
p= 0.695, LSD (0.05)= 16.904

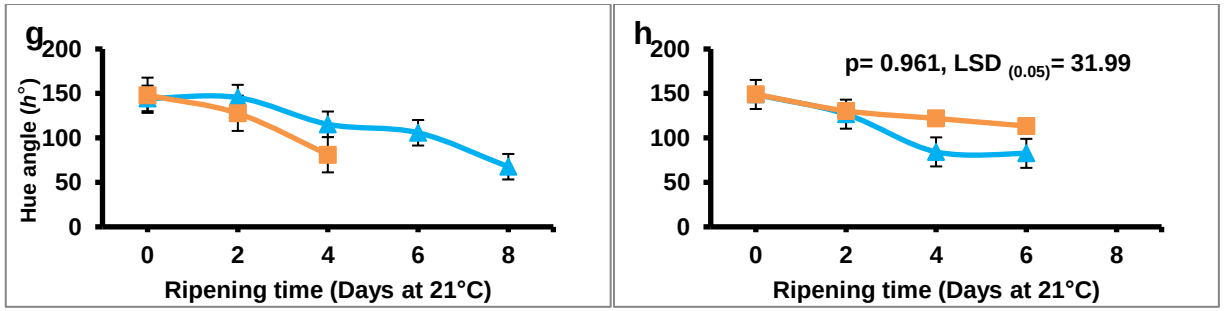



Figure 4.2 Effect of tree girdling and harvest maturity on 'Hass' avocado fruit visual colour (**a-b**), lightness (**c-d**), chroma (**e-f**), and hue angle (**g-h**) after 28 days cold storage (5.5°C) and ripening at 21°C. Error bars indicate ±SE of means at p≤ 0.05.

*4.3.3. Chlorophyll and carotenoids*

In this study, an interaction between girdling and harvest maturity had a significant effect on chlorophyll-a ($p< 0.001$) and chlorophyll-b ($p= 0.027$) during ripening (Figure 4.3). During ripening, early 'Hass' avocado fruit harvested from girdled trees showed a sharp decrease in exocarp chlorophyll-a, which was significantly different from control fruit after 4 days at 21°C (Figure 4.3a). In addition, the exocarp chlorophyll-b fruit harvested from girdled trees showed a sharp decrease trend in early harvest fruit with treatments showing significant difference after 4 days at 21°C (Figure 4.3a). Similar results were reported by Davie et al. (1995b) in 'Hass' avocado and Arakawa et al. (1996) in 'Fuji', 'Jonathan', 'Jonagold' and 'Hokuto' apple. Furthermore, mid-harvested 'Hass' avocado fruit showed a decreasing content of both chlorophyll-a and -b with treatments showing no significant difference after 6 days at 21°C (Figure 4.3b and d). In terms of total carotenoids, tree girdling and harvest maturity showed no significant interaction ($p= 0.840$) during ripening (Figure 4.3e-f). In the early harvest, 'Hass' avocado fruit from girdled treatment showed a decrease in exocarp total carotenoids but lower content for the fruit from girdled trees when compared with fruit from control trees (Figure 4.3e-f). In the mid-harvest, 'Hass' avocado exocarp total carotenoids also showed a decreasing trend with no significant difference between treatments throughout the ripening period (Figure 4.3e-f).

In our findings, girdled treatment resulted in high chlorophyll-a and -b content at day 0 of ripening at 21°C in early and mid-harvest. During the early stage of fruit growth, vegetative and reproduction organs compete for available carbohydrates (Li et al., 2003). However, fruit are strong source-sink; therefore, most carbohydrates are translocated towards them for repartitioning and synthesis of metabolites (Arakawa et

al., 1996, Davie et al., 1995a, Choi et al., 2010). Therefore, girdling treatment reduces the basipetal movement of these carbohydrates through phloem, consequently, most carbohydrates accumulate above the girdle region (Davie and Stassen, 1997, Choi et al., 2010). In this study, this could be the main reason for high chlorophyll-a and -b content in fruit from girdled trees. In 'Hass' avocado fruit, exocarp colour development depends upon chlorophyll degradation, concomitantly, anthocyanin synthesis and accumulation during ripening (Cox et al. (2004). In this study, chlorophyll-a and -b showed a negative correlation with total anthocyanin and cyanidin 3-*O*-glucoside (Table 4.1). However, tree girdling treatment did not promote chlorophyll degradation and anthocyanin interrelation during ripening of early harvested fruit due to lower exocarp visual colour (Figure 4.3a). Therefore, we could confirm that chlorophyll degradation in this study could be related to other external factors such as chilling injury (Mir et al., 2001).

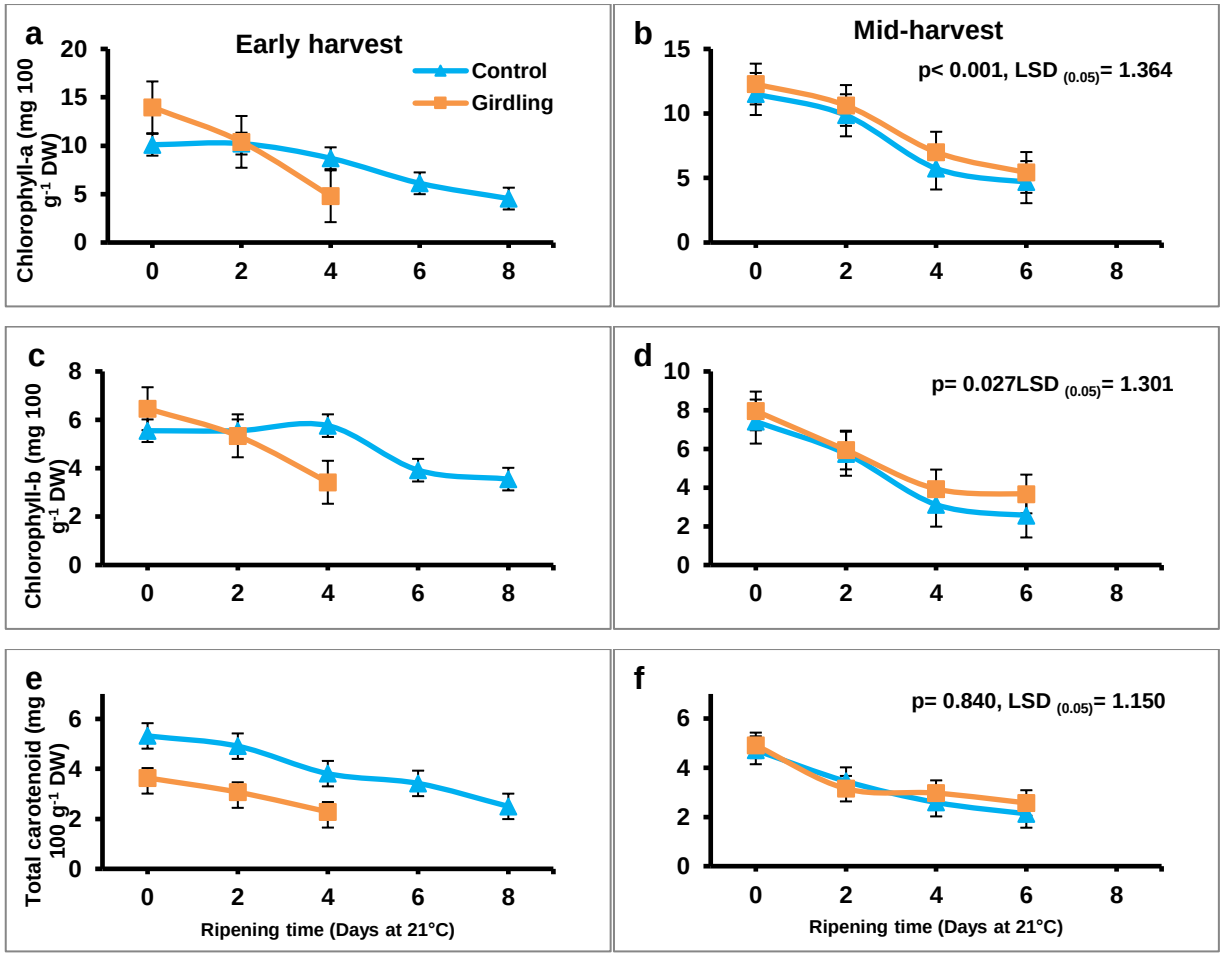



Figure 4.3 Effect of tree girdling and harvest maturity on 'Hass' avocado fruit chlorophyll-a (**a-b**), chlorophyll-b (**c-d**), and total carotenoids (**e-f**), content after 28 days cold storage (5.5°C) and ripening at 21°C. Error bars indicate ±SE of means at p≤ 0.05

*4.3.4. Total anthocyanin and cyanidin 3-O-glucoside concentration*

In this study, total anthocyanin (p= 0.555) and cyanidin 3-*O*-glucoside (p= 0.135) were not significantly affected by the interaction between girdling and harvest maturity during ripening (Figure 4.4a-b). The early harvest 'Hass' avocado fruit from girdled trees showed lower total anthocyanin and cyanidin 3-*O*-glucoside after 4 ripening days at 21°C (Figure 4.4a). However, mid-harvested 'Hass' avocado fruit from girdled and control trees showed no significant total anthocyanin difference during ripening (Figure 4.4b). Furthermore, mid-harvested 'Hass' avocado fruit from girdled fruit showed lower cyanidin 3-*O*-glucoside concentration when compared with the control, especially at day 6. Cyanidin 3-*O*-glucoside is responsible for the desirable purple to black exocarp colour of 'Hass' avocado fruit during ripening (Cox et al., 2004, Donetti and Terry, 2014). In 'Hass' avocado exocarp, anthocyanin accumulation can be influenced by fruit harvest maturity (Donetti and Terry, 2014). Various studies have reported that tree girdling influences anthocyanin synthesis and accumulation in numerous fruit crops (Keskin et al., 2013, Shu et al., 2016, Michailidis et al., 2020). For instance, 'Pione' grapevine tree branch girdling increased anthocyanin accumulation and skin colouration (Fujishima et al., 2005). This finding was contrary to the result found in the present experiment. In our experiment, early matured 'Hass' avocado fruit from girdled trees showed a decrease in total anthocyanin and cyanidin 3-*O*-glucoside after 4 ripening days. In contrast, mid-harvested 'Hass' avocado fruit from girdled and control trees showed no significant difference relating to total anthocyanin and cyanidin 3-*O*-glucoside during ripening.

It has been reported that an increase in anthocyanin, specifically cyanidin 3-*O*-glucoside is related to glucose and sucrose concentration (Teng et al., 2005, Liu et al., 2017). In particular, uridine diphosphate (UDP-Gluc) a product resultant from the addition of phosphate to glucose and sucrose, get attached to an anthocyanidin structure to produce a cyanidin 3-*O*-glucoside (Gu et al., 2019). An increase in cyanidin 3-*O*-glucoside concentration has been reported to be linked with sucrose metabolism, whereby, sucrose sugar is used as a precursor for the UDP-Gluc molecule that promotes colour development. We suppose that sucrose content needs to be higher during early harvest maturity to promote exocarp colour change in 'Hass' avocado fruit during postharvest ripening.

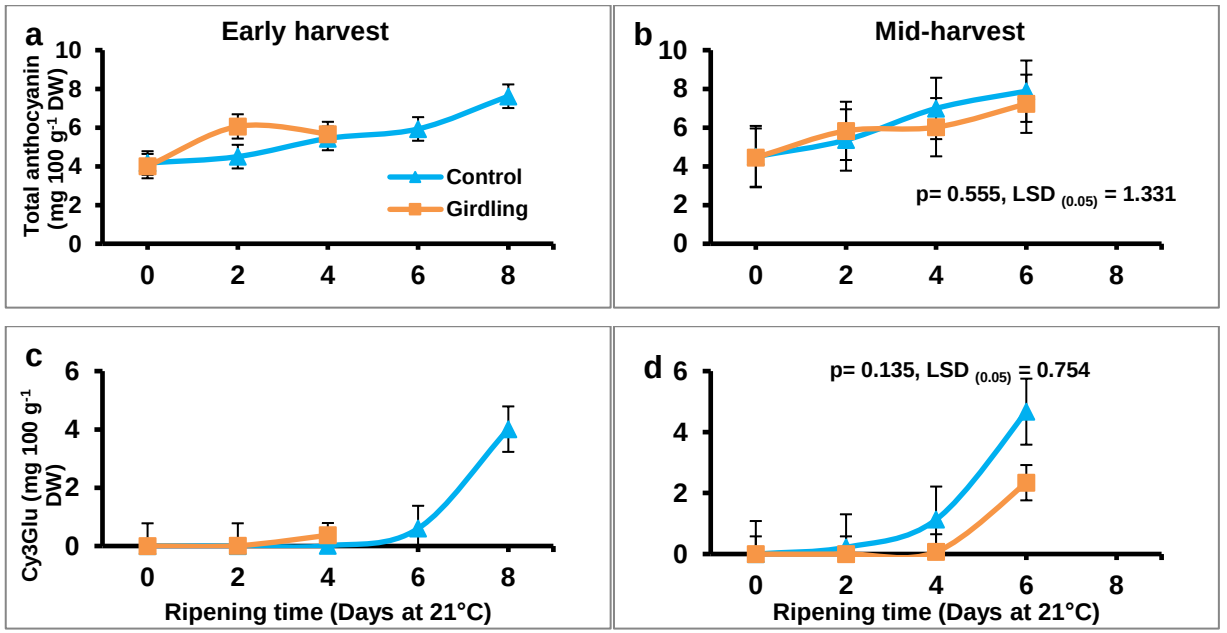



Figure 4.4 Effect of tree girdling and harvest maturity on 'Hass' avocado fruit total anthocyanin (**a-b**) and cyanidin 3-*O*-glucoside (Cy3Glu) (**c-d**) concentration after 28 days cold storage (5.5°C) and ripening at 21°C. Error bars indicate ±SE of means at p≤ 0.05

*4.3.5. Pearson correlation analysis*

Pearson correlation was conducted to determine the relationship among exocarp colour parameters with pigments degradation and biosynthesis during ripening of 'Hass' avocado fruit (Table 4.1). Significant negative correlation was found between visual colour and L* (r= -0.877**), C* (r= -0.497*) and $h^o$ (r= -0.919**), indicating the relationship between subjective and objective colour parameters. Similarly, visual colour had a significant negative correlation with chlorophyll-a (r= -0.855**) and chlorophyll-b (r= -0.808**) (Table 4.1). Furthermore, visual colour showed significant positive correlation with total anthocyanin (r= 0.792**) and cyanidin 3-*O*-glucoside (r= 0.796**), highlighting their considerable contribution to exocarp colour development during ripening.

