EFFECT OF SUCROSE AND CALCIUM PULSING ON EARLY SEASON 'HASS' AVOCADO FRUIT EXOCARP COLOUR CHANGE DURING RIPENING

by

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DECLARATION

I, <u>Chuene Dipuo Rebecca,</u> declare that the research report hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Horticulture) has not been submitted previously by me or anybody for a degree at this or any other University. In addition, this is my work in design and in execution, and related materials contained herein have been duly acknowledged.

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DEDICATION

I dedicate this mini-dissertation to my lovely kids (Mapula and Philan Chuene)

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- My partner Mr NI Nobela for his support, love and for highly valuing my dreams.

- Above all, I thank Almighty God for his grace and mercy. It is by his grace that I have managed to come this far. *Prov.3 verses 6: In all thy ways acknowledge him, and he shall direct thy paths.*

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ABSTRACT

Avocado fruit 'Hass' exocarp changes colour from green to purple and black during ripening. However, uniform purple or black exocarp colour is not achieved during ripening, leading to consumers' rejection of fruit for not meeting quality standards. Avocado 'Hass' fruit harvested early remain green or develop a multicoloured appearance, concurrently devaluing their commercial value; and, therefore, unattractive to consumers. Therefore, this study aimed to investigate the role of sucrose and calcium postharvest pulsing on early matured 'Hass' avocado exocarp colour change during ripening. In this study, early matured 'Hass' avocado fruit were harvested from Halls and Sons, Mataffin farm (25°25'39.13" S, 30°55'52.84" E), Nelspruit, South Africa with 10 cm pedicel at commercial dry matter content (22%). Thereafter, the fruit were transported to the University of Mpumalanga laboratory. In the laboratory, the study was divided into two experiments: Experiment 1 'Hass' fruit were continuously infused through the pedicel with different sucrose concentrations; 0 (control), 0.2 and 0.5 mM L^{-1}. Experiment 2 fruit were infused with different calcium chloride (CaCl$_2$) concentrations; 0 (control), 2 and 3 mM L^{-1}. In both experiments, treated and untreated fruit were stored at 5.5°C for 28 days. After removal from cold storage, fruit were ripened at room temperature (±25°C) and evaluated every other day for firmness, subjective colour (visual colour), objective colour parameters (lightness-L^*, chroma-C^* and hue angle-$h°$), external chilling injury and ripening percentage. The results showed that Su (0.2 mM L^{-1}) pulsing extended the ripening period by one day, corresponding with maintained fruit firmness. Furthermore, Su (0.2 mM L^{-1}) treated fruit reduced the 'Hass' avocado fruit chilling injury index (CII) during cold storage. With respect to colour change, Su (0.5 mM L^{-1}) treated fruit developed purple colour when compared with Su

(0.2 mM L^{-1}), which only developed to olive colour on the final ripening day (day 6). In addition, the results showed that all sucrose concentrations had a significant decreasing effect ($P < 0.05$) on objective colour parameters (L^*, C^* and $h°$) and increasing visual colour rating. With respect to CaCl$_2$ treatments, fruit firmness decline was significantly delayed, which resulted in extended ripening time. Moreover, CaCl$_2$ (2 and 3 mM L^{-1}) significantly decreased ($P < 0.05$) pericarp objective colour parameters (L^*, C^* and $h°$) and increased visual colour rating and developed purple colour on the final ripening day (days 6 and 8, respectively). In addition, pulsing with CaCl$_2$ (2 and 3 mM L^{-1}) concentration reduced chilling injury during storage compared with control fruit. In conclusion, the results of this study indicated that Su and CaCl$_2$ applied as postharvest treatments may contribute to avocado colour development by increasing anthocyanin accumulation. However, future research is required to investigate whether these treatments affect anthocyanin biosynthesis at the gene level.

Keywords: Calcium chloride, chilling injury, exocarp colour, 'Hass' avocado, sucrose

CHAPTER 01

GENERAL INTRODUCTION

1.1. Background

The 'Hass' avocado fruit is more widely sold and consumed when compared with other avocado cultivars. This is attributed to its attractive exocarp colours, nutritious content, medium-size and nutty taste (Wien *et al.*, 2013). A major distinguishing characteristic of the 'Hass' avocado cultivar is that its exocarp colour changes from emerald green to purple, then black as it ripens (Mathaba *et al.*, 2015). In general, the purchase decision for 'Hass' avocado fruit is mainly influenced by colour development during ripening (Donetti and Terry, 2012). Unfortunately, this ripening guide does not always meet the consumer's expectations. There has been inconsistent exocarp colour development of South African 'Hass' avocado fruit during ripening (Mathaba *et al.*, 2017). Importing countries and consumers have complained and questioned the quality of South African 'Hass' avocado fruit. These challenges can reduce market demand and revenue for the South African Avocado Industry (SAAI). Currently, the SAAI is investigating alternative postharvest treatments that can promote anthocyanin accumulation, ultimately improving 'Hass' avocado exocarp colour development.

According to Shikwambana *et al.* (2021), 'Hass' avocado fruit exocarp colour change results from increased anthocyanin biosynthesis and accumulation, particularly cyanidin 3-*O*-glucoside. The anthocyanin biosynthesis pathway and signalling molecules have been studied. Studies have reported that several signalling molecules can influence anthocyanin biosynthesis and accumulation, including sucrose and calcium (Teng *et al.*, 2005). In particular, sucrose has been reported to regulate anthocyanins biosynthesis and control the expression of related genes.

Solfanelli *et al.* (2006) found that exogenous sucrose treatment triggered an increase in anthocyanin synthesis, subsequently, improved colour development for *Arabidopsis thaliana* flower.

In plants, calcium (Ca) is a signal element that regulates physiological and biochemical processes (Gao *et al.*, 2019). A study by Solhjoo *et al.* (2017) demonstrated that preharvest spraying 'Red Delicious' apple tree with $CaCl_2$ (5 g L^{-1}) significantly increased fruit weight, sugar and anthocyanin concentrations. In 'Ruegen F7-4' strawberry, calcium application significantly increased total phenolics content, especially anthocyanin (Xu *et al.*, 2014). There is limited information on the role of sucrose and calcium postharvest treatment in improving colour development in 'Hass' avocado.

1.2. Problem statement

Avocado 'Hass' fruit is lucrative in South Africa; therefore, producing and supplying high-quality fruit is essential (Naamani, 2011). However, producers struggle to achieve promised-fruit-quality (PFQ) for the lucrative market. Recently, the early season 'Hass' avocado fruit have been showing desynchronization of fruit softening with exocarp colour development during ripening, a conundrum affecting promised-fruit-quality (PFQ) in South Africa (Mathaba *et al.*, 2015). In general, if PFQ is compromised, it negatively affects the industry's competitiveness in lucrative overseas markets. The exocarp colour change in 'Hass' avocado fruit occurs due to increased total anthocyanin, concomitantly decreasing chlorophyll content (Cox *et al.*, 2004; Donetti and Terry, 2012). Cox *et al.* (2004) reported that cyanidin 3-*O*-glucoside is the main anthocyanin responsible for the purple colour in 'Hass' avocado fruit, with the biosynthesis involving a calcium-sucrose complex (Mathaba

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et al., 2017). In general, early harvested avocado fruit are low in sugars and calcium, assumable, poor exocarp colour change (Mathaba *et al.*, 2015). Therefore, investigating and understanding the role of sucrose and calcium 'Hass' avocado fruit through postharvest pulsing treatment in order to improve exocarp colour change is crucial. In early season 'Hass' avocado fruit, improved exocarp colour change would provide an advantage in terms of quality as colour is used as a quality attribute (Pervaiz *et al.*, 2017). Furthermore, increased fruit quality will positively affect the fruit market locally and internationally.