Table 4.1 Pearson correlation coefficient between chromaticity colour parameter (L*, C* and *h*°) and visual colour of 'Hass' avocado fruit exocarp colour measurement/firmness and total anthocyanin and cyanidin 3-*O*-glucoside concentrations in response interaction between girdling and harvest time, during ripening at 21°C

| | **L*** | **C*** | ***h*°** | **Vis-colour** | **Firmness** | **Chl-a** | **Chl-b** | **Total carot** | **Total ant** | **Cy3Glu** |
|---|---|---|---|---|---|---|---|---|---|---|
| L* | 1 | | | | | | | | | |
| C* | 0.509** | 1 | | | | | | | | |
| *h*° | 0.675** | 0.378* | 1 | | | | | | | |
| Vis-colour | -0.877** | -0.497** | -0.919** | 1 | | | | | | |
| Firmness | 0.796** | 0.362* | 0.821** | -0.902** | 1 | | | | | |
| Chl-a | 0.697** | 0.371* | 0.817** | -0.855** | 0.875** | 1 | | | | |
| Chl-b | 0.659** | 0.306* | 0.763** | -0.808** | 0.759** | 0.845** | 1 | | | |
| Total carot | 0.500** | $0.225^{ns}$ | 0.554** | -0.608** | 0.707** | 0.621** | 0.694** | 1 | | |
| Total ant | -0.662** | -0.389* | -0.699** | 0.792** | -0.781** | -0.714** | -0.681** | -0.579** | 1 | |
| Cy3Glu | -0.766** | -0.373* | -0.670** | 0.796** | -0.688** | -0.647** | -0.587** | -0.404* | 0.672** | 1 |

L* = Lightness, C* = Chroma, *h*° = hue angle, Vis-colour = visual colour, Total ant = Total anthocyanin, Cy3Glu = Cyanidin 3-*O*-glucoside, Chl-a = Chlorophyll-a, Chl-b = Chlorophyll-b, Total carot = Total carotenoids, * = $p<0.05$ and ** = $p<0.001$, ns = not significant

## 4.4. Conclusion

In this study, interaction effect between girdling and harvest maturity had a negative influence on colour (visual and chromaticity colour parameters), chlorophyll degradation total anthocyanin, cyanidin 3-*O*-glucoside and total carotenoids of ‘Hass’ avocado fruit during ripening. This study revealed that ‘Hass’ avocado fruit did not change exocarp colour to the desirable purple or black colour during ripening for early harvested fruit despite the girdling treatment. Supposedly, the combined treatment effect between girdling and harvest maturity did not promote sufficient pre-harvest sucrose exocarp accumulation. In ‘Hass’ avocado fruit, the exocarp sucrose content at harvest could be the potential biochemical marker determining final exocarp colour change during ripening. Further research is needed to determine the optimum girdling time that promotes sucrose accumulation, assumable, postharvest exocarp colour development.

# CHAPTER 5

# THE EXOGENOUS GLUCOSE INFUSION ON 'HASS' AVOCADO FRUIT EXOCARP COLOUR DEVELOPMENT DURING POSTHARVEST RIPENING


## Abstract

Downgrading and rejection of fruit due to insufficient purple exocarp colour during ripening have limited the profitability of lucrative 'Hass' avocado (*Persea americana* Mill) fruit. Thus, this study investigated whether glucose infusion through the pedicel can trigger anthocyanin pigment synthesis and accumulation of early harvested 'Hass' avocado exocarp, thereby resulting in improved colour development during ripening. Detached 'Hass' avocado fruit were continuously infused through the pedicel with distilled water and different glucose concentrations; 0.05, 0.13, 0.28 mM and control, thereafter, stored at 5.5°C for 28 days. After storage, the fruit were ripened at 25±2°C and assessed for fruit quality; firmness loss, subjective (visual colour), chromaticity parameters (lightness-L*, chroma-C* and hue-*h*°) and exocarp pigments (chlorophyll, carotenoids, anthocyanin and cyanidin 3-*O*-glucoside). The results showed that infusion with 0.05 and 0.13 mM glucose concentration resulted in higher accumulation of anthocyanin and cyanidin 3-*O*-glucoside when compared with control, distilled water and 0.28 mM. Concomitantly, improved exocarp colour development (visual colour) after 8 days of ripening. This study valorized the possible function of $C_6$ sugars in 'Hass' avocado fruit during postharvest ripening. Thus, production practices that enhance carbohydrates accumulation in the exocarp of avocado fruit could be applied to control postharvest poor exocarp colouration.



**Keywords:** Anthocyanin, chlorophyll, cyanidin 3-*O*-glucoside, firmness, visual colour

## 5.1. Introduction

During ripening, chlorophyll is degraded and anthocyanin is synthesized and accumulated. Several authors have reported that cyanidin 3-*O*-glucoside, anthocyanin biosynthesis and accumulation confers ripe ‘Hass’ avocado fruit the purple and dark black exocarp colour (Cox et al., 2004, Ashton et al., 2006, Donetti and Terry, 2014). Therefore, the poor exocarp colouration conundrum of 'Hass' avocado fruit can be controlled during ripening by triggering the pathway responsible for anthocyanin biosynthesis. Peng et al. (2016) found that the increase in anthocyanin concentration was related to the enzymatic activity responsible for the biosynthetic pathway phenylalanine ammonia-lyase and uridine diphosphate glucose flavonoid 3-*O*-glucosyltransferase. Anthocyanin synthesis is induced by glucose by modifying cytosolic sucrose content (McKibbin et al., 2006, Xu et al., 2014). Shi et al. (2014) observed a strong correlation between anthocyanin biosynthesis and soluble sugar levels in Chinese bayberry 'Dongkui' fruit. The authors found that higher anthocyanin concentration was correlated with an increased expression of enzymes involved in the biosynthesis of anthocyanin and genes related to sugar metabolism. The phosphorylation of the uridine diphosphate (UDP) glucose molecule is required for the regulation of genes encoding anthocyanin biosynthesis (Chen et al., 2013, Wang et al., 2019). Due to the importance of UDP-glucose as a precursor in secondary metabolites and subsequently, anthocyanin biosynthesis, we investigated whether postharvest treatment with glucose can improve avocado fruit colour by triggering the flavonoid pathway.

The avocado fruit consists of seed, mesocarp and exocarp (which contain a proportion of carbohydrates). Tesfay et al. (2012) and Tesfay (2009) reported on the function of

carbohydrates in ‘Hass’ avocado. According to the authors, carbohydrate carbon seven ($C_7$) dominates the mesocarp at different stages of an avocado life cycle and serves as an energy source and antioxidant. The $C_7$ sugar in avocado fruit 'Hass' was reported to be the main energy source after harvest. To date, $C_7$ sugars are unknown to play any role in exocarp colour development of ‘Hass' avocados. There is a possibility that carbon-six ($C_6$) carbohydrates are also involved. Hence, it is reasonable to investigate the role of $C_6$ sugar in avocado during ripening. Hu et al. (2016) report that sucrose, glucose and fructose are critical for anthocyanin biosynthesis. The exogenous supply of sucrose stimulated anthocyanin biosynthesis in leaf disks, leaves and suspension cultures (Larronde et al., 1998). Weiss (2000) reported that sugars, light and plant hormones interact to induce anthocyanin biosynthesis and expression of structural genes in ‘Petunia hybrid’ corollas.

In *Arabidopsis thaliana* flower and ‘Orin’ apple fruit, sucrose and biosynthesis pathway for uridine diphosphate glucose (UDP-gluc) contributed to the biosynthesis of cyanidin-3-galactoside anthocyanin pigment (Sivitz et al., 2008, Ban et al., 2009). This work investigated whether glucose infused through fruit pedicel can trigger anthocyanin biosynthesis and accumulation in the exocarp tissue of early harvested ‘Hass’ avocado, resulting in improved exocarp colour development during ripening.

## 5.2. Materials and Methods

### *5.2.1. Plant materials*

In April 2019, early matured ‘Hass’ avocado fruit were harvested with 10 cm pedicel at commercial standard using dry matter (21% DM) maturity from Nico Swart Trust

commercial farm at Kiepersol (Hazyview, Mpumalanga, South Africa, GPS: 25°29'19'' S; 31°13'67'' E). Harvested 'Hass' with 10 cm pedicel were carefully placed in crates and immediately transported to the Agricultural Research Council - Tropical and Subtropical Postharvest Laboratory in Nelspruit (Mpumalanga, South Africa, GPS: 25°45'18''S; 30°96'97'' E).

*5.2.2. Postharvest laboratory procedures, experimental design and treatments*

Upon arrival at the laboratory, fruit pedicel were re-cut to 5 cm length and 15 mL tubes were interleaved on the apex of the fruit with 5 cm pedicles inside the tube and at the bottom (where the apex of the fruit and silicon tube meet) as previously described by Bertling and Tesfay (2011). Bostik prestik and petroleum jelly were applied to prevent leakages. Five treatments were: control fruit with pedicel and infused with distilled water and glucose concentrations [in millimole (mM)]: 0.05, 0.13 and 0.28 mM. Ninety fruit per treatment were continuously infused with 10 mL/fruit of the above treatments. Controls were also included in which fruit with pedicel were not treated with any solution. Thereafter, distilled water, glucose infused and control fruit were stored at 5.5°C for up to 28 days. After cold storage, the treated and control fruit were kept at ambient temperature 25°C for ripening. The experiment was conducted as a completely randomized design (CRD) with three replications per treatment.

Methodologies for evaluation of fruit firmness (***cf. section* 3.2.6**), exocarp colour (***cf. section* 3.2.7**), chlorophyll and carotenoids (***cf.* section 3.2.9**), as well as total anthocyanin (***cf. section* 3.2.10**) are as explained in Chapter 3.

*5.2.3. Statistical analysis*

Analysis of variance (ANOVA) was performed using GenStat 18th version (VSN International, UK). A *p*-value was calculated at 95% confidence interval around the difference between treatments using the Least Significant Difference (LSD). The relationship between firmness, chromaticity parameters (L*, C* and *h*°) and exocarp pigments (chlorophyll, carotenoids, anthocyanin and cyanidin 3-*O*-glucoside) were evaluated by Pearson's correlation.

## 5.3. Results and Discussion

*5.3.1. Fruit firmness*

In 'Hass' avocado fruit, firmness is the main measure of eating quality (Ahmad et al., 2013). In this study, no significant (p= 0.590) difference in firmness was observed between treatments at 25°C (Figure 5.1). A general decreasing trend was observed for all treatments at 25°C. The decrease in fruit firmness correlated negatively and significantly (p< 0.01) with the exocarp colour development as measured by visual rating (r= -0.85) and correlate positively with chromaticity parameters; L* (r= 0.89), C* (r= 0.94) and *h*° (r= 0.81) (Table 5.1), as has been previously reported by Cox et al. (2004). The control fruit ripened rapidly after cold storage and softened to full ripeness within 6 days (Figure 5.1). However, glucose infusion and distilled water extended the ripening period by 2 days. These results were in accordance with the study of Bertling and Tesfay (2011); whereby, pedicel infusion with seven-carbon ($C_7$) sugar significantly maintained the D-mannoheptulose and perseitol sugar pool in 'Hass' avocado mesocarp, resulting in increased shelf-life. Moreover, the present findings are still in accordance with the study by Mathe (2018), who reported that water infusion

and $C_7$ (D-mannoheptulose and perseitol) and sucrose improved fruit quality attributes, flesh firmness, fresh mass retention and respiration rate. The firmness of glucose infused fruit and distilled water were higher when compared to control after the same ripening period (Figure 5.1). After 6 and 8 days, the firmness of infused treatments were not significantly different, however, 0.13 mM and distilled water infused fruit maintained higher fruit firmness followed by 0.05 and 0.28 mM. These results are consistent with previous studies on sugar and water infusion through pedicel for ‘Fuerte’ and ‘Hass’ avocado fruit (Blakey et al., 2009, Bertling and Tesfay, 2011, Mathe, 2018). These studies found that infusion with sugars such as D-mannoheptulose, perseitol and sucrose and water improved the fruit quality of ‘Fuerte’ and ‘Hass’ avocado by decreasing respiration rate, retained firmness and extended shelf-life. In this study, extended shelf-life due to glucose and distilled water infusion could be attributed to improved water balance and maintained turgidity of the fruit. Furthermore, fruit infused with D-mannoheptulose and water were found to be firmer than those of control fruit (Bertling and Tesfay, 2011). This was probably due to glucose infusion resulting in increased fruit water potential which contributed to reduced ethylene production causing fruit to ripen slower than those of control fruit.