1.3. Motivation for the study

The increased demand for avocado has led to increased cultivation area and supply (Naamani, 2011). The increasing 'Hass' avocado fruit supply has resulted in international buyers and consumers becoming more familiar with avocados; and, thus, more discerning regarding fruit quality (Arias Bustos, 2016). Many markets are already distinguishing between avocado fruit from different origins; and, therefore, pay low prices for fruits from countries with a poor quality reputation (Arias Bustos, 2016). The South African 'Hass' avocado fruit continues to show poor exocarp colour development, which compromises the quality in the international market (Mathaba *et al.*, 2015). Therefore, the current study would evaluate the potential use of postharvest sucrose and calcium pulsing treatment to enhance 'Hass' avocado fruit exocarp colour change during ripening. The results would also benefit avocado growers, distributors and ripeners by providing new scientific knowledge and new innovative techniques to improve Hass avocado fruit exocarp colour development during ripening.

1.4. Aim and objectives

1.4.1. Aim

The study aimed to investigate the effect of postharvest sucrose and calcium pulsing on early-season 'Hass' avocado fruit exocarp colour change during ripening.

1.4.2. Objectives of this study were to:

I. Determine whether sucrose pulsing would have an effect on early-season 'Hass' avocado fruit exocarp colour change during ripening.

II. Determine whether calcium pulsing would have an effect on early-season 'Hass' avocado fruit exocarp colour change during ripening

1.5. Hypotheses

I. Sucrose pulsing would have an effect on early-season 'Hass' avocado fruit exocarp colour change during ripening.

II. Calcium pulsing would have an effect on early-season 'Hass' avocado fruit exocarp colour change during ripening.

CHAPTER 02

LITERATURE REVIEW

2.1. Introduction

South African early harvested 'Hass' avocado fruit continue to show poor exocarp colour development during ripening, thereby compromising the promised quality (Mathaba *et al.*, 2017). In early-season 'Hass' avocado, improved exocarp colour change provides quality advantages and consumer acceptance (Rico-Londoño, 2021). Several pre- and postharvest factors were investigated to evaluate their effect on 'Hass' exocarp colour development (Mathaba *et al.*, 2015). The results showed that both pre- and postharvest factors such as production site (Tzaneen and Hazyview), orchard topography (lower and upper slopes), fruit tree canopy position (inside and outside canopy), fruit exocarp minerals and postharvest factors such as 1-methylcyclopropene (1-MCP) and ripening temperature have minimal influence on 'Hass' avocado fruit exocarp colour development during ripening (Mathaba *et al.*, 2015; Mathaba *et al.*, 2016; Mathaba *et al.*, 2017). These studies concluded that the exocarp colour of 'Hass' avocado fruit during ripening was a complex combination of physiological and environmental factors. In 'Hass' avocado fruit exocarp, cyanidin 3-*O*-glucoside is the main anthocyanin involved in purple colour change (Cox *et al.*, 2004). According to studies, cyanidin 3-*O*-glucoside synthesis involves a sucrose molecule and calcium complex (Teng *et al.*, 2005). In general, early harvest 'Hass' avocado fruit are low in sugars and calcium, assumable, poor exocarp colour change (Mathaba *et al.*, 2015). Therefore, this review aims to discuss the effects of sucrose and calcium treatment on exocarp colour development.

2.2. Exocarp colour

Colour is a key indicator of fruit quality and ripeness and a determinant of consumer acceptance and marketability (Hewett, 2006). According to Kassim *et al.* (2013), fruit colour is due to the distribution and concentration of exocarp pigments such as anthocyanins, chlorophyll, and carotenoids. Several factors affect pigment biosynthesis, including light, temperature, ethylene, and cultural practices (Downey *et al.*, 2006). In addition, Hass' avocado fruit colour development is associated with a decrease in chlorophyll content; concomitantly, cyanidin 3-*O*-glucoside concentration increases (Cox *et al.*, 2004). Accumulation of anthocyanin and degradation of chlorophyll is attributed to ripening temperatures and other external stimuli (Ashton *et al.*, 2006; Kassim *et al.*, 2013).

2.2.1. Colour biosynthesis and physiology

The chlorophyll content of 'Hass' avocado fruit degrades as it ripens (Cox *et al.*, 2004). Chlorophyll degradation has been suggested to have the following putative pathways. An early step of chlorophyll-a and chlorophyll-b degradation seems to be the removal of phytol to form a chlorophyllide by chlorophyllase (Lee and Min, 2010). Secondly, the chlide-a is removed from an Mg atom to form a pheophorbide-a (pheide) by Mg-dechelating substances (MDS), which are heat stable and low molecular weight compounds (Kunieda *et al.*, 2005; Suzuki *et al.*, 2005). Finally, pheide-a is degraded to fluorescent chlorophyll catabolites, which are primary colourless catabolites, via a red chlorophyll catabolite by both pheide and oxygenase and red chlorophyll catabolite reductase (Green *et al.*, 2003).

It has also been reported that 'Hass' avocado fruit exocarp contains carotenoids (β-carotene and all-trans-lutein) (Villa-Rodriguez *et al.*, 2020). Beta-carotene is the most common carotene that gives carrots, sweet potatoes and other vegetables their

orange colour (Kato *et al.*, 2004). In fruits and vegetables, β-carotene is found to be bound to either chlorophylls or xanthophylls, forming chlorophyll-carotenoid complexes, which absorb light in the orange or red light spectrum and give rise to green, purple, or blue coloration (Wieruszewski, 2002). Xanthophylls have one or more oxygen atoms; lutein is one of the most common xanthophylls (Fernandes *et al.*, 2018). Carotenoids such as the xanthophylls are involved in photosynthesis by participating in energy transfer in the presence of chlorophyll in plants (Thomas and Johnson, 2018).

In 'Hass' avocado fruit, chlorophyll and carotenoids degradation occur concomitantly with anthocyanin accumulation (Cox *et al.*, 2004). Anthocyanins are a diverse class of flavonoids composed of anthocyanidin backbone with sugar and acyl conjugates (Stommel *et al.*, 2009). Pelargonidin, cyanidin, and delphinidin are the primary anthocyanidins; they differ by the number of hydroxyl groups at their B-rings (Zhao *et al.*, 2014). Structural and regulatory genes primarily control anthocyanin biosynthesis (Xu *et al.*, 2014). Regulatory genes encode transcription factors that modulate structural gene expression (Gonzali *et al.*, 2009). The structural anthocyanin biosynthetic pathway genes function under the control of a regulatory complex called the MYB-bHLH-WD40 (MBW) complex, consisting of MYB, basic helix-loop-helix (bHLH) and WD40 repeat families (Chen *et al.*, 2019).