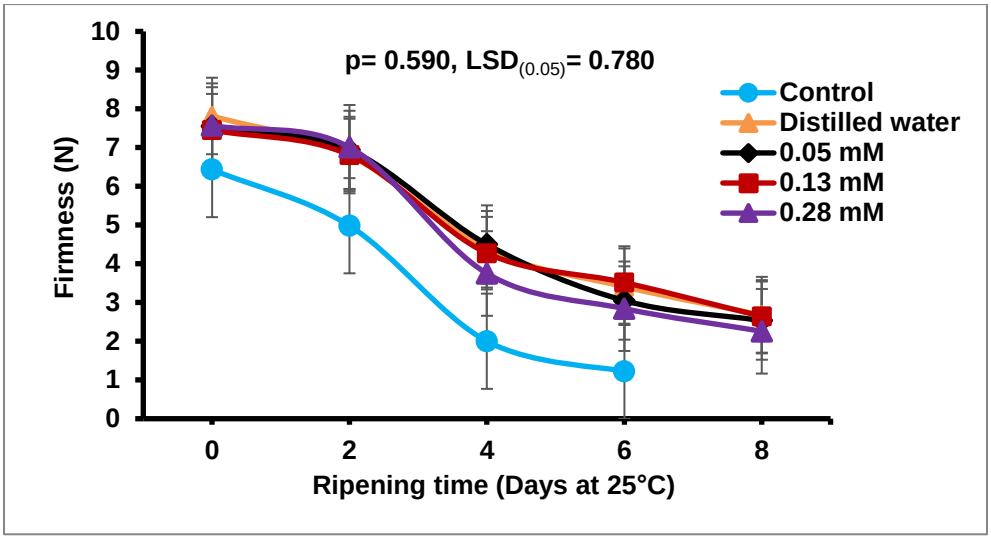



Figure 5.1 Effect of distilled water and glucose infusion through pedicel on the firmness of 'Hass' avocado fruit during ripening. Error bars indicate ±SE of means at p≤ 0.05

*5.3.2. Exocarp colour development*

Colour change as measured by visual rating significantly (p= 0.012) increased for all treatments at 25°C (Figure 5.2a). As shown in this figure, visual colour increased slightly during the initial 4 days at 25°C but then increased sharply until the end of ripening (6 days for control) and 8 days for infused treatments. However, 'Hass' avocado fruit infused with glucose showed improved exocarp colour development when compared with control and distilled water as evident from day 4 until day 8. After 6 ripening days at 25°C, control fruit developed to olive green (visual colour = 3.42) when compared with other treatments. After day 8 at 25°C, the visual colour of fruit infused with glucose at 0.05, 0.13 and 0.28 mM developed purple colour (visual colour = 5.25), violet (visual colour = 4.92 and 4.42), respectively (Figure 3.1, 5.2 and 5.3). Whereas fruit infused with distilled water only showed traces of olive-green colour (visual colour = 3.92) on day 8 at 25°C. The visual colour significantly and negatively correlated well with firmness (r= -0.81) (Table 5.1), as has been found by other researchers (Cox et al., 2004, Donetti, 2011). In this study, the visual colour correlated

positively and significantly with anthocyanin content, suggesting that colour change was to a certain extent influenced by glucose infusion. According to Bolouri-Moghaddam et al. (2010), glucose acts as a signalling molecule and functions as a precursor for regulating anthocyanin biosynthesis.

Moreover, the values of chromaticity parameters (L*, C* and *h*°) decreased with ripening days at 25°C for all treatments during the colour development and ripening (Figure 5.2a-d). The reduction in L*, C* and *h*° values of 'Hass' avocado (Figure 5.2b-d) are consistent with Cox et al. (2004) and Donetti and Terry (2011). However, control fruit measured lower C* value and higher *h*° values when compared with glucose and distilled water infused fruit after 6 days. All glucose infused fruit showed lower L* and *h*° values after 6 and 8 days when compared with distilled water. This could be an indication of the positive effect of glucose in promoting 'Hass' avocado fruit exocarp colour development during ripening. The change in exocarp colour was due to chlorophyll degradation concomitant with the biosynthesis and accumulation of anthocyanin (Cox et al., 2004). In addition, chromaticity parameters (L*, C* and *h*°) were highly correlated with total chlorophyll (r= 0.72, 0.76 and 0.68, respectively) and anthocyanin (r= -0.73, -0.67 and -0.73, respectively) which was also promoted by glucose infusion (Table 5.1). Several studies have revealed that anthocyanin pigments that accumulate in fruit crops were mediated by carbohydrates (Das et al., 2012, Huang et al., 2019). It has been reported that glucose molecule is phosphorylated by hexokinase (HXK1) to constitute uridine diphosphate (UDP) glucose molecule, which functions as the precursor in anthocyanin biosynthesis (Yang et al., 2013, Xu et al., 2014)

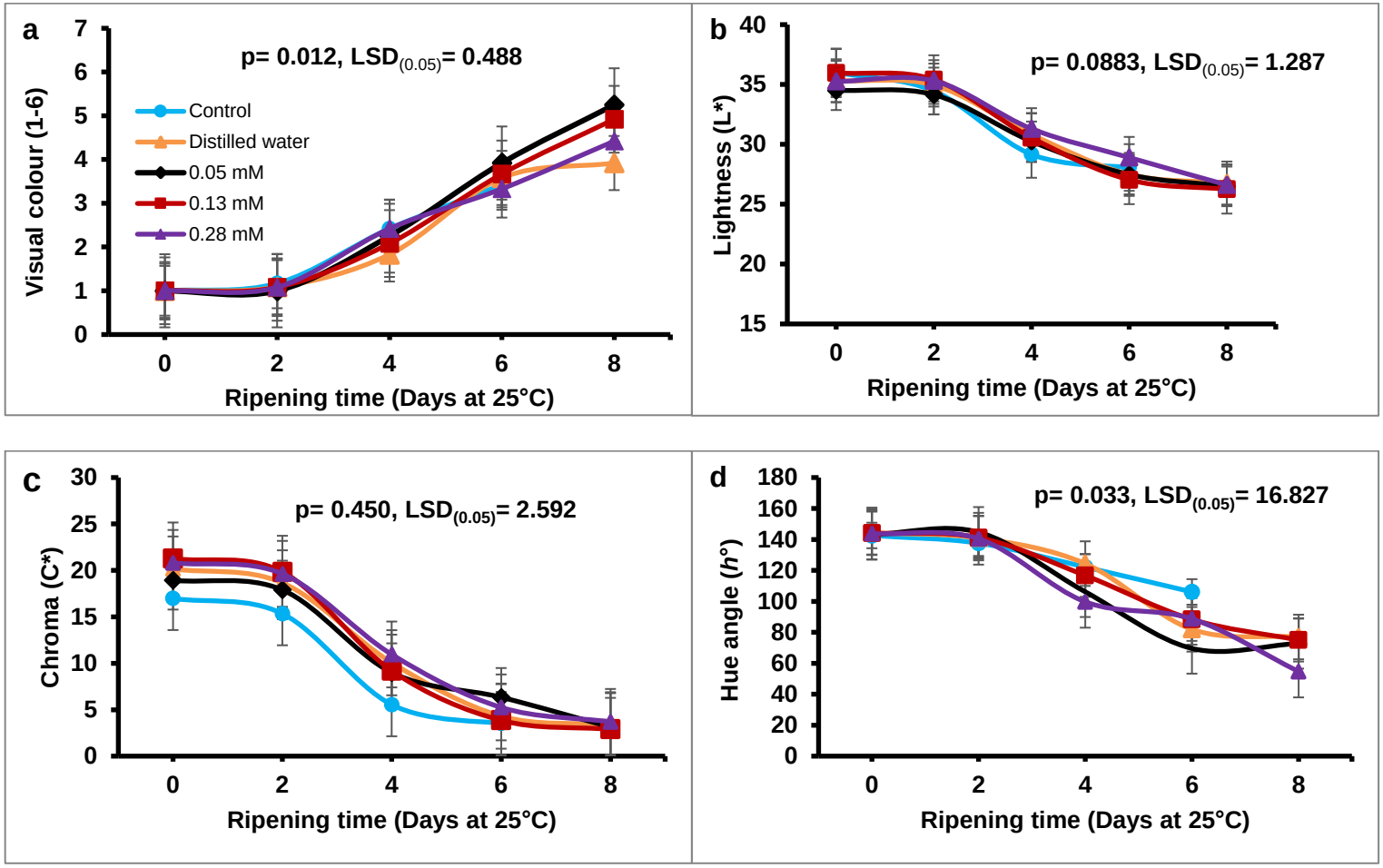



Figure 5.2 Effect of distilled water and glucose infusion through pedicel on subjective (visual colour) (**a**) and chromaticity parameters (L*, C* and *h*°, **b**, **c** and **d**, respectively) of 'Hass' avocado fruit during ripening. Error bars indicate ±SE of means at p≤ 0.05

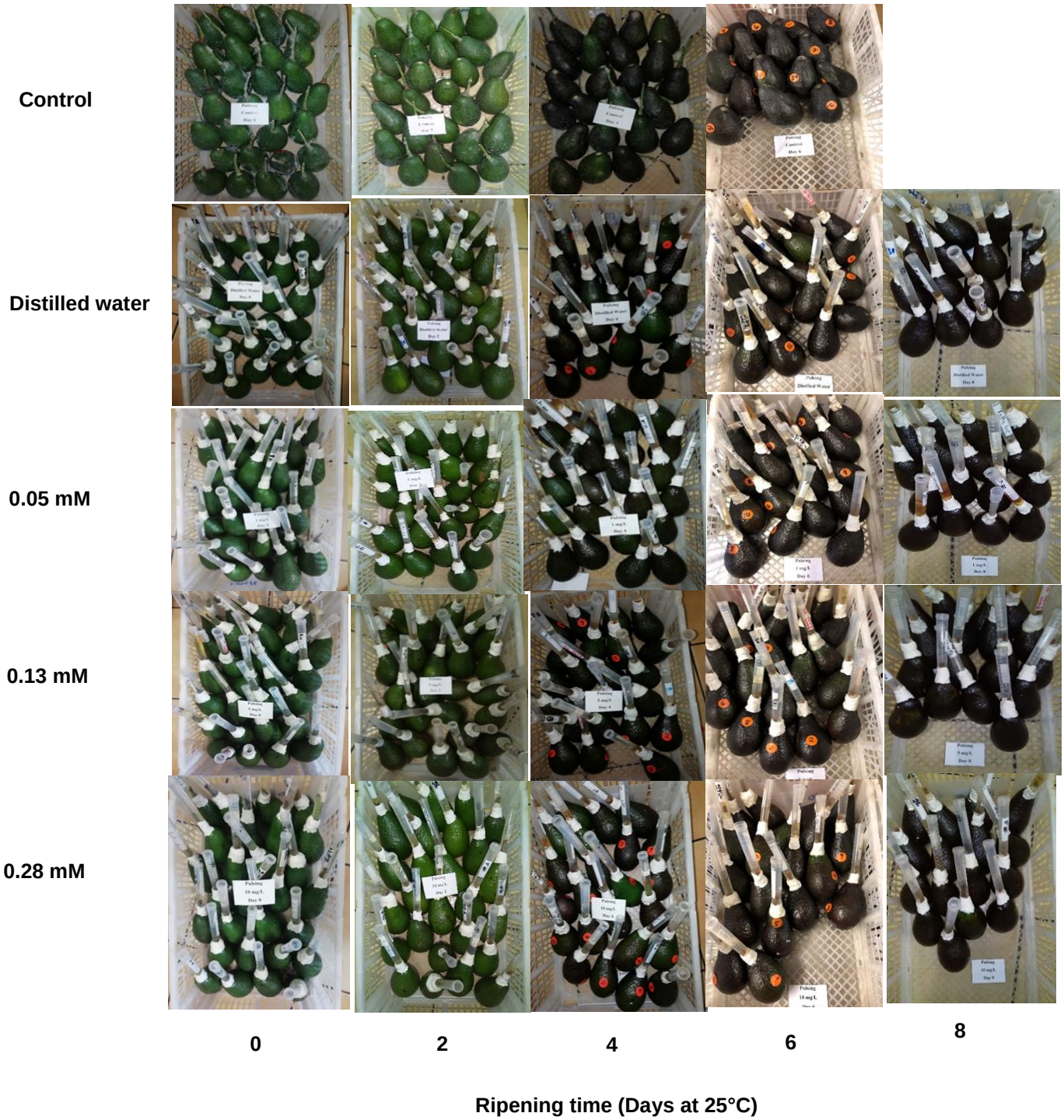



Figure 5.3 Effect of distilled water and glucose infusion through pedicel on exocarp colour development of 'Hass' avocado fruit during ripening

*5.3.3. Total chlorophyll and carotenoids content*

Total chlorophyll ($p= 0.002$), chlorophyll-a ($p= 0.002$), chlorophyll-b ($p= 0.004$) and total carotenoids ($p< 0.001$) significantly declined with ripening days at 25°C for all treatments (Figure 5.4). These findings were in agreement with observations by Ashton et al. (2006). We observed that visual colour assessment was consistent with both chlorophyll and carotenoids degradation and was negatively correlated with total chlorophyll ($r= -0.68$) and total carotenoids ($r= -0.71$) (Table 5.1). However, from day 0 to 2 at 25°C, control fruit showed a slower decrease in total chlorophyll, chlorophyll-a, chlorophyll-b and total carotenoids but the content decreased rapidly after 4 days, reaching somewhat lower values at day 6 (Figure 5.4). Interestingly, infusion with glucose and distilled water delayed the rate of chlorophyll degradation compared to control. Fruit infused with 0.05 mM showed greater delayed chlorophyll and carotenoids degradation than those infused with 0.13 and 0.28 mM. This can be ascribed to high water potential from infusion treatment, which ended up with reduced abscisic acid (ABA) and ethylene production (Li and Huang, 2011, Blakey, 2011). In addition, Iqbal et al. (2017) reported that ABA functions by suppressing ethylene biosynthesis which deregulates and delays fruit ripening. Supposedly, glucose and distilled water infused maintained chlorophyll and carotenoids degradation by decreasing the level of ABA. Therefore, reduced ABA level in the exocarp would further impede ethylene production which in turn limited ethylene dependent catabolic pathways associated with chlorophyll and carotenoids breakdown. In addition, maintaining exocarp chlorophyll and carotenoids breakdown could have improved antioxidant capacity thus, protecting the fruit from oxidative damage during storage.

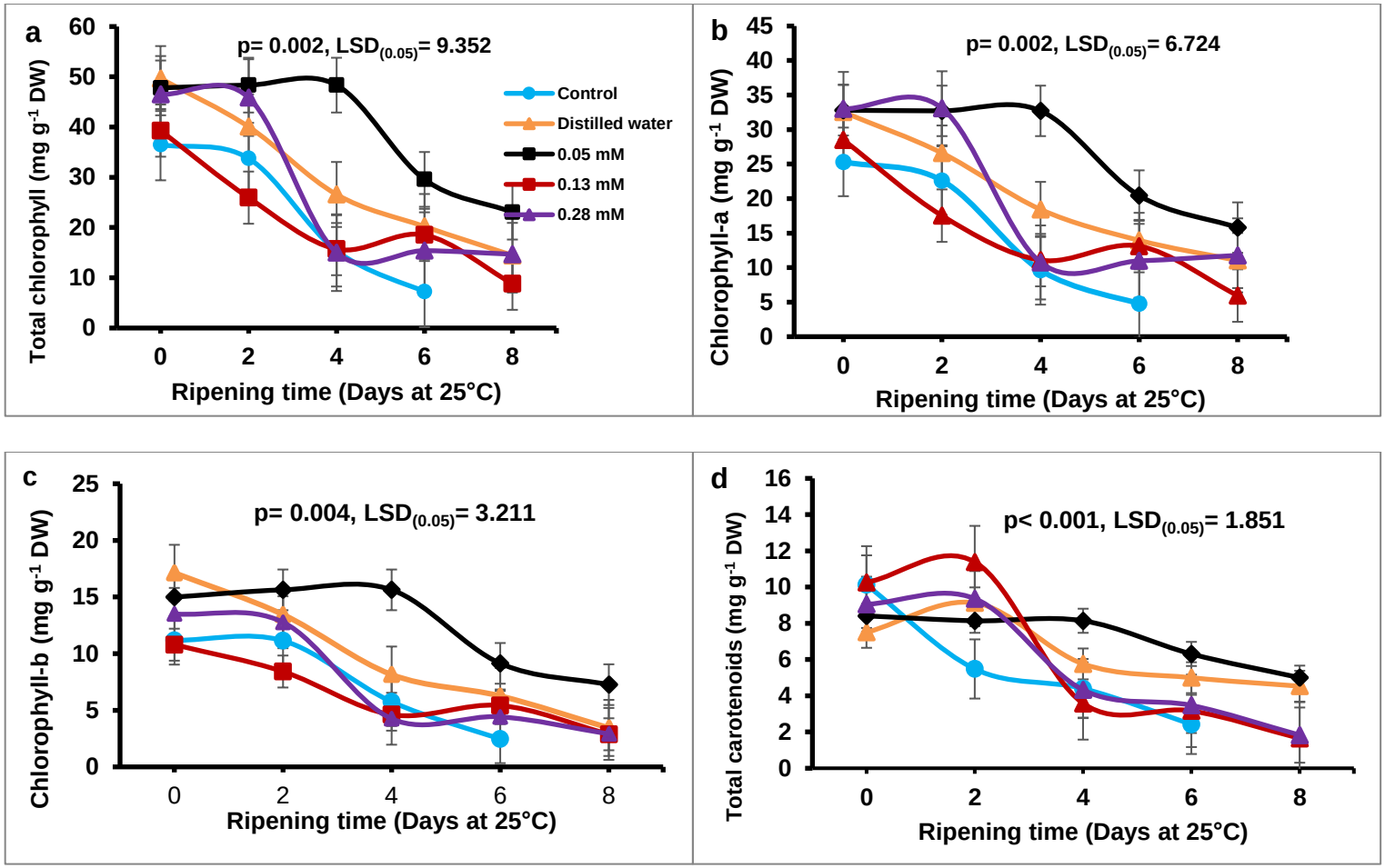



Figure 5.4 Effect of distilled water and glucose infusion through pedicel on total chlorophyll (**a**), chlorophyll-a (**b**), chlorophyll-b (**c**) and total carotenoids (**d**) of 'Hass' avocado fruit during ripening. Error bars indicate ±SE of means at p≤ 0.05

*5.3.4. Total anthocyanin and cyanidin 3-O-glucoside concentration*

In 'Hass' avocado fruit exocarp, cyanidin 3-*O*-glucoside is the main anthocyanin responsible for the purple and black colour during ripening (Cox et al., 2004). In this study, we observed a significant ($p < 0.001$) increase in total anthocyanin concentration for all treatments during ripening. However, fruit infused with 0.13 mM showed enhanced anthocyanin concentration when compared with all other treatments, especially during the first 6 days at 25°C. After 8 days at 25°C, anthocyanin concentrations for fruit all glucose infused fruit were higher than those for control and distilled water (Figure 5.5a). There was a significant ($p < 0.01$) and a positive correlation between total anthocyanin and cyanidin 3-*O*-glucoside ($r = 0.84$), which was consistent with findings in previous studies (Cox et al., 2004, Donetti and Terry, 2014).

Cyanidin 3-*O*-glucoside concentration increased significantly ($p < 0.001$) as the ripening time increased among treatments (Figure 5.5b). The concentration of cyanidin 3-*O*-glucoside remained unchanged from day 0 to 2 at 25°C then increased in fruit infused with 0.05, 0.13 mM glucose and distilled water increased after 2 to 8 days at 25°C and were higher with glucose than distilled water (Figure 5.5b). The increase in cyanidin 3-*O*-glucoside of fruit infused with glucose was in accordance with several studies which reported that exogenous sugar treatment can induce anthocyanin accumulation; however, the effect may differ in different crops (Hu et al., 2016, Ai et al., 2016). The de novo biosynthesis of anthocyanin is associated with glycosylation of uridine diphosphate glucose (UDP-gluc) molecules. In this study, an increase in cyanidin 3-*O*-glucoside concentration due to glucose infusion could be attributed to the provision of UDP-glucose. According to Das et al. (2012), sucrose, glucose and

fructose, induced enhancement of anthocyanin biosynthesis and accumulation through the altered expression of regulatory and structural genes, which is featured by reprogramming of the signal transduction pathways. In ‘Red Delicious’ apple fruit, Hu et al. (2016) found that glucose regulated anthocyanin biosynthesis by acting as a signalling molecule in the regulation of gene govern by MdHXK1 and MdbHLH3 protein kinase. In ‘Mirage rose’ *Petunia* hybrid flower, Ai et al. (2016) found that sucrose enhanced anthocyanin content through enhancement of transcription factors responsible for the induction of genes involved in anthocyanin biosynthesis. The present study only explored the influence of glucose by postharvest infusion technique on the colour change of ‘Hass’ avocado fruit during ripening but did not elucidate how this sugar affects anthocyanin biosynthesis.

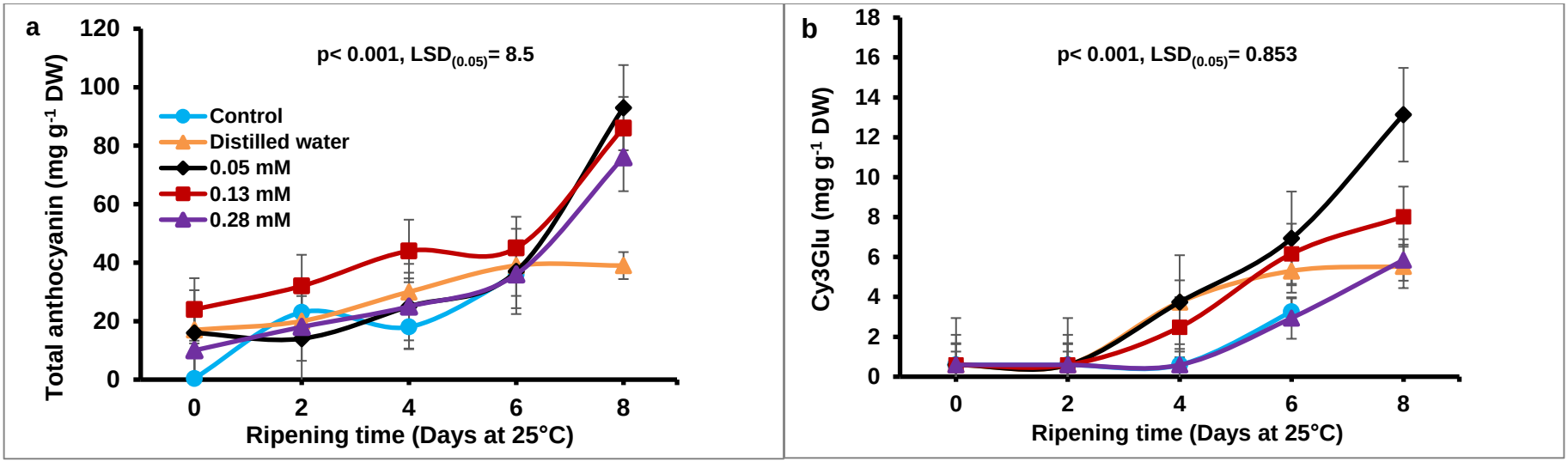



Figure 5.5 Effect of distilled water and glucose infusion through pedicel on total anthocyanin (**a**) and cyanidin 3-*O*-glucoside concentration (**b**) of 'Hass' avocado fruit during ripening. Error bars indicate ±SE of means at p≤ 0.05

*5.3.5. Correlation analysis*

Pearson correlation coefficients between firmness, colour attributes and exocarp pigments associated with glucose concentration infusion treatments were calculated and listed in Table 5.1. The firmness exhibited a significant ($p < 0.01$) and strong negative correlation to visual colour ($r = -0.85$), total anthocyanin ($r = -0.60$) and cyanidin 3-*O*-glucoside ($r = -0.63$). These changes indicate that 'Hass' avocado fruit becomes soft and darker as the ripening time progresses. Similar *h*° values had a significant negative correlation with visual colour ($r = -0.95$), total anthocyanin ($r = -0.73$) and cyanidin 3-*O*-glucoside ($r = -0.79$). Several studies have reported that change in exocarp colour (*h*°) relates to variation in cyanidin 3-*O*-glucoside (Cox et al., 2004, Ashton et al., 2006, Donetti, 2011). Total chlorophyll had positive correlation with chlorophyll-a ($r = 0.99$) and chlorophyll-b ($r = 0.97$). Ashton et al (2006) reported that 'Hass' avocado fruit exhibit higher chlorophyll content due to high content of chlorophyll-a and chlorophyll-b. The chlorophyll content negatively correlated with total anthocyanin ($r = -0.59$) and cyanidin 3-*O*-glucoside concentration ($r = 0.51$). Cox et al. (2004) found that chlorophyll breakdown of 'Hass' avocado fruit coincides with anthocyanin accumulation resulting in exocarp colour development.

Table 5.1 Pearson correlation coefficient between chromaticity colour parameter (L*, C* and *h*°) and visual colour of 'Hass' avocado fruit exocarp colour measurement/firmness and total anthocyanin, cyanidin 3-*O*-glucoside concentrations in response to distilled water and glucose infusion through pedicel during ripening at 25°C

| | L* | Visual colour | C* | *h*° | Total anth | Cy3Glu | Chl-a | Chl-b | Total Chl | Total caret |
|---|---|---|---|---|---|---|---|---|---|---|
| **Firmness** | **0.89**** | **-0.85**** | **0.94**** | **0.81**** | **-0.60**** | **-0.63**** | **0.80**** | **0.77**** | **0.80**** | **0.81**** |
| **L*** | | **-0.93**** | **0.97**** | **0.89**** | **-0.73**** | **-0.80**** | **0.71**** | **0.71**** | **0.72**** | **0.78**** |
| **visual colour** | | | **-0.89**** | **-0.95**** | **0.84**** | **0.87**** | **-0.67**** | **-0.68**** | **-0.68**** | **-0.71**** |
| **C*** | | | | **0.85**** | **-0.67**** | **-073**** | **0.76**** | **0.74**** | **0.76**** | **0.80**** |
| ***h*°** | | | | | **-0.73**** | **-0.79**** | **0.61**** | **0.65**** | **0.63**** | **0.67**** |
| **Total ant** | | | | | | **0.84**** | **-0.55**** | **-0.57**** | **-0.56**** | **-0.59**** |
| **Cy3Glu** | | | | | | | **-0.44**** | **-0.44**** | **-0.45**** | **-0.51**** |
| **Chl-a** | | | | | | | | **0.94**** | **0.99**** | **0.81**** |
| **Chl-b** | | | | | | | | | **0.97**** | **0.74**** |
| **Total Chl** | | | | | | | | | | **0.79**** |

L* = Lightness, C* = Chroma, *h*° = hue angle, Total ant = Total anthocyanin, Cy3Glu = Cyanidin 3-*O*-glucoside, Chl-a = Chlorophyll-a, Chl-b = Chlorophyll-b, Total Chl = Total chlorophyll, Total caret = Total carotenoids, * = $p<0.05$ and ** = $p<0.01$

## 5.4. Conclusion

In 'Hass' avocado fruit, exocarp colour development during ripening is a result of an inadequate accumulation of cyanidin 3-*O*-glucoside. The result in this study showed that continuous glucose infusion at 0.05 and 0.13 mM through the fruit pedicel resulted in an increased total anthocyanin and cyanidin 3-*O*-glucoside concentration; concomitantly, the fruit developed to the purple (visual colour = 5) exocarp colour after 8 days at 25°C. Production practices that enhance sugar accumulation in avocado 'Hass' fruit exocarp may be important to control postharvest poor exocarp colouration.

# CHAPTER 6

# THE RELATIONSHIP BETWEEN DIFFERENT SIZE 'HASS' AVOCADO FRUIT AND EXOCARP COLOUR DEVELOPMENT, PIGMENTS, ANTIOXIDANTS AND SUGARS DURING RIPENING


## Abstract

The aim of this research work was to study the effect of fruit size; small- (< 200 g) and large-sized (> 201 g) of 'Hass' avocado fruit (*Persea americana* Mill.) exocarp colour development, pigments (chlorophyll, carotenoids, anthocyanin and cyanidin 3-*O*-glucoside), antioxidants (2,2-diphenyl-1-picrylhydrazyl-DPPH, phenol, ascorbic acid and flavonoids) and sugars concentration (D-mannoheptulose and perseitol) during ripening. Small- and large-sized fruit were stored at 5.5°C for up to 28 days thereafter, ripened at 25°C and assessed for fruit quality. In this study, small-sized fruit exhibited a significantly higher visual colour rating (≈ 5 purple/black) when compared with large-sized fruit, which showed lower visual colour rating (≈ 3 olive green) after 4 ripening days at 25°C. Furthermore, exocarp total chlorophyll and carotenoids content were significantly lower in small-sized after day 4 of ripening than large-sized fruit. The results also indicated that total anthocyanin and cyanidin 3-*O*-glucoside were significantly higher in small-sized fruit across all ripening days at 25°C when compared with large-sized fruit. Exocarp antioxidants measured by (2,2-diphenyl-1-picrydrazyl-DPPH, ascorbic acid, and total flavonoids) and sugars (D-mannoheptulose and perseitol) were highly concentrated in small-sized fruit after 4 ripening days at 25°C when compared with large-sized fruit. Furthermore, positive correlations ($p < 0.001$) were observed between visual colour and total anthocyanin ($r = 0.853$), cyanidin 3-*O*-glucoside ($r = 0.822$), DPPH ($r = 0.723$), total phenol ($r = 0.729$), flavonoid ($r = 0.775$)

and negative correlation between visual colour and total chlorophyll (r= -0.694), carotenoids (r= -0.670), D-mannoheptulose (r= -0.592) and perseitol (r= -0.664). In conclusion, the small-sized ‘Hass’ avocado fruit had higher exocarp visual colour and higher pigments concentration. In addition, higher antioxidants activities and sugar concentration compared with large-sized fruit, which correlated with improved exocarp colour development during ripening.