The anthocyanin biosynthesis pathway is an extension of the general flavonoid pathway, which starts with the chalcone synthase (CHS) mediated synthesis of naringenin chalcone from 4-coumaroyl-CoA and malonyl-CoA (Deng *et al.*, 2013). Thereafter, naringenin chalcone is isomerized by chalcone isomerase (CHI) to naringenin. Flavanone 3-hydroxylase (F3H) converts naringenin into dihydrokaempferol that can be further hydroxylated by flavonoid 3′-hydroxylase

(F3′H) or flavonoid 3′,5′-hydroxylase (F3′5′H) into two other dihydroflavonols, dihydroquercetin or dihydromyricetin, respectively. In addition, the three dihydroflavonols are converted into colourless leucoanthocyanidins by dihydroflavonol 4-reductase (DFR); subsequently, coloured anthocyanidins by anthocyanidin synthase (ANS). Finally, sugar molecules are attached to anthocyanidins by various members of the glycosyltransferase enzyme family. For instance, flavonoid 3-O-glucosyltransferase (UFGT) might be further acylated with aromatic acyl groups by acyltransferases. In general, chalcone synthase (CHS) is the initial key enzyme of flavonoid biosynthesis. Whereas flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′,5′-hydroxylase (F3′5′H) are the primary enzymes responsible for the diversification of anthocyanins by determining their B-ring hydroxylation pattern; and consequently, colour (Tanaka and Brugliera, 2013).

2.2.2. Chilling injury and its effect on colour development

Chilling injury (CI) is a physiological dysfunction that occurs when tropical and subtropical crops are exposed to low or non-freezing temperatures below a critical level (Woolf *et al.*, 2003). Symptoms appear as mesocarp discolouration, surface darkening, and pitting (Pesis *et al.*, 2002). However, symptoms severity has been shown to depend on fruit maturity, cultivar susceptibility and cold storage duration (Dixon *et al.*, 2008). Mathaba *et al.* (2016) found that early-season 'Hass' avocado fruit were highly susceptible to CI compared with late season. In addition, 'Hass' avocado fruit harvested during February were highly susceptible to CI when compared with fruit harvested during October or December after storage at 2°C for 6 weeks (Dixon *et al.*, 2008). Furthermore, storage at 3°C or below induced CI in 'Hass' avocado fruit (Woolf *et al.*, 2003). Lütge *et al.* (2010) investigated ultra-low temperature shipping and cold chain management of 'Fuerte' avocado grown in the

KwaZulu-Natal midlands. The study revealed that exposing 'Fuerte' avocado fruit to 2 and 5.5°C storage temperature for 28 days resulted in internal and external CI development.

Studies have reported that chilling injury stress alters membrane fluidity by oxidative stress, inducing peroxidation and membrane lipid breakdown (Kong *et al.*, 2018). It also causes plant metabolic changes by activation of signal transduction that regulates stress-responsive genes such as lipoxygenase (LOX), fatty-acid desaturase (FAD) and heat shock proteins (HSPs) to cope with stress (Lukatkin *et al.*, 2012). The major negative effect of cold stress is inducing severe membrane damage, ;altering the photosynthetic electron transfer machinery, and generating oxidative stress (Janská *et al.*, 2010).

In 'Hass' avocado fruit, the development of CI symptoms resulted in variable exocarp colouration during ripening (Mathaba *et al.*, 2015). Mathaba *et al.* (2015) further showed that 'Hass' avocado fruit harvested in the early season had higher CI symptoms, exhibiting poor exocarp colour development during ripening. However, cold stress is an important factor in inducing pigments that act as antioxidants. Carotenoids biosynthesis increased during cold stress of 'Baladi' lemon fruit and showed an increased yellow colouration (Lo'ay and Dawoods, 2019). Moreover, anthocyanin accumulation in '*Fragaria*' strawberry (Zhang *et al.*, 2018), 'Vinhao' grape (Zhang *et al.*, 2015) and 'David' peach (Leng and Qi, 2003) resulted from cold stress.

Anthocyanins are synthesised from phenylalanine ammonia lyase (PAL), which is the first enzyme of the polyphenol biosynthesis pathways (Jeong *et al.*, 2016). In general, phenylalanine ammonia lyase (PAL) expression and activity has already

been associated with chilling tolerance in several fruit species like tomato (*Solanum lycopersicum*) and watermelon (*Citrullus lanatus*) (Rivero *et al.*, 2001). In 'Fortune' mandarin fruit peel, PAL induction of expression was observed under cold stress and attributed to a rapid adaptive response of the tissue to low temperature (Sanchez-Ballesta *et al.*, 2000). The expressions of anthocyanin biosynthetic genes; C4H, F3H, DFR, ANS, and UFGT, were all enhanced under chilling in purple kale (*Brassica Oleracea*) (Zhang *et al.*, 2012). Under low temperatures, MdMYBA binds specifically to the ANS and activates anthocyanin accumulation in 'Tsugaru' apple skin (Ban *et al.*, 2007).

It has been demonstrated that chilling stress can also impair chlorophyll biosynthesis (Zhao *et al.*, 2020). Chlorophyll-a and chlorophyll-b (green pigments) are accumulated in the chloroplasts and mediate photosynthetic light reactions. Both chlorophylls are degraded by oxidative stress. It was found that chlorophyll-a is more easily degraded by oxidative stress than chlorophyll-b in 72 rice (*Oryza sativa*) cultivars (Kasajima, 2017). The transcription gene levels associated with chlorophyll biosynthesis, photosynthesis and chloroplast development were altered by chilling in rice (*Oryza sativa*) (Gong *et al.*, 2014; Wu *et al.*, 2016). In 'Baladi' lemon fruit, chlorophyll pigment degradation occurred under cold stress, and carotenoids synthesis increased (Lo'ay and Dawoods, 2019).

Carotenoids are antioxidants that confer higher CI tolerance for many fruits and vegetables (Rey *et al.*, 2020). Oxidative stress is a secondary response of plant cells to CI caused by an increase in reactive oxygen species (ROS) production due to low-temperature stress and the inability of the plant to counteract this proliferation of ROS (Lado *et al.*, 2019). Among carotenoids, lycopene is reported as an efficient singlet oxygen quencher (Cantrell *et al.*, 2003). Accumulation of this carotenoid in

the flavedo of CI-tolerant red grapefruit (*Citrus paradisi* Macf) was associated with lower oxidative damage (membrane structure and lipid peroxidation), higher singlet oxygen scavenging capacity (SOAC), and higher catalase activity (Lado *et al.*, 2016). Grapefruit (*Citrus paradisi* Macf), white cultivars accumulating low peel carotenoid levels were more prone to CI development than red grapefruit (*Citrus paradisi* Macf) with moderate to high levels (Lado *et al.*, 2015).

2.2.3. De-synchronisation of colour development with firmness during ripening

Fruit ripening is a highly coordinated developmental process regulated by several genes and hormones (Klee and Giovannoni, 2011). The main changes associated with ripening include colour (loss of green colour and increase in non-photosynthetic pigments that vary depending on species and cultivar) and firmness (softening by cell wall degrading activities and alterations in cuticle properties) (Giovannoni, 2004). During ripening of climacteric fruits, there is an increase in ethylene production (Zhang *et al.*, 2018). Production of ethylene regulates enzymes associated with colour pigments while triggering the enzymes associated with cell membrane catabolism in the cell wall (Tucker *et al.*, 2017). As a result, the fruit develops colour while softening (Meyer *et al.*, 2017). However, in the case of 'Hass' avocado, it appears as if there is no association between these two processes (Mathaba *et al.*, 2015).