**Keywords:** Avocado fruit size, small sized fruit, large sized fruit, exocarp pigments, sugar concentration

## 6.1. Introduction

In the field, ‘Hass’ avocado trees produce high fruit yields of different sizes; small and large-size fruit. Economically, small-sized fruit are unviable; therefore, often transferred to the processing unit for the production of refinery by-products such as oil (Trujillo-Mayol et al., 2020). However, large-size fruit are regarded as high quality, therefore, graded and sorted and sent to both local and export markets. Literature exists on the relationship between fruit size, colour development, pigments and antioxidants for several crops such as; tomato (Arias et al., 2000), guava (Srimat et al., 2011) and papaya (Iamjud et al., 2016). In ‘Hass’ avocado fruit, it is currently unknown whether fruit size plays a role in poor exocarp colouration prevalence during ripening. In addition, there are currently no studies reported on the relationship between the physiology of ‘Hass’ avocado fruit exocarp colour developments during ripening. Thus, we hypothesized that ‘Hass’ avocado fruit exocarp colour development, anthocyanin concentration, antioxidant, sugar concentration varies with fruit size. The purpose of this work was to study two groups of statistically different avocado fruit sizes and their difference in pigments, antioxidants and sugars concentration in the exocarp during ripening.

## 6.2. Materials and Methods

### *6.2.1. Plant materials*

Early matured ‘Hass’ avocado fruit were harvested at their commercial dry matter (21% DM) from Nico Swart Trust avocado orchard at Kiepersol (Hazyview, Mpumalanga, South Africa, GPS: 25°29’19’’ S; 31°13’67’’ E). Harvested fruit were transported to the Agricultural Research Council - Tropical and Subtropical Postharvest Laboratory in Nelspruit (Mpumalanga, South Africa, GPS: 25°45’18’’S; 30°96’97’’ E).

### *6.2.2. Laboratory procedures, experimental design and treatments*

At the laboratory, fruit were grouped into two categories by their weight; small-sized (< 200 g) and large-sized (> 201 g), their diameter and length were also measured using a vernier calliper (Topacc LCD 150 mm Electronic Digital). Subsequently, fruit were stored at 5.5°C for up to 28 days, thereafter, ripened at 25°C. Ninety fruit were assigned to each category and the experiment was conducted as a completely randomised design (CRD) using 25 fruit as replication per category. The selected 25 fruit were continuously evaluated every other day (0, 2, 4 days at 25°C).

Methodologies for evaluation of fruit firmness (***cf. section* 3.2.6**), exocarp colour (***cf. section* 3.2.7**), total chlorophyll and carotenoids (***cf. section* 3.2.9**) and total

anthocyanin (***cf. section* 3.2.10**), total phenolic/phenol (***cf. section* 3.2.12**) and sugars (***cf. section* 3.2.11**) were done as explained in Chapter 3.

### *6.2.3. Determination of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability*

A method by Karioti et al. (2004) with slight modification was used to essay free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). A sample of 0.5 gram of fine powder avocado exocarp was mixed with 10 mL of 80% (v/v) methanol incubated at 4°C overnight, subsequently, 50 µL of the extract was mixed with 1 µL DPPH solution (0.1 mM in 95% methanol). The mixture was incubated in the dark for 30 minutes. The absorbance of the mixture was read at 517 nm against blank using a spectrophotometer (UV-1800, Shimadzu, Cor. Japan). The radical scavenging ability was determined using the following equation.

%DPPH (RSA) = [((Abs(ref) - Abs (sample))/Abs (ref)] × 100

Where Abs (ref.) = absorbance of reference (reacting mixture without the test sample), while Abs (sample) = Absorbance of reacting mixture with the test sample.

### *6.2.4. Determination of ascorbic acid*

A method described by Boonkasem et al. (2015) with slight modification was used to determine ascorbic acid. A sample of 0.5 gram of fine powder avocado exocarp was mixed with 10 mL of 1% metaphosphoric acid (v/v). The mixture was centrifuged at 10 000 × g for 5 minutes at 4°C. Subsequently, 1 mL of the extracted solution was mixed with 9 mL of 2,6-dichlorophenolindophenol dye then incubated in the dark for

10 minutes. The absorbance was read at 515 nm using a spectrophotometer (UV-1800, Shimadzu, Corp. Japan) and expressed as mg $g^{-1}$ ascorbic acid (AsA) DW.

### *6.2.5. Determination of total flavonoids*

A method described by Eghdami and Sadeghi (2010) with little modification was used to determine total flavonoid. A sample of 2 grams of fine powder avocado exocarp was mixed with 10 mL of 99.8% methanol and vortexed for 60 seconds then incubated at 4°C overnight. Subsequently, 1 mL of the extract solution was mixed with 0.03 mL of 5% $NaNO_2$ and allowed to react at room temperature for 5 minutes, followed by adding 0.03 mL of 10% $AlCl_3$ and allowed to react for 6 minutes. Following this, 0.2 mL of 1 mM NaOH was added into the mixture and diluted with 1 mL distilled water. The absorbance was read at 510 nm using a spectrophotometer (UV-1800, Shimadzu, Cor. Japan) and expressed as mg $g^{-1}$ quercetin equivalent (QuE) DW.

### *6.2.6. Statistical analysis*

The analysis of variance (ANOVA) was conducted using Statistix 10 data analysis software (Statistix, Vision 10$^{th}$, Analytical Software, USA). All-pairwise comparisons were completed utilizing the Least Significant Difference method (LSD; $p \leq 0.05$). To determine relationships between the measured variables, the data was subjected to a Pearson correlation test in Statistix 10.

## 6.3. Results and Discussion

### *6.3.1. Whole fruit characteristics, firmness and colour development*

All morphometric measurements of the fruit such as weight, diameter and length showed significant ($p < 0.001$) differences between small-sized and large-sized fruit, indicating a clear fruit size variation (Figure 6.1). All large-sized fruit samples were significantly higher in weight, diameter and length when compared with small-sized fruit. In general, the reduction in cell division which is promoted by alteration in abscisic acid, cytokinin hormones and sugar metabolisms is the main reason for fruit size variation in 'Hass' avocado cultivar (Richings et al., 2000, Taylor and Cowan, 2001). According to Mathaba et al. (2017), firmness and exocarp colour are two reliable factors that determine 'Hass' avocado fruit ripeness. In this research work, changes in firmness between small- and large-sized avocado fruit during 4 ripening days at 25°C are presented (Figure 6.2). In this study, fruit firmness decreased continuously until fully ripened at day 4, irrespective of size. Our results were consistent with Donetti (2011), who found that higher ripening temperature induced a faster decrease in ''Hass' avocado fruit firmness. According to Goulao and Oliveira (2008), a decrease in 'Hass' avocado fruit firmness is ascribed to cell wall-related enzymatic activities such as polygalacturonase (PG) and pectin methylesterase (PME) and cellulase that degrade cell walls and promotes turgidity loss. In this study, firmness values between small- and large-sized avocado groups did not differ significantly ($p = 0.269$) during ripening at 25°C. Similarly, Trujillo-Mayol et al. (2020) reported no significant differences in 'Hass' avocado fruit firmness between small-, medium- and the large-sized during ripening. The change in firmness in this study coincided with exocarp colour development, whereby visual colour rating significantly ($p < 0.001$) increased with ripening days at 25°C (Figure 6.3a). This study findings agreed with Cox et al. (2004) and Donetti (2011), who found that 'Hass' avocado fruit firmness and exocarp

colour development were negatively correlated with each other during ripening. A study by El-Kereamy et al. (2003) reported that cell wall enzymatic activities, chlorophyll degradation and anthocyanin biosynthesis are regulated by an ethylene-dependent system.

The highest visual colour rating was observed for the small-sized group when compared with large-sized fruit after 4 days at 25°C. Small-sized 'Hass' avocado fruit exocarp colour changed from green (≈ 1 visual colour rating) to purple (≈ 5 visual colour rating), whereas large-sized fruit only changed to olive green (≈ 3 visual colour rating) after 4 ripening days at 25°C (Figure 6.3 and 6.4). Similar decreasing trends were observed for colour chromaticity parameters (L*, C* and *h*°) during days at 25°C (Figure 6.3b-d). Previous studies in 'Hass' avocado also found that colour chromaticity parameters (L*, C* and *h*°) decrease during ripening at different ripening temperatures (Cox et al., 2004, Donetti and Terry, 2011). The results obtained in this study showed that small-sized fruit exocarp colour chromaticity (L*, C* and *h*°) values were lower throughout the ripening period when compared with a large-sized group. This result implied that small-sized fruit had darker exocarp colour when compared with large-sized fruit after 4 ripening days at 25°C. This was further supported by lower colour chromaticity (L*, C* and *h*°) values, concurrently, lower chlorophyll concentration and high anthocyanin accumulation, which conferred darker exocarp colour as reported by (Cox et al., 2004).

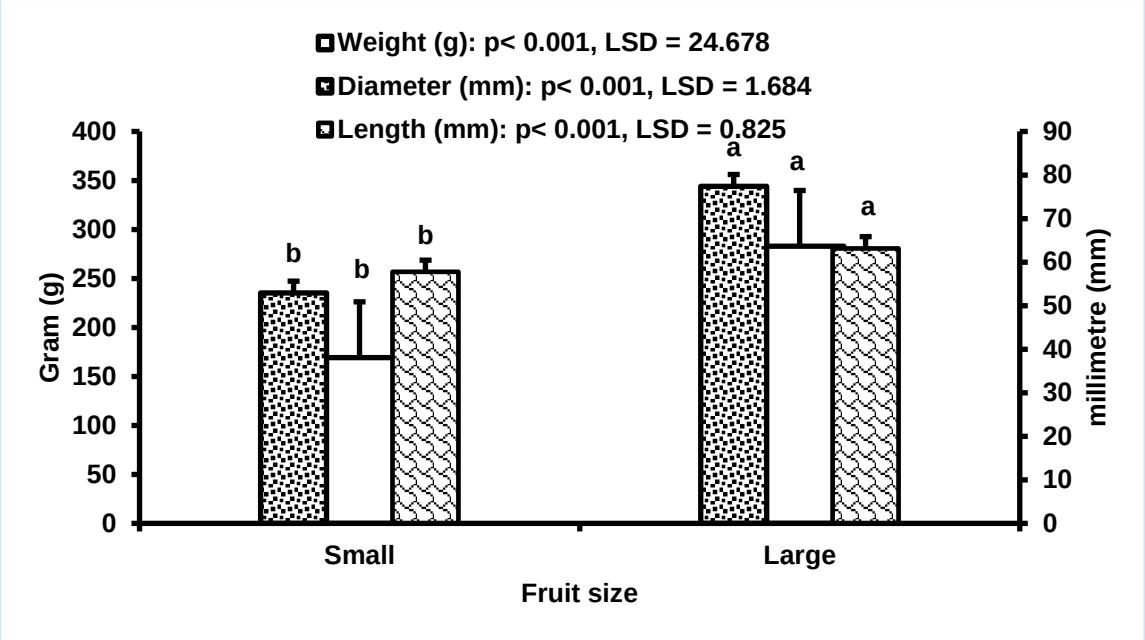



Figure 6.1 Morphometric measurement of whole fruit, weight, diameter and length between small- and large-sized. Values are means of 25 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters represent significant different values according to the LSD test (p< 0.05)

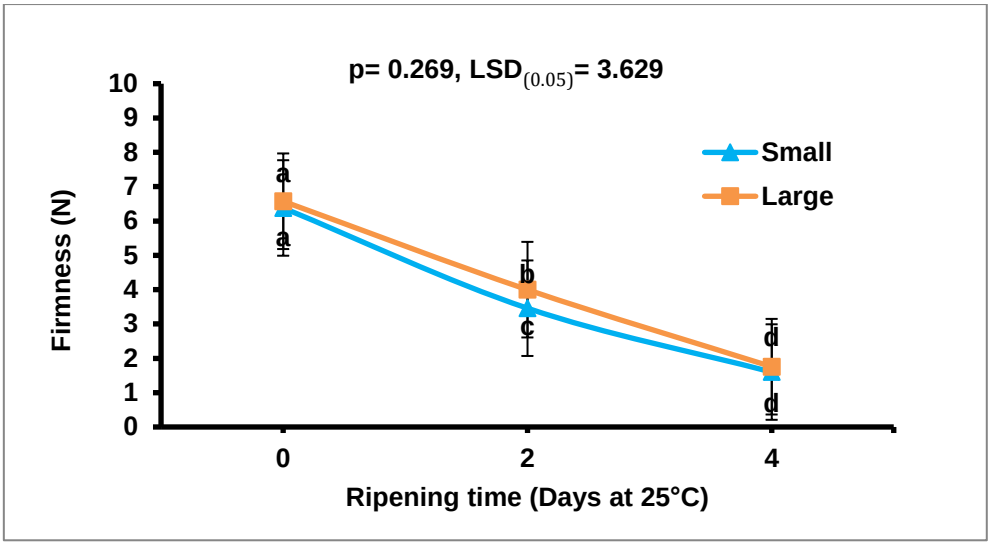



Figure 6.2 Changes in firmness (N) of small- and large-sized fruit during ripening at 25°C. Results are presented as means of 25 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters on the same day represent significantly different letters on the same day represent significantly different values according to the LSD test (p< 0.05)