During ripening, the exocarp colour of 'Hass' avocado changes from green to purple and black (Cox *et al.*, 2004) and shows a reduced firmness (Sierra *et al.*, 2019). Moreover, Cai *et al.* (2006) observed changes in chlorophyll concentrations in relation to fruit firmness. However, early-season 'Hass' avocado fruit shows a desynchronized firmness decline with exocarp colour in South Africa (Mathaba *et al.*, 2015). An increase in ethylene resulted in increased cell wall degrading enzymes

such as pectinase, cellulase, and polygalacturonase (PG), leading to the disintegration of cell wall structures in 'Hass' avocado fruit (Sierra *et al.*, 2019). In 'Toyonoka' strawberries, ethylene up-regulated several genes involved in cell wall modification, such as FaPG1 (*Fragaria x ananassa* polygalacturonase1), FaGal1 (*Fragaria x ananassa* Galacturonase1) and FaGal2 (*Fragaria x ananassa* Galacturonase2) (Villarreal *et al.*, 2016). A study on 'Hongyang' kiwifruit softening showed that ABA could activate the onset of fruit softening and accelerate ethylene synthesis to accelerate softening (Gan *et al.*, 2020). Abscisic acid treatment has been found to stimulate the expression of ACS and ACO in 'Alisa Craig' tomato fruit (Zhang *et al.*, 2009), thus promoting ACC conversion to ethylene.

2.3.   Sucrose and calcium treatments for colour development

2.3.1. Sucrose mechanism – association with biosynthesis of pigments (for colour development)
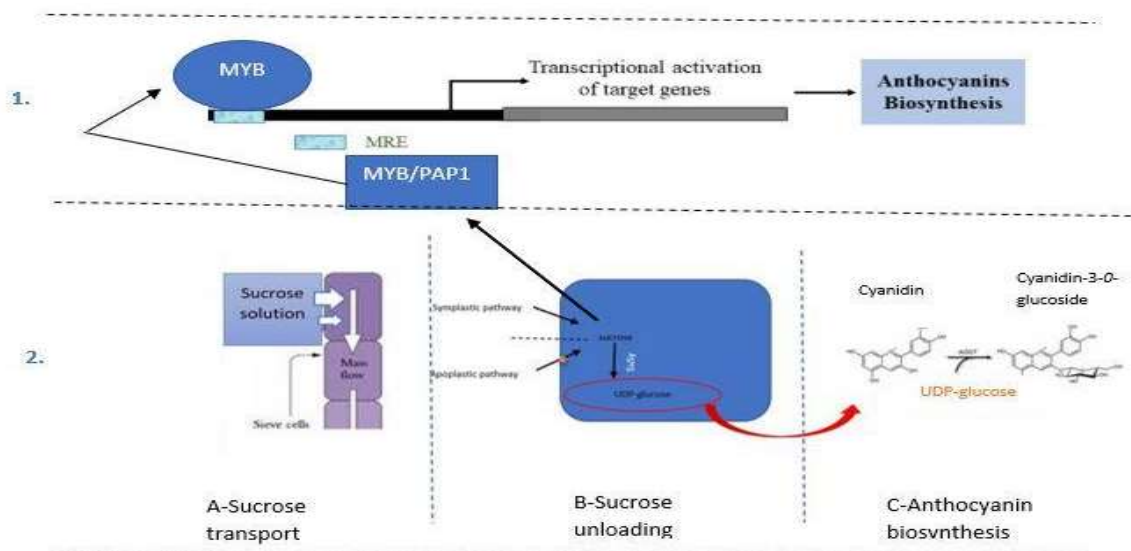

Figure 2.1: Physiological mechanism of sucrose in anthocyanin production model adapted from (Lemoine, 2000; Liu *et al.*, 2012; Janse van Rensburg and Van den ende, 2018)

Figure 2.1. shows the physiological mechanism of sucrose triggering anthocyanin biosynthesis. Initially, sucrose solution (**A**) is translocated in the pedicel phloem of the fruit through mass flow. Secondly, phloem unloading (**B**) occurs, whereby, sucrose influx occurs via symplastic and apoplastic pathways. Furthermore, sucrose is unloaded in the cytoplasm and acts as a signalling molecule (**B**). Therefore, sucrose is converted to a UDP-glucose molecule with the aid of sucrose synthase enzyme. Concurrently, it activates the transcription factor, MYB. MYB is recognised by MRE (MYB recognition elements), which then binds to the target gene's promoter. The transcriptional activation of target genes promotes anthocyanin biosynthesis. Finally, the resulted UDP-glucose reacts with the non-colourful anthocyanidin (cyanidin) to form the purple pigment, cyanidin 3-*O*-glucoside.

In plants, sucrose moves from the source (leaves) to the fruit through the phloem to the inner exocarp, where unloading occurs (Aoki *et al.*, 2012). The sucrose transport can either be apoplastic or symplastic (Hocking *et al.*, 2016). With the apoplastic transport, sucrose is transported into the cell by sucrose transporters (SUTs) and through active diffusion with the symplastic pathway (Zanon *et al.*, 2015). The sucrose synthase enzyme converts unloaded sucrose to Uridine diphosphate glucose (UDP-glucose) in the cytoplasm (Park *et al.*, 2010). The enzyme usually resides in the cytoplasm of both photosynthetic and non-photosynthetic cells (Bieniawska, 2007). In '*Fragaria*' strawberry, UDP-glucose has been shown to function in anthocyanin biosynthesis. Anthocyanidin (cyanidin) reacts with UDP-glucose to produce cyanidin 3-*O*-glucoside, a pigment responsible for the purple colour (Shi *et al.*, 2021).

Sucrose has been shown to increase MYB75 levels, a transcription factor required for DFR expression in 'Mirage rose' petunia (Ai *et al.*, 2016). According

to Solfanelli *et al.* (2006), a sucrose-specific signalling pathway activates the transcription factor, PAP1. In the *Arabidopsis thaliana* plant, PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1) has been reported to activate the phenylpropanoid pathway (Borevitz *et al.*, 2000). Zvi *et al.* (2012) found that anthocyanin production in 'Pariser Charme' rose was enhanced by PAP1 transcription. In the 'Xanthi' tobacco plant, Xie *et al.* (2006) found that the PAP1 overexpression led to an increase in anthocyanin in the leaves, flowers, stems, and roots. Similarly, Rowan *et al.* (2009) found that PAP1 overexpression in 'Mirage rose' petunia flowers was associated with increased anthocyanin levels.

Exogenous sucrose application increased PAP1 level and anthocyanin biosynthetic genes such as dihydroflavonol reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) in *Arabidopsis thaliana* seedling (Solfanelli *et al.*, 2006). Moreover, the expression of structural genes, including phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS), DFR and, UFGT, were associated with sucrose application in 'Purple Majesty' potato (Payyavula *et al.*, 2013). Similarly, exogenous sucrose and endogenous sugar accumulation have been shown to increase the expression of early and late anthocyanin biosynthesis genes, including CHS, DFR, and anthocyanidin synthase (ANS) in 'Vinhao' grape, 'Mirage rose' petunia and 'Comet' radish (Hara *et al.*, 2004; Ai *et al.*, 2016). Previously, studies have demonstrated that 7% sucrose can induce anthocyanin in 'Kauai Rose' torenia shoots by degrading chlorophyll contents, whereas anthocyanin induction did not occur when the shoots were retransferred to 1.5% sucrose (Naing *et al.*, 2021).