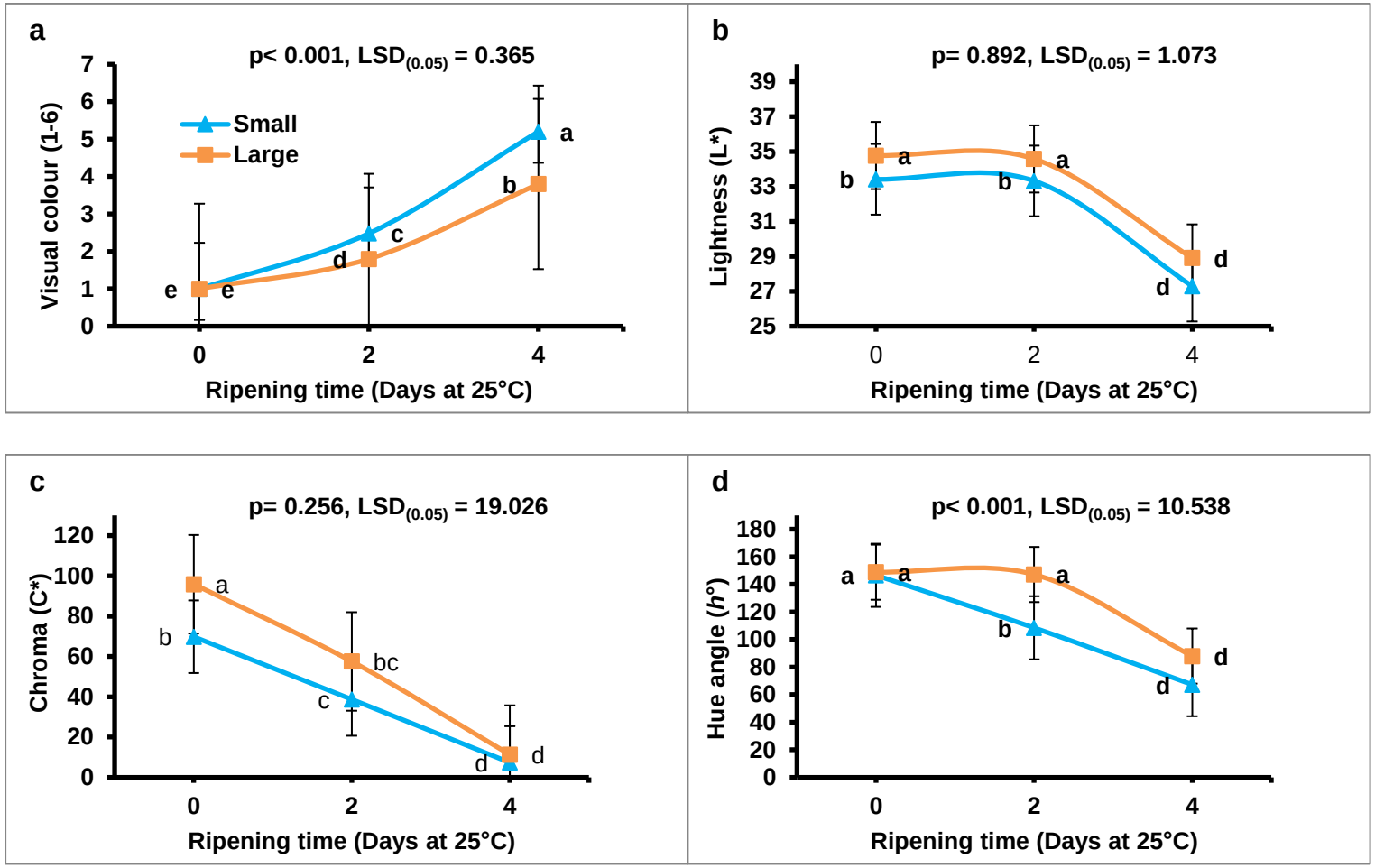



Figure 6.3 Change in visual colour (**a**), L* values (**b**), C* values (**c**) and *h*° values (**d**) of small- and large-sized fruit during ripening at 25°C. Results are presented as means of 25 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters on the same day represent significantly different values according to the LSD test (p< 0.05)


Figure 6.4 The differences between small- (**A**) and large-sized (**B**) colour development after 4 days ripening at 25°C

*6.3.2. Change in exocarp colour pigments during ripening*

Changes in chlorophyll and carotenoids concentration in small- and large-sized avocado fruit exocarp are shown in Figure 6.5. In general, there was significant ($p < 0.001$) differences in chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids between small- and large-sized fruit during ripening at 25°C. However, chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids concentration decreased with ripening days at 25°C for both small-sized and large-sized fruit avocado fruit (Figure 6.5a-d). These results were in agreement with the findings of Ashton et al. (2006) and Donetti (2011), whereby chlorophyll-a, chlorophyll-b, total chlorophyll and carotenoids concentrations decreased in the skin and flesh of 'Hass' avocado fruit exponentially as the fruit ripened. During the initial day at 25°C, small-sized fruit showed significantly higher total chlorophyll concentration when compared with large-sized fruit (Figure

6.5c). According to Ashton et al. (2006), high chlorophyll concentration in ‘Hass avocado fruit exocarp is attributed to higher chlorophyllides concentration. However, after 4 ripening days at 25°C, small-sized fruit showed significantly lower chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids concentration when compared to the large-sized group. According to Cox et al. (2004), reduction in ‘Hass’ avocado fruit exocarp chlorophyll concentration is likely to be correlated with colour development during ripening.

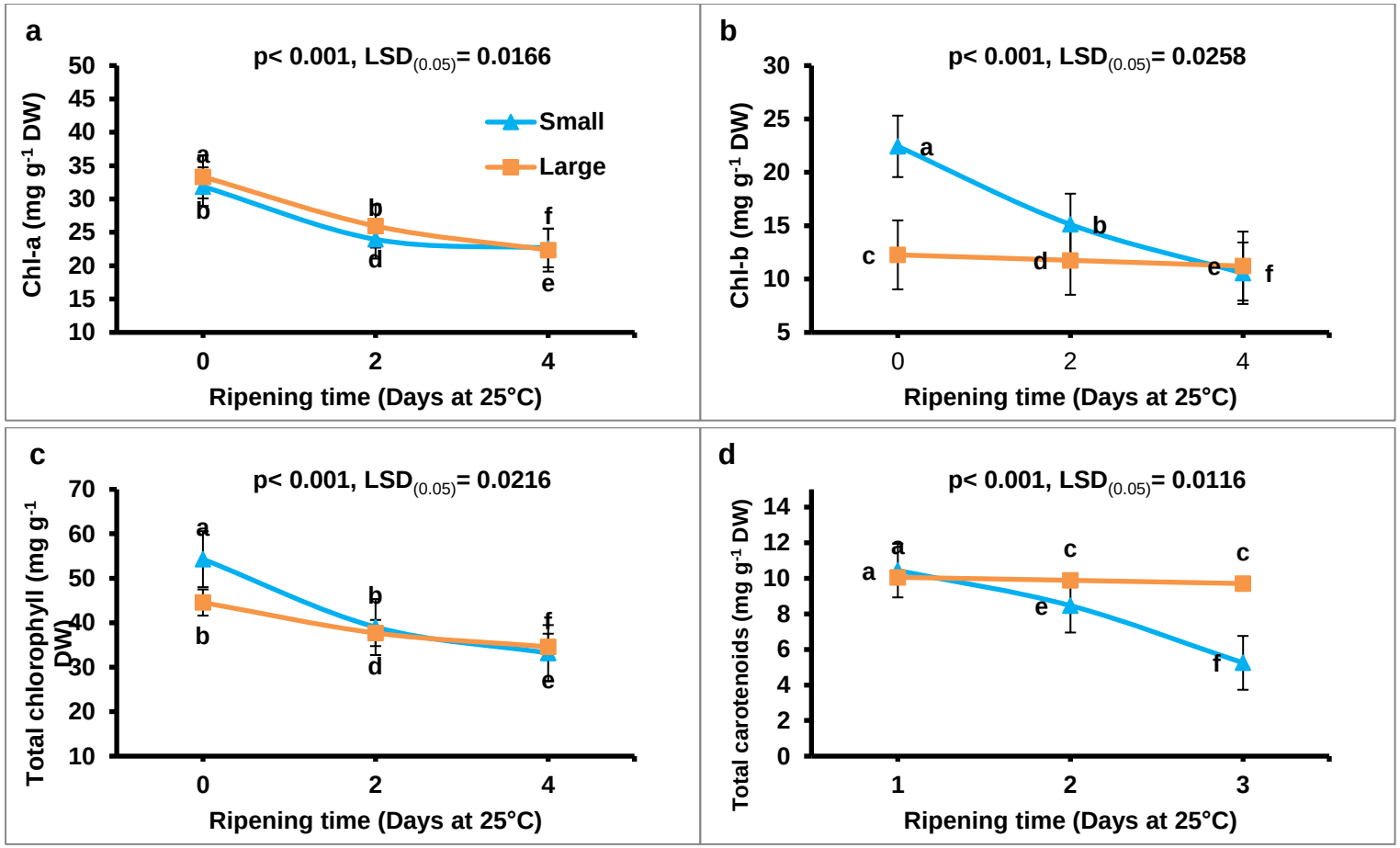



Figure 6.5 Change in chlorophyll-a (**a**), chlorophyll-b (**b**), total chlorophyll (**c**) and total carotenoids (**d**) concentration between small- and large-sized fruit during ripening at 25°C. Results are presented as means of 12 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters on the same day represent significantly different values according to the LSD test (p< 0.05)

Furthermore, chlorophyll and carotenoids breakdown coincided with total anthocyanin and cyanidin 3-*O*-glucoside concentration increase, resulting in 'Hass' avocado fruit exocarp colour development during ripening (Figure 6.6). This was in agreement with previous studies by Cox et al. (2004) and Donetti and Terry (2011), whereby, 'Hass' avocado fruit exocarp colour development was strongly influenced by chlorophyll degradation, concomitantly, an increase in total anthocyanin and cyanidin 3-*O*-glucoside. In this study, significant differences ($p < 0.001$) in total anthocyanin and cyanidin 3-*O*-glucoside were found between small- and large-sized groups (Figure 6.6a-b). The total anthocyanin concentration of small-sized fruit was significantly higher than large-sized fruit across all ripening days at 25°C. Similarly, small-sized fruit displayed significantly higher exocarp cyanidin 3-*O*-glucoside concentration from ripening day 2 to day 4 at 25°C. We did not evaluate polyphenol related enzymatic activities in this study. However, our results were consistent with the findings of Trujillo-Mayol et al. (2020). These authors found that small-sized displayed high enzymatic activities of phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and polyphenol oxidase (PPO) when compared with medium and large-sized avocado fruit. According to He et al. (2010), cyanidin 3-*O*-glucoside biosynthesis is regulated by the expression and activities of phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) and anthocyanidin synthase (ANS) enzymes. In addition to regulating cyanidin 3-*O*-glucoside, an enzyme such as PAL is also involved in the biosynthesis pathway of phenolic compounds (Villa-Rodríguez et al., 2011). In the present study, the increase in 'Hass' avocado fruit exocarp cyanidin 3-*O*-glucoside concentration was significantly ($p < 0.001$) positively related to the increase in total phenol ($r = 0.652$) (Table 6.2) during ripening.

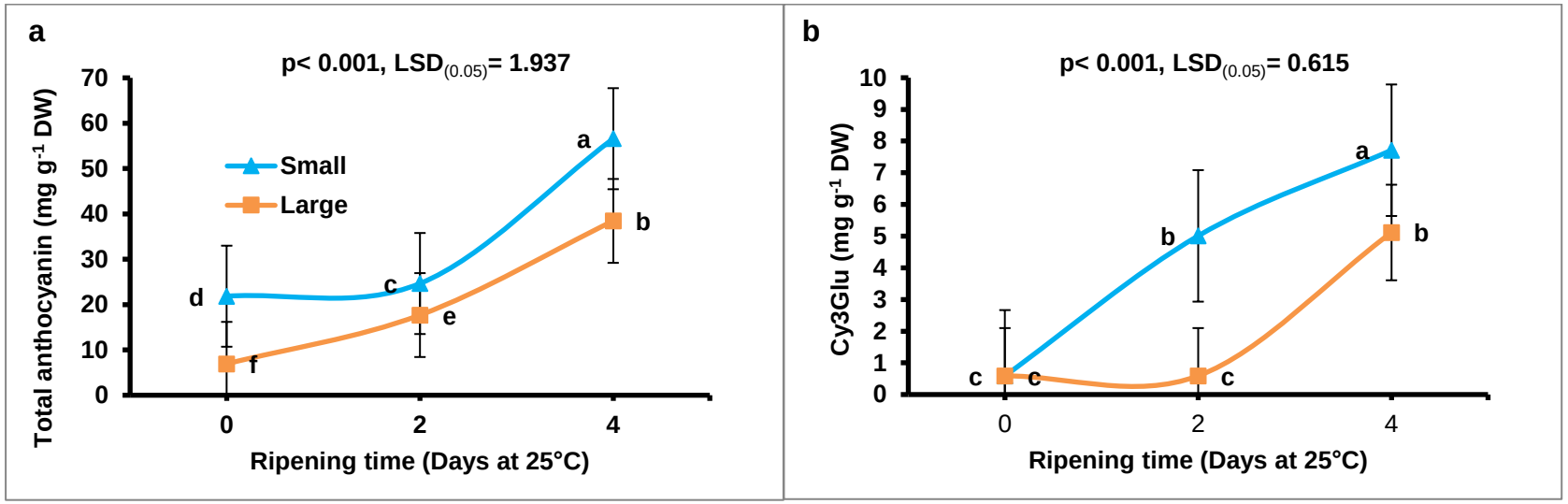



Figure 6.6 Change in total anthocyanin (**a**) and cyanidin 3-*O*-glucoside (Cy3Glu) (**b**) concentration between small- and large-sized fruit during ripening at 25°C. Results are presented as means of 12 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters on the same day represent significantly different values according to the LSD test (p< 0.05)

*6.3.3. Exocarp antioxidants capacity*

Table 6.1 shows the change in 'Hass' avocado fruit exocarp antioxidants capacity between small-sized and large-sized avocado fruit during ripening at 25°C. In this study, DPPH radical scavenging ability increased in both small- and large-sized fruit with ripening days at 25°C. Significant ($p < 0.001$) differences in DPPH radical scavenging ability were observed between small- and large-sized at 25°C. In this study, small-sized fruit showed significantly higher DPPH radical scavenging ability after 4 ripening days at 25°C when compared with large-sized fruit. These results corroborate with Trujillo-Mayol et al. (2020), whereby, 'Hass' avocado exocarp extracts of small- and medium-sized fruit had higher DPPH radical scavenging ability when compared with large-sized. In general, the colour of the fruit is consistent with their antioxidant content and consequently with their antioxidant capacity. Several studies have reported that avocado fruit exhibit high anti-radical ability with high antioxidant capacity (Bertling et al., 2007, Tesfay et al., 2010).