2.3.2. Calcium mechanism association with biosynthesis of pigments (for colour development)
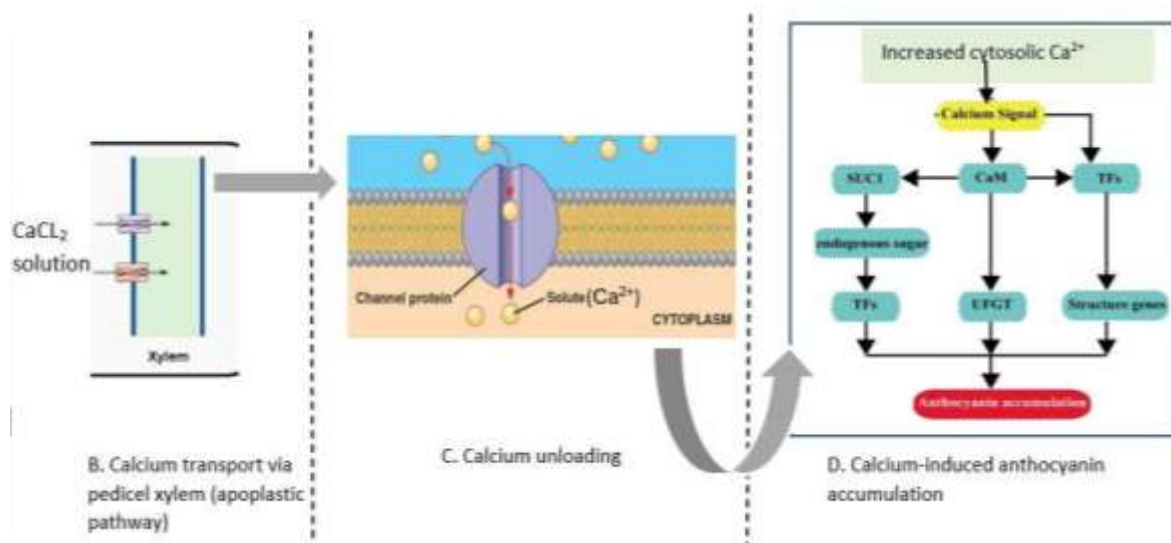
Figure 2.2: Physiological mechanism of calcium in anthocyanin production. Model adapted from (Yu *et al.*, 2020; Yang and Jie, 2005)

Figure 2.2 shows the physiological mechanism of calcium triggering anthocyanin biosynthesis. Calcium solution (**A**) is translocated through the pedicel into the fruit, where unloading occurs. Calcium influx into the cell occurs apoplectically, via channel proteins (**B**). Furthermore, calcium influx increases the cytosolic calcium (**C**). The increased level of Ca^{2+} is recognised by some calcium-sensors or calcium-binding proteins such as calmodulin (CaM). CaM activates anthocyanin transcriptional factors, anthocyanin biosynthetic gene and sucrose transporter (SUC1), resulting in increased endogenous sugar that activates the anthocyanin transcription factors, therefore increasing anthocyanin accumulation.

In general, fruits are architecturally isolated organs connected to trees by pedicel/peduncle, where sap carries various nutrients and is fed to fruit via xylem and phloem (Song *et al.*, 2018). Generally, calcium transport is believed to be

exclusively through the xylem or apoplast pathway (Yang and Jie, 2005). However, calcium is relatively immobile in the phloem or symplastic pathway (Hocking *et al.*, 2016). Therefore, calcium (Ca) is taken up by the pedicel and transported to the fruit in a mainly apoplastic way to avoid interference with its function as a secondary messenger (Hocking *et al.*, 2016). The Ca molecule moves through the channel protein into the cytoplasm (Gilliham *et al.*, 2011). Calcium transport into the cytoplasm results in increased cytoplasmic calcium concentration. Therefore, cytosolic calcium ion concentration changes play a key second messenger role in signal transduction (Rudd and Franklin-Tong, 1999). The increased Ca^{2+} level is recognised by some calcium-sensors or calcium-binding proteins such as calmodulin (Tuteja and Mahajan, 2007).

Moreover, calmodulin binds with calcium to activate several protein kinases, such as calcium-dependent protein kinases (CDPKs) (Schulz, 2013). Numerous CDPKs families have been characterised in crops such as *Arabidopsis thaliana*, rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) (Asano *et al.*, 2005; Kong *et al.*, 2013). Calmodulin-binding protein60 (CBP60s) was the first CDPK type identified in maize (*Zea mays*). It was reported to be responsible for regulating PAP1 transcription factor expression and controlling anthocyanin biosynthetic genes (Asano *et al.*, 2005).

According to Tuteja and Mahajan (2007), CDPKs regulate the function of many genes like anthocyanin biosynthetic genes. In the 'Vinhao' grape cultivar, anthocyanin biosynthetic gene expression, DFR and UFGT were enhanced by calcium treatment (10 mM $CaCl_2$) (Martins *et al.*, 2018). The expression of anthocyanin biosynthetic genes, DFR, ANS and UFGT, were stimulated by calcium treatment in 'Ruegen F7-4' red fruit-bearing strawberry inbred line (Xu *et al.*, 2014).

Shin *et al.* (2013) found that calcium-induced *Arabidopsis thaliana* anthocyanin accumulation was reduced by adding calcium antagonist (endomembrane Ca^{2+} channel blocker) into the Murashige and Skoog (MS) growth medium, showing that calcium is required for anthocyanin production.

In the findings of Sudha and Ravishankar (2003), calcium applied to carrots (*Daucus carota*) callus cultures enhanced anthocyanin levels. Furthermore, exogenous Ca^{2+} addition has been shown to play a role in hydrogen-rich water (HRW)-regulated anthocyanin biosynthesis in 'Comet' radish sprouts hypocotyls (Zhang *et al.*, 2018). Solhjoo *et al.* (2017) showed that spraying with $CaCl_2$ (5 g L^{-1}) in 'Red Delicious' apples significantly increased anthocyanin concentrations. In 'Ruegen F7-4' strawberry, calcium application boosted the total phenolics content, especially anthocyanin (Xu *et al.*, 2014). In *Arabidopsis thaliana*, change in endogenous Ca^{2+} levels modulated sucrose-induced sugar uptake, which in turn regulated anthocyanin accumulation (Shin *et al.*, 2013). Similarly, Zhi *et al.* (2017) found that immersing 'Dongzao' jujube fruits with 10 g L^{-1} $CaCl_2$ increased their anthocyanin content.

2.4.   Effect of sucrose and calcium treatment on mitigation of chilling injury

Sucrose

According to Caverzan *et al.* (2016), low temperature induces reactive oxygen species (ROS) accumulation, including superoxide radical and hydrogen peroxide. To alleviate the toxic effects of the overproduced reactive oxygen species (ROS), plants have evolved a complex antioxidant system (Gill *et al.*, 2010). Sucrose has been shown to reduce spinach leaves (*Spinacia oleracea* cv. Amaranthus) chilling injury during cold storage by improving the proline content (Karim and Yusof, 2021).

Proline acts as an Osmo protectant, and stabilises proteins and scavenger free radicals, including hydroxyl radicals (Kaur and Asthir, 2015). Additionally, proline has been shown to buffer in cellular redox potential and as a major constituent of cell wall structural proteins, which may provide mechanical support for cells (Ozden *et al.*, 2009).

According to Lee *et al.* (2016), sucrose regulates the production of other antioxidants such as anthocyanin in *Arabidopsis thaliana*. Anthocyanin is a phenolic compound synthesised through phenylalanine as a precursor and phenylalanine ammonia-lyase (PAL) as a rate-limiting enzyme (Vogt, 2010). It has been suggested that PAL is related to the increase in secondary metabolites within buckwheat sprouts (*Fagopyrum esculentum* M.) treated with an elicitor as sucrose (Jeong *et al.*, 2016). Similarly, sucrose improved antioxidant activities in 'Youxiu' broccoli and reduced the chilling injury (Xu *et al.*, 2016). In *Arabidopsis thaliana*, treatment with sucrose reduced ROS stress during extended cold temperatures (Rosenwasser *et al.*, 2011).

According to Uemura and Steponkus (2003), sucrose may function directly as a protective agent at higher concentrations and serve as substrates or signals for stress-induced alterations at low concentrations in *Arabidopsis thaliana*. Contrarily, high sucrose concentration results in increased ethylene production in 'Hongyang' kiwifruit (Fei *et al.*, 2020). In Pesis *et al.*'s (2002) study, chilling injury symptoms, expressed as mesocarp discoloration, resulted in ethylene biosynthesis in the tissue under a low-temperature environment of 5°C in 'Ettinger' avocado fruit. Furthermore, treatment with the ethylene action inhibitor 1-methylcyclopropene (1-MCP), either before or after cold storage reduced fruit firmness loss associated with the development of CI injury symptoms in 'Rojo Brillante' persimmon (*Diospyros kaki* L.) (Orihuel-Iranzo *et al.*, 2010). Yu *et al.* (2017) demonstrated that two varieties of

'Zajiao' and 'Yulu' peach fruit treated with 1-MCP exhibited higher chilling tolerance than the untreated control, which was mainly due to that 1-MCP suppressed sucrose degradation, leading to alleviate ROS production.

Calcium

In plants, calcium plays a role as second messenger signal transduction involved in physiological processes and the responses to various stresses (Tuteja and Mahajan, 2007). Under cold stress, induced transient elevations cytosolic calcium is sensed by different calcium binding proteins, consequently, initiating various physiological responses in the cell (Sanders *et al.*, 2002). In 'Hass' avocado fruit stored at 5.5°C, calcium application resulted in reduced ethylene production and chilling injury symptoms (Bill *et al.*, 2014). Li *et al.* (2020) suggested that postharvest Ca^{2+} application significantly increased Ca^{2+} and calmodulin content; and concomitantly, endogenous Gamma-aminobutyric acid (GABA) content in 'Nanguo' pear fruit, which in turn delayed fruit browning after low-temperature storage. In plant tissues, GABA is an acid that accumulates rapidly in response to biotic or abiotic stress and acts as an intracellular signalling molecule (Roberts, 2007).

Recent studies have reported that calcium chloride ($CaCl_2$) application can trigger antioxidant system activity and maintain ROS homeostasis to increase cold tolerance of postharvest vegetables and fruits (Hou *et al.*, 2021). For instance, in 'Nanguo' pear fruit $CaCl_2$ application effectively alleviated peel browning caused by chilling injury on account of membrane lipid peroxidation inhibition and a higher activity and expression of superoxide dismutase (SOD) (Zhang *et al.*, 2019). Wei and Zhao (2020) also found that $CaCl_2$ treatment could alleviate chilling injury symptoms

in winter 'Dongzao' jujube fruit by promoting the SOD, CAT, and peroxidase (POD) activities to scavenge ROS. In green peppers (*Capsicum annuum L.*), $CaCl_2$ treatment significantly suppressed ROS levels by regulating activities of SOD, POD, and CAT, consequently, enhanced chilling tolerance (Zhang *et al.*, 2021).

2.5. Research gap

Avocado fruit 'Hass' is characterised by a change in exocarp colour from emerald green to purple during ripening. However, poor exocarp colour change has been evidenced in the South African early season 'Hass' avocado (Mathaba *et al.*, 2015). The early season 'Hass' avocado fruit have been showing desynchronization of fruit softening with exocarp colour development during ripening, a conundrum affecting promised-fruit-quality. Studies were conducted to investigate the contributing factors. To date, it has been concluded that the environment had a minimal impact on how colour developed in 'Hass' avocado fruit (Mathaba *et al.*, 2015, Mathaba *et al.*, 2016). Subsequently, the purple colour development is dependent on anthocyanin accumulation (Cox *et al.*, 2004). Researchers have shown that colour change and anthocyanin accumulation can be regulated by calcium and sucrose in other crops like strawberry, grapes, and *Arabidopsis thaliana* (Gollop, 2002; Shin *et al.*, 2013; Xu *et al.*, 2014). However, there is no information as to whether calcium and sucrose can induce colour change in 'Hass' avocado fruit.

CHAPTER 03

THE EFFECT OF POSTHARVEST SUCROSE-PULSING ON EARLY SEASON

'HASS' AVOCADO FRUIT EXOCARP COLOUR CHANGE DURING RIPENING

3.1. Introduction

Sugars are important for various aspects of plant development. Besides providing energy and carbon structural components for plant growth, sugars also function as signalling molecules in many developmental processes (Granot *et al.* 2013). Increasing evidence indicate that the role of sucrose as a signalling regulator deserves to be recognized (Ruan *et al.* 2012). As a postharvest treatment, sucrose has been applied to various fruits and found to enhance different quality parameters such as colour (Li *et al.*, 2019). However, there is no empirical evidence on sucrose use to enhance 'Hass' avocado fruit exocarp colour change during ripening. Therefore, the purpose of this study was to determine sucrose effect as a post-harvest treatment on early season 'Hass' avocado fruit exocarp colour change during ripening.

3.2. Materials and Methods

3.2.1. Experimental sites

Early matured 'Hass' avocado fruit were harvested with 10 cm pedicel at commercial dry matter content (22%) from Halls and Sons, Mataffin farm (25°25'39.13" S, 30°55'52.84" E), Nelspruit, South Africa. The farm receives an annual rainfall of <1000 mm, with maximum (28°C) and minimum temperatures (15°C) and soils are classified as sandy soils. Harvested fruit with pedicels were carefully transported at

ambient temperature to the University of Mpumalanga postharvest laboratory (25.4371°S, 30.9818°E) for treatment, storage, and analysis.

3.2.2. Experimental procedures and design

The experiment was conducted as a completely randomised design with three replications. The treatments were two concentrations of sucrose namely, 0.2 and 0.5 mM L^{-1}. In the laboratory, 10 cm fruit pedicels were re-cut to 5 cm in length and 15 mL silicon tubes were placed on the apex of the fruit. Bostik Prestik and Vaseline were applied to prevent leakages (Figure 3.1). Thereafter, fruit with silicon tubes were pulsed with 0.2 and 0.5 mM L^{-1} sucrose solution and the control fruit were not pulsed. Subsequently, fruit were packed into commercial carton boxes each containing 12 fruit and stored at 5.5°C for 28 days. After 28 days of cold storage, fruit were ripened at 21°C.