Pigmented compounds such as chlorophyll, carotenoids and anthocyanin contribute most of the antioxidant potential of the 'Hass' avocado fruit. In this study, relationships were found between DPPH and total chlorophyll ($r = -0.928$) and total carotenoids ($r = -0.762$). These results suggested that chlorophyll and carotenoids pigments contributed to antioxidant potential of unripe avocado fruit. It is further reported that the increase in phenolic compounds such as anthocyanin also contributes to the antioxidant potential of the fruit (Cömert et al., 2020). In this study, DPPH was highly correlated with cyanidin 3-*O*-glucoside ($r = 0.736$), suggesting that high antioxidant

potential in avocado exocarp at ripe stage could relate to cyanidin 3-*O*-glucoside biosynthesis and accumulation.

Moreover, there were no significant (p= 0.793) differences in 'Hass' avocado fruit exocarp ascorbic acid concentration between small- and large-sized during ripening at 25°C (Table 6.1). In general, exocarp ascorbic acid concentration decreased for both small- and large-sized fruit with ripening days at 25°C. This tendency was consistent with the observation of Mahendran and Prasannath (2008), whereby ascorbic acid concentration in 'Hass' avocado fruit was affected by storage temperature, duration and atmospheric composition. However, ascorbic acid concentration did not differ significantly in the exocarp of small- and large-sized fruit after 4 ripening days at 25°C (Table 6.1). It has been reported that ascorbic acid provides the basic antioxidant system in avocado mesocarp, which plays a role in scavenging reactive species (ROS) (Tesfay et al., 2010). According to Tesfay et al. (2009), the reduction in ascorbic acid concentration might be attributed to its ability to donate electrons for the regeneration of other antioxidants compounds. Supporting this, in the current study, ascorbic acid was negatively correlated with DPPH, total phenol and total flavonoid (Table 6.2). It has been reported that an avocado exocarp contains substantially higher phenol compounds and antioxidants (Tesfay et al., 2010). Thus, phenol accumulation enhances the antioxidants pool, thereby improving postharvest fruit quality (Tesfay et al., 2011).

According to Balasundram et al. (2006), phenolic compounds are predominantly responsible for determining the colour, taste and are involved in response to stress conditions. For instance, Kuti (2004) reported that the high antioxidant of purple

‘Opuntia’ cactus pear fruit was associated with phenolic compounds. In this study, exocarp total phenol concentration showed an increasing trend with ripening days at 25°C. However, no significant (p= 0.355) differences were observed between small- and large-sized during ripening at 25°C. Our results were different from those reported by Trujillo-Mayol et al. (2020), whereby, total phenolic concentration varied substantially between ‘Hass’ avocado exocarp extract of small-, medium- and large-sized fruit. In general, the total phenols concentration obtained in this study were lower than those obtained by Wang et al. (2016) and Trujillo-Mayol et al. (2020). According to Donetti (2011), avocado fruit differs in its response to the postharvest condition due to preharvest factors such as harvest time and fruit size. Supposedly, the lower total phenol concentration obtained in this study for both small- and large-sized could be attributed to oxidative destruction under the influence of cold storage at 5.5°C, subsequently, ripening temperature at 25°C.

In the case of exocarp total flavonoids, significant (p= 0.041) differences were observed between small- and large-sized during ripening at 25°C (Table 5.1). Small-sized fruit tended to show significantly higher total flavonoids concentration when compared with large-sized fruit during ripening at 25°C. Similar results were reported by Trujillo-Mayol et al. (2020), whereby, total flavonoids concentration in small-sized ‘Hass’ avocado fruit exocarp extract be significantly higher when compared with medium- and large-sized fruit. In plants, a variability in polyphenols and flavonoids is attributed to the biotic and abiotic conditions (Wang et al., 2016). The higher total flavonoids in small-sized fruit as found in this study could be due to tree and environmental stress including heavy bearing, tree age, warm growing conditions and canopy temperature previously reported by Taylor and Cowan (2001).

Table 6.1 Change in DPPH radical scavenging ability, ascorbic acid, total phenol and total flavonoids concentration in the exocarp of small-sized and large-sized avocado fruit during ripening at 25°C

| Fruit size | Days at 25°C | DPPH radical scavenging ability (%) | Ascorbic acid (mg $g^{-1}$ AsA) | Total phenol (mg $g^{-1}$ GAE) | Total flavonoids (µg $g^{-1}$ QuE) |
|---|---|---|---|---|---|
| Small-sized | 0 | 8.07 e | 1.52 a | 21.11 d | 43.48 d |
| | 2 | 44.13 c | 0.99 b | 30.07 b | 87.49 bc |
| | 4 | 67.80 a | 0.71 cd | 35.81 a | 137.97 a |
| Large-sized | 0 | 38.46 d | 1.62 a | 23.67 c | 31.46 d |
| | 2 | 41.81 c | 1.06 b | 32.13 b | 82.99 c |
| | 4 | 49.84 b | 0.65 d | 36.27 a | 103.11 b |
| p value | | <0.001 | 0.793 | 0.355 | 0.041 |
| LSD $_{(0.05)}$ | | 3.305 | 0.214 | 1.535 | 17.721 |
| CV% | | 9.69 | 23.99 | 6.28 | 26.71 |

DPPH (2,2-diphenyl-1-picrylhydrazyl), AsA (ascorbic acid), GAE (Gallic acid equivalents), QuE (quercetin equivalents). Results are presented as the means of 12 fruit. Means with different letters in the same column represent significant different values according to the LSD test ($p< 0.05$)

*6.3.4. Sugar concentration in the exocarp and seed*

In this study, D-mannoheptulose and perseitol were the most abundant sugars found in the 'Hass' avocado fruit exocarp during ripening at 25°C (Figure 6.7a-b). In general, small- and large-sized fruit varied significantly in D-mannoheptulose concentration ($p< 0.001$) but not perseitol ($p= 0.164$) during ripening at 25°C (Figure 6.7a-b). However, D-mannoheptulose and perseitol concentrations decreased for both small- and large-sized fruit during ripening at 25°C. These results were directly in line with previous

findings in avocado fruit by other researchers (Liu et al., 1999, Tesfay, 2009, Bertling and Tesfay, 2011, Blakey et al., 2012). These researchers found D-mannoheptulose and perseitol sugars concentrations declined to minimal levels as avocado fruit ripens. Our results were in agreement with this observation. During the initial days at 25°C, small-sized fruit had significantly higher D-mannoheptulose and perseitol concentrations when compared with large-sized fruit. However, D-mannoheptulose and perseitol concentration declined with no significant difference observed between small- and large-sized fruit after 4 ripening days at 25°C.

Previous studies showed that avocado fruit contains a considerable quantity of D-mannoheptulose and perseitol (Bertling and Bower, 2005, Bertling et al., 2007, Tesfay et al., 2010). D-mannoheptulose sugar is believed to be directly proportional to antioxidant capacity, while perseitol serves as a storage compound (Tesfay et al., 2010). However, little is known about their involvement in colour development during ripening. Although the six-carbon ($C_6$) sugar compounds (sucrose, glucose and fructose) were not detected in the exocarp due to their lower concentrations in this study, their relationship with anthocyanin accumulation has been reported. Richings et al. (2000) found that small avocado fruit had a lower concentration of sucrose and higher glucose level, higher respiration rate and abscisic acid metabolism. In general, phenolic compounds such as anthocyanin occur as flavonoid *O*-glucoside because one or more flavonoids hydroxyl groups are attached to a sugar molecule (Nakatsuka et al., 2008). According to Bowles et al. (2006), flavonoid *O*-glucoside is generally synthesized by nucleotide diphosphate dependent glycosyltransferase. It has been reported that a uridine diphosphate (UDP) glucose molecule in the form of UDP-glucose, UDP-galactose, UDP-arabinose and UDP-xylose which is liberated from

glucose, galactose and xylose sugar monomers is glycosylated to the flavonoid by uridine 5'-diphosphate (UDP) carbohydrate-dependent glycosyltransferase (UGTs) (Nakatsuka et al., 2008). The succession of this glycosylation results in anthocyanin biosynthesis and ensures stability (Yonekura-Sakakibara et al., 2012). In apple, an increase in galactose before harvest permitted glycosylation and anthocyanin biosynthesis and accumulation (Redgwell et al., 1997). Jeong and Huber (2004) reported that arabinose, galactose and xylose were the dominant sugar monomers at the full ripe stage. Unfortunately, the main sugar attached to the flavonoid in 'Hass' avocado has not yet been identified. In this study, there was a weak negative correlation between D-mannoheptulose and perseitol sugar and total phenol and total flavonoid. It is possible that although D-mannoheptulose and perseitol are abundant in the exocarp of avocado fruit, however, not play a role in exocarp colour development during ripening. However, their concentrations are directly proportional to the concentration of soluble sugars. We assume that the decrease in both D-mannoheptulose and perseitol concentration in this study may indicate that both sugars contribute towards antioxidant production in the exocarp during ripening. Thus, higher antioxidant activity in the avocado exocarp could also be associated with higher D-mannoheptulose and perseitol concentrations. In addition, our results showed a strong negative relationship between D-mannoheptulose and perseitol with DPPH (Table 6.2).

Figure 6.8 shows the differences in sugar concentration between the seeds from small- and large-sized. In this study, significant differences in sugars concentration were detected between seeds from small- and large-sized fruit. In the present study, D-mannoheptulose, perseitol, glucose and sucrose were detected in fruit. Similar

results were reported previously by Tesfay et al. (2010), whereby both six-carbon ($C_6$) and seven-carbon ($C_7$) sugars were dominant in avocado fruit seed. In the present study, all detected sugars were significantly higher in the seed of small-sized fruit when compared with large-sized. Moreover, seeds from small-sized fruit showed significantly higher glucose concentrations than seeds from large-sized fruit. These results were in agreement with findings by Taylor and Cowan (2001). According to Taylor and Cowan (2001), the increase of invertase and reduced sucrose synthase (SuSy) activity in the seed of small-sized fruit is associated with sucrose depletion and increase in glucose as a constituent of total soluble sugars. Previously, Liu et al. (1999) reported that sugars stored in the exocarp and mesocarp of avocado fruit are broken down while carbohydrates stored in the seed remain unbroken. The relationship between seed sugar concentration and exocarp colour development is predominantly related to sugar partitioning. It has been reported that soluble sugars are the major source of carbon for seed growth and development (Liu et al., 1999). Therefore, the seed in avocado fruit is pronounced as a strong sink of carbohydrate when compared with mesocarp and exocarp (Wolstenholme, 1985), especially $C_6$ sugars required for exocarp anthocyanin biosynthesis. In this study, small-sized fruit showed high seed sugar concentration when compared with seed from large-seed, as result showed high exocarp sugar accumulation; therefore, improved exocarp colour development during ripening.

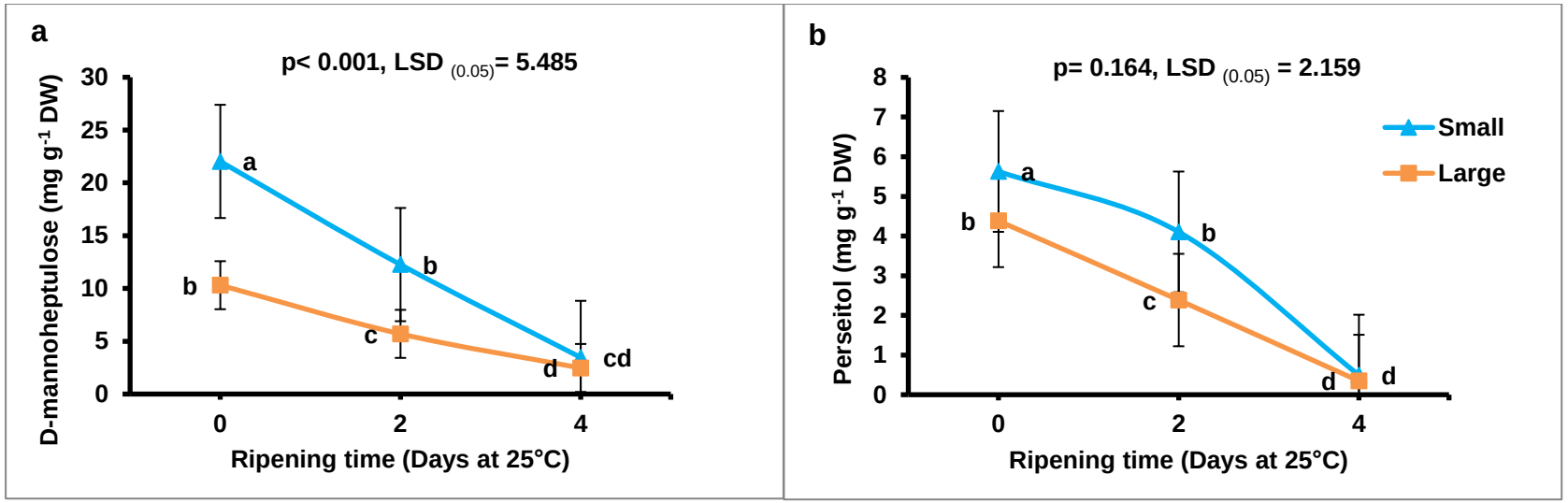



Figure 6.7 Change in D-mannoheptulose (**a**) and perseitol (**b**) concentration between small- and large-sized fruit during ripening at 25°C. Results are presented as means of 10 fruit. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters on the same day represent significantly different values according to LSD test (p< 0.05)