A. Insertion of pedicel into test tubes
B. Sealing of the test tubes
C. Application of treatment solution

Figure 3.1: Showing procedures applied for the infusion of the treatment solutions

3.2.3. Data collection

Fruit were assessed every other day (0, 2, 4, 6 and 8 days) during ripening at 25°C until 'eating ripe' firmness was reached. At each interval, 12 fruit were sampled and

examined for firmness, subjective and objective colour parameters and external chilling injury during ripening.

Determination of fruit firmness

As described by Kohne *et al.* (1998), fruit firmness was measured by a non-destructive method using a hand-held densimeter (Model: 53524, Bareiss, Oberdischingen, Germany) with a 5 mm tip was used to measure fruit firmness on a scale of 85-90 DM (hard, unripe; $\approx$ 8.06 N) to <60 DM (soft, ready to eat; $\approx$ 5.05 N) densimeter units. Each fruit was measured three times around the circumference and the average reading was recorded (Figure 3.2). The final firmness value was expressed in newton (N).

Figure 3.2: Measuring the firmness of the 'Hass' avocado fruit

Determination of exocarp colour change

Visual colour of the fruit was measured subjectively using exocarp colour rating scale [emerald-green (1), forest-green (2), olive (3), purple (4) and black (5)] as previously described by Mathaba *et al.* (2015). Chromameter (Minolta CR-400 Corp, Ramsey, NJ, USA) with 8 mm diameter light path aperture was used to measure objective exocarp colour change (Figure 3.3). The device was calibrated before use with a Minolta standard white tile. Each fruit was measured in three parts around the equatorial region. By averaging three measurements of the fruit equatorial region, three colour parameters were measured, *L** value (lightness or brightness), a* (redness or greenness), and b* (yellowness or blueness). The value of hue angle (h^o) and chroma (*C**) were calculated from a* and b* using the formula: 180 + [tan (a*/b*)] and $C\mathord{*}= (a\mathord{*}{}^2 + b\mathord{*}{}^2)^{1/2}$ as previously described by McGuire (1992).

Figure 3.3: Measuring the objective colour parameters of the 'Hass' avocado fruit

Determining ripening percentage

The ripening percentage was calculated as the number of fruits that reached the 'eating soft' stage, which corresponded to an average densimeter reading of less than 60 DM (5.05 N) during shelf-life. The ripening percentage was calculated as follows

Ripening percentage (%) = ($\sum R_f /T_f$) x 100

Where, R_f = ripe fruit, T_f = total number of fruits evaluated

Determination of external chilling injury

Exocarp spotting (lenticel damage) and discrete patches (external chilling injury) on 'Hass' avocado fruit during ripening were visually assessed using a scale of 0 to 3 and was expressed in percentage whereby 0 = 0% (indicating no chilling symptoms), 1 = 10%, 2 = 25% and 3 = 50% (Indicating severe chilling symptoms) according to the International Avocado Quality Manual (White *et al.*, 2007).

Chilling injury index (CII) = $\sum$ (CI level × number of fruits with chilling injury symptoms) ÷ total number of fruits evaluated.

3.2.4. Data analysis

The data were subjected to analysis of variance (ANOVA) using a windows software GenStat® version 16th (VSN International, Hemel Hempstead, UK). Means were separated using Duncan Multiple Range Test at a 5% level of significance.

3.3. Results and Discussion

Fruit firmness

In this study, the treatments had a highly significant effect ($P < 0.001$) on early season 'Hass' avocado fruit firmness during ripening (Figure 3.4). In general, early season 'Hass' avocado fruit firmness decreased with ripening time (Figure 3.4). However, firmness decreasing trend was rapid in control when compared with Su (0.5 mM L^{-1}) and Su (0.2 mM L^{-1}) treatments. According to Xanthopoulos (2017), most fruits lose water through the transpiration process during storage and ripening. Ultimately, water loss can elicit senescence responses such as ethylene production and membrane permeability changes (Blakey *et al.*, 2009). Lallum *et al.* (2004) showed that 'Hass' avocado fruit moisture loss increased ethylene accumulation. Moreover, a decrease in fruit moisture has been shown to contribute to firmness reduction in 'Centurion' rabbiteye blueberries (Allan-Wojtas *et al.*, 2001). Therefore, we assume that the increased softening in control fruit may be attributed to increased water loss during ripening contrary to sucrose treatments.

In fruit treated with Su (0.2 mM L^{-1}), a delayed in firmness reduction was observed, thereby, resulting in extended ripening time (Figure 3.4). Exogenous sucrose application has been shown to increase the expression of two ethylene biosynthesis genes; DcACS1 and DcACO1 in detached 'Hongyang' kiwifruit. Furthermore, sucrose was able to enhance the expression of ethylene receptor (ETR) genes, *Solanum lycopersicum* Ethylene Receptor 3 (SlETR3) and *Solanum lycopersicum* Ethylene Receptor 4 (SlETR4) and ethylene signalling in 'Fenniang' tomato fruit (Li *et al.*, 2016). Therefore, rapid firmness loss with the postharvest sucrose (0.5 mM L^{-}

[1]) treatment application may be ascribed to high sucrose content effect on ethylene synthesis resulting in fruit softening.
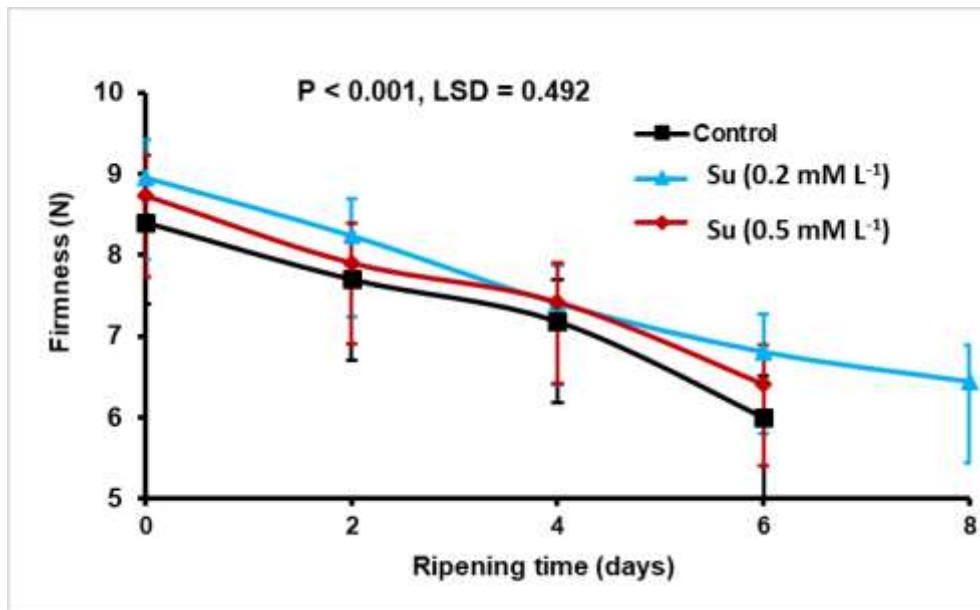
Figure 3.4: Effect of sucrose infusion through pedicel on the firmness of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ± SE of means at $P \leq 0.05$

Exocarp subjective and objective colour change

Avocado fruit 'Hass' is characterised by a change in exocarp colour from green to purple/black as the fruit ripens (Mathaba *et al.*, 2015). In this study, there was a significant difference ($P < 0.001$) in visual colour due to studied treatments during ripening (Figure 3.5a). As shown in Figure 3.5a, visual colour increased throughout ripening irrespective of treatment. Control fruit showed higher visual colour throughout ripening time followed by Su (0.5 mM L^{-1}). Moreover, 'Hass' avocado fruit treated with Su (0.2 mM L^{-1}) changed to olive green ≈ 3.67 visual colour after day 8.

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Nonetheless, no significant difference visual colour rating was observed between sucrose treatments during ripening, both treatments developed olive exocarp colour (rating 3) (Figure 3.5 and Plate 3.1).

In terms of objective exocarp colour change, treatments showed a significant difference for L^*, C^* and $h°$ (P < 0.001) during ripening (Figure 3.5b-d). The objective exocarp colour parameters (L^*, C^* and $h°$) decreased for all the treatments during ripening. However, Su (0.2 mM L^{-1}) pulsed fruit showed higher L^* values when compared with Su (0.5 mM L^{-1}) treated and control fruit from day 0-6. Higher L^* values for fruit pulsed with Su (0.2 mM L^{-1}) corresponded to green colour maintenance and delayed firmness decline. An increase in exocarp colour correlated negatively and significantly with firmness of control (r= -0.682), Su (0.5 mM L^{-1}) (r= -0.878) and Su (0.2 mM L^{-1}) (r = -0.712) (Table 3.1). Chromaticity colour parameters were non-significant (P > 0.05) and positively correlated with firmness regardless of treatment (Table 3.1). Naing *et al.* (2021) demonstrated that 7% sucrose can induce anthocyanin in torenia shoots by degrading chlorophyll contents, whereas anthocyanin induction did not occur when the shoots were retransferred to 1.5% sucrose (Naing *et al.*, 2021).
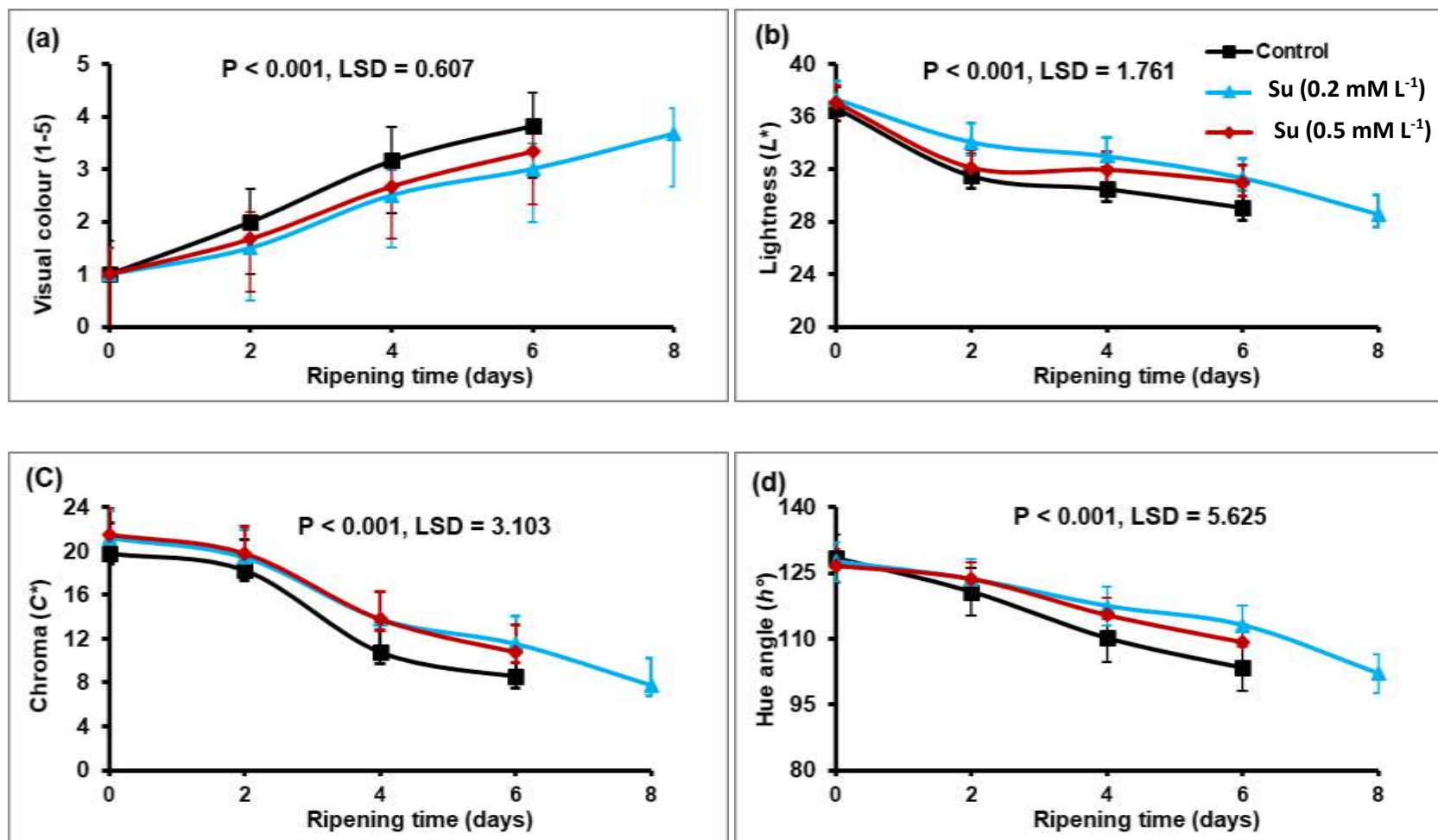
Figure 3.5: Effect of sucrose infusion through pedicel on subjective (visual colour) (a) and chromaticity parameters (L^* -Lightness, C^*- Chroma and $h°$- Hue angle), (b), (c) and (d), respectively) of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ±SE of means at $P \leq 0.05$

	Day 0	**Day 2**	**Day 4**	**Day 6**	**Day 8**
Control					
Su (0.2 mM					
Su (0.5 mM					

Plate 3.1: Visual colour change for control and sucrose treatments during ripening

Table 3.1: Pearson correlation coefficient between objective colour parameters (L^*, C^* and h°) and subjective (visual colour) of 'Hass' avocado fruit exocarp colour measurement/firmness during ripening as influenced by control, Su (0.2 mM L^{-1}) and Su (0.5 mM L^{-1}) treatment.

	Control	Su (0.2 mM L^{-1})	Su (0.5 mM L^{-1})
Correlations		R^2	
Firmness × visual colour	-0.682***	-0.878***	-0.712**
Firmness × L^*	0.722^{ns}	0.832^{ns}	0.687^{ns}
Firmness × C^*	0.663^{ns}	0.854^{ns}	0.651^{ns}
Firmness × h^o	0.732^{ns}	0.818^{ns}	0.667^{ns}
Visual colour × L^*	-0.588***	-0.887***	-0.676***
Visual colour × C^*	-0.744***	-0.895***	-0.893***
Visual colour × h^o	-0.903***	-0.851***	-0.899***

*Represent significant difference at *P < 0.05, **