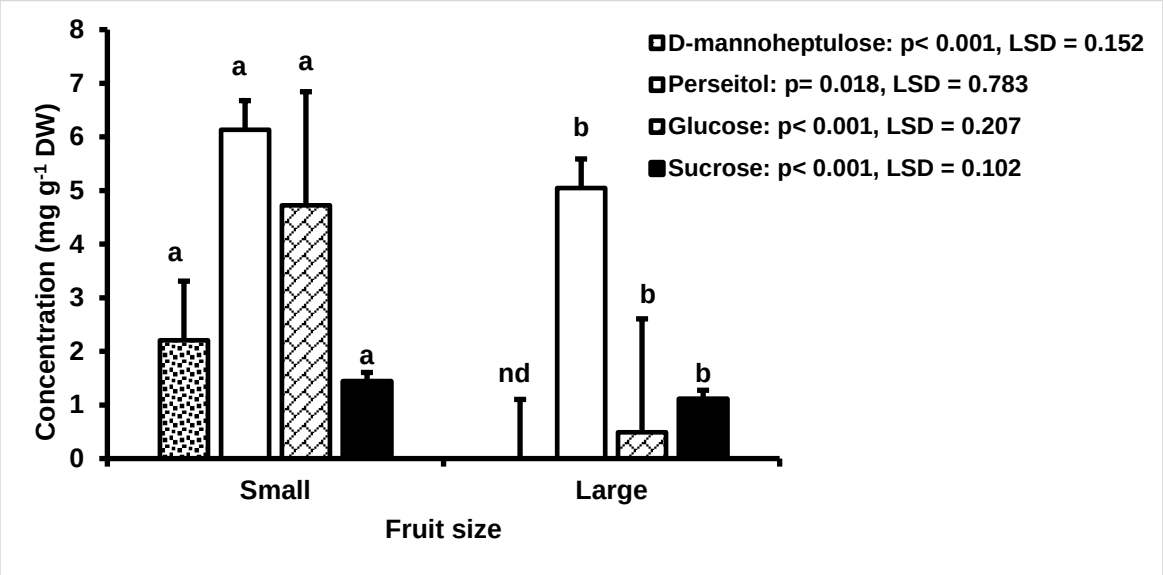



Figure 6.8 The difference in sugar concentration between seeds from small- and large-sized fruit. Results are presented as means of 5 seeds. Error bars indicate ±SE of means at p≤ 0.05 and means with different letters represent significantly different values according to the LSD test (p< 0.05) and nd = not detected

*6.3.5. Pearson correlation analysis*

Table 6.2 shows that colour parameters such as visual colour and all chromaticity parameters (L*, C* and *h*°) had a high correlation with total anthocyanin and cyanidin 3-*O*-glucoside, with visual colour being the colour parameter with the highest correlation. Similarly, visual colour had a high correlation with total phenol (r= 0.729, Table 6.2) and total flavonoids (r= 0.775) (Table 6.2). These results supported the notion that the increase in the antioxidant pool enhances postharvest fruit quality (Tesfay et al., 2010). Cyanidin 3-*O*-glucoside positively correlated with DPPH (r= 0.736), total phenol (r= 0.652) and total flavonoids (r= 0.723). This depicts an essential role of anthocyanin on the antioxidant pool of avocado exocarp. Furthermore, negative weak correlation was observed between cyanidin 3-*O*-glucoside and D-mannoheptulose (r= -0.464) as well as perseitol (r= -0.559). These findings suggest that D-mannoheptulose and perseitol concentration in the exocarp of avocado may not contribute to the biosynthesis of cyanidin 3-*O*-glucoside; concurrently, colour development.

Table 6.2 Pearson correlation coefficient between firmness, chromaticity colour parameters (L*, C* and *h*°) and visual colour and total anthocyanin, cyanidin 3-*O*-glucoside, chlorophyll, carotenoids, DPPH, ascorbic acid, total phenol, flavonoids and D-mannoheptulose and perseitol

| Variables | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Firmness | 1 | | | | | | | | | | | | | | | | |
| 2. Vis-col | -0.911*** | 1 | | | | | | | | | | | | | | | |
| 3. L* | 0.693*** | -0.774*** | 1 | | | | | | | | | | | | | | |
| 4. C* | 0.862*** | -0.765*** | 0.736*** | 1 | | | | | | | | | | | | | |
| 5. *h*° | 0.844*** | -0.851*** | 0.589*** | 0.731*** | 1 | | | | | | | | | | | | |
| 6. Chl-a | 0.875*** | -0.745*** | 0.426** | 0.797*** | 0.734*** | 1 | | | | | | | | | | | |
| 7. Chl-b | 0.412** | -0.416** | 0.192ns | 0.195ns | 0.433** | 0.423** | 1 | | | | | | | | | | |
| 8. Total chl | 0.772*** | -0.694*** | 0.370** | 0.599*** | 0.698*** | 0.854*** | 0.832*** | 1 | | | | | | | | | |
| 9. Total Car | 0.662*** | -0.670*** | 0.529*** | 0.562*** | 0.628*** | 0.590*** | 0.423** | 0.603*** | 1 | | | | | | | | |
| 10. T-Anth | -0.850*** | 0.853*** | -0.695*** | -0.773*** | -0.823*** | -0.754*** | -0.229ns | -0.593*** | -0.803*** | 1 | | | | | | | |
| 11. Cy3Glu | -0.841*** | 0.822*** | -0.645*** | -0.740*** | -0.772*** | -0.787*** | -0.360* | -0.688*** | -0.834*** | 0.849*** | 1 | | | | | | |
| 12. DPPH | -0.718*** | 0.723*** | -0.449** | -0.526*** | -0.710*** | -0.708*** | -0.864*** | -0.928*** | -0.762*** | 0.644*** | 0.736*** | 1 | | | | | |
| 13. AsA | 0.601*** | -0.479*** | 0.257* | 0.488*** | 0.540*** | 0.657*** | 0.302* | 0.576*** | 0.428** | -0.590*** | -0.536*** | -0.527*** | 1 | | | | |
| 14. T-Phen | -0.823*** | 0.729*** | -0.384** | -0.651*** | -0.741*** | -0.870*** | -0.598*** | -0.875*** | -0.513*** | 0.691*** | 0.652*** | 0.765*** | -0.694*** | 1 | | | |
| 15. T-Flav | -0.807*** | 0.775*** | -0.489*** | -0.734*** | -0.757*** | -0.790*** | -0.421** | -0.725*** | -0.705*** | 0.79*** | 0.723*** | 0.711*** | -0.557*** | 0.773*** | 1 | | |
| 16. *D*-mann | 0.601*** | -0.592*** | 0.299* | 0.405** | 0.590*** | 0.623*** | 0.820*** | 0.852*** | 0.404** | -0.454** | -0.464** | -0.811*** | 0.527*** | -0.818*** | -0.561*** | 1 | |
| 17. Pers | 0.743*** | -0.664*** | 0.493*** | 0.582*** | 0.660*** | 0.682*** | 0.595*** | 0.758*** | 0.466** | -0.629*** | -0.559*** | -0.706*** | 0.572*** | -0.802*** | -0.649*** | 0.735*** | 1 |

Vis-col = visual colour, L* = Lightness, C* = Chroma, *h*° = hue angle, Chl-a = Chlorophyll-a,Chl-b = Chlorophyll-b, Total Chl = Total chlorophyll, Total car = Total carotenoids,T- anth = Total anthocyanin, Cy3Glu = Cyanidin 3-*O*-glucoside, DPPH (2,2-diphenyl-1-picrylhydrazyl), AsA = ascorbic acid, T-phen = Total phenol, T-flav = Total flavonoid, D-mann = D-mannoheptulose and pers = perseitol, * represent significant difference at *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001 and ns = not significant

## 6.4. Conclusion

This study indicated that colour development, exocarp pigments, antioxidants activity and sugar concentration varied between two groups of different size avocado fruit. The small-sized ‘Hass’ avocado fruit had greater visual colour and higher concentration of pigments (total anthocyanin and cyanidin 3-*O*-glucoside) in addition to higher antioxidants activities (DPPH and total flavonoids) and sugar concentration (D-mannoheptulose and perseitol) compared with large-sized fruit during ripening. While D-mannoheptulose concentration in the exocarp was higher than in the seed of small- and large-sized fruit, whereas perseitol concentration was higher in seed than in the exocarp.

# CHAPTER 7

## SUMMARY, CONCLUSION, LIMITATIONS AND FUTURE RESEARCH

In Chapter 3, the effect of crop load adjustment was examined on exocarp colour of 'Hass' avocado during ripening at three harvest times (early, mid- and late maturity). In this study, early season fruit showed poor exocarp colour during ripening because they were still maturing when compared with mid- and late season fruit. However, reducing crop load to moderate or low loads significantly improved fruit maturity by advancing dry weight. Therefore, fruit harvested from adjusted crop loads [moderate (50%) and low (25%)] showed an improvement in exocarp colour as well as an increase in anthocyanin concentration as the fruit ripened.

In this study, moderate (50%) and low (25%) crop load adjustments improved early-season avocado exocarp colour development when compared with normal crop load (100%) during ripening. In mid-harvest, 'Hass' avocado fruit from low crop load exhibited the desired exocarp colour compared to those from moderate (50%) and normal crop loads (100%). The results showed that crop load adjustment improved 'Hass' avocado fruit maturity, exocarp sugar accumulation, phenolic concentration in turn improving exocarp anthocyanin biosynthesis and accumulation; ultimately, influencing colour development during ripening.

In this Chapter, we presented results supporting our hypothesis that crop load adjustment increase carbohydrates availability for fruit development, therefore advancing maturity and improve harvest and postharvest fruit quality. The results seem inconclusive due to the lack of growing season agro-climatic data, vegetative productivity, yield and whole fruit quality. Also, $C_6$ carbohydrates were not detected;

however, they are known to be responsible for anthocyanin biosynthesis. Therefore, we assume that $C_6$ were present in small quantities not detected by High-Pressure Liquid Chromatography (HPLC) and require Gas Chromatography-Mass Spectrometer (GC-MS). The study showed that crop load adjustment had a positive impact on 'Hass' avocado fruit exocarp colour development during ripening. In future studies, best crop load adjustment time should be established for ‘Hass’ avocado commercial yield and fruit quality. In addition, different crop load adjustment methods should be considered such as the flower and panicle and the use of chemical.

Three months before early harvest, branch girdling was performed on ‘Hass’ avocado tree in Chapter 4. In this study, fruit harvested from girdled trees showed poor exocarp colour development as compared to fruit harvested from non-girdled trees, regardless of harvest maturity. During the early and mid-harvest, avocado 'Hass' fruit harvested from girdled and control trees did not significantly differ in visual exocarp colour. However, ‘Hass’ avocado fruit harvested from girdled trees showed low anthocyanin concentration; and therefore, poor exocarp colour development during ripening, irrespective of harvest maturity. The results were unexpected since girdling increases the accumulation of carbohydrates, and carbohydrates are precursors for anthocyanin, which is the pigment compound that is responsible for ‘Hass’ avocado exocarp purple colouration. However, girdling ‘Hass’ avocado fruit trees in February could have led to a reduction in $C_6$ sugar accumulation to the exocarp. Low glucose and sucrose concentration in exocarp affects enzyme-rate-linked anthocyanin biosynthesis, accumulation and colour change, especially for early season girdled and control fruit. Furthermore, girdling during this period appears to partition carbohydrates for seed and mesocarp growth, ultimately leading to an increase in fruit size. Therefore, exocarp carbohydrates accumulation appeared to be neglected, causing

the exocarp to become thinner, deteriorating faster and becoming susceptible to physiological disorders such as chilling injury, vascular browning, and body rot during ripening (data not shown).

In a study by Bertling et al. (2008), it was found that girdling avocado trees during late fruit growth and development negatively affects carbohydrate balance and accumulation. The results of this research confirm that girdling avocado trees in February has a negative effect on fruit quality, including exocarp colour development, regardless of maturity. The limitations of this study in chapter 4 are that girdling time and phenological growth stage were not considered for the experiment, and that girdling was only performed once, rather than continuously. These factors should be investigated and considered when girdling avocado fruit trees in the future.

In Chapter 5, we investigated the role of $C_6$ carbohydrates in avocado fruit development and ripening since they were not detected in Chapter 3 and Chapter 4 in exocarp from 'Hass' avocado. Chapter 5 presents a study that investigates whether postharvest glucose infusion through pedicels can improve avocado exocarp colour development during ripening. In this study, glucose concentration (0.05 and 0.13 mM) resulted in purple 'Hass' avocado fruit after 8 ripening days at 25°C when compared with the control. In 'Hass' avocado fruit infused with glucose, anthocyanin and cyanidin 3-*O*-glucoside concentration significantly increased during ripening. These results suggest that glucose infusion provided UDP-gluc donor, which promoted anthocyanin biosynthesis, thus increasing cyanidin 3-*O*-glucoside and improving exocarp colour development.

The results of glucose infusion supported the initial hypothesis that glucose serves as a signal molecule for anthocyanin biosynthesis. However, our research work only used

glucose as a sugar source, but we recommended the use and elucidation of other important sugars such as sucrose, galactose, arabinose, rhamnose and xylose.

Apart from elucidating the effect of postharvest glucose pulsing, fruit size seemed to play a role in determining ‘Hass’ avocado fruit exocarp colour during ripening. In Chapter 6, we investigated the relationship between ‘Hass’ avocado fruit size, exocarp colour related pigments, bioactivity compounds with antioxidant capacity and sugars during ripening. In this study, small fruit developed the desired purple to black exocarp colour after 4 days of ripening. These results were confirmed by lower chromaticity exocarp colour parameters (L*, C* and *h*°), chlorophyll content, degradation and total anthocyanin and cyanidin 3-*O*-glucoside biosynthesis and accumulation.

We found that small-sized fruit exhibited higher antioxidant capacity as quantified by DPPH, ascorbic acid and total flavonoids, ultimately; improved exocarp colour change during ripening. These findings suggested that small-sized ‘Hass’ avocado fruit have a higher physiological ability to scavenge free radicals when exposed to oxidative stress when compared with large-sized fruit. In addition, small-sized ‘Hass’ avocado fruit accumulated higher sugars (D-mannoheptulose, perseitol, glucose and sucrose) in both the exocarp and seed. In considering this evidence, an avocado fruit requires high exocarp sugars, bioactive compounds with antioxidant properties and pigment biosynthesis and accumulation in order to yield a purple exocarp colouration during ripening.

Ultimately, avocado fruit with different sizes also differs in maturity, bioactive compounds with antioxidant capacity, postharvest quality, ripening and exocarp colour change. To prevent uneven ripening and variable exocarp colour development, fruit size should be carefully considered during harvest, packaging and transport to market.

This study has the limitation that only one season and fruit were grouped into two categories. Future research should focus on manipulating the enzymes, genes and factors controlling anthocyanin concentration in large-sized 'Hass' avocado fruit to manipulate avocado exocarp colour during ripening.

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PhD thesis

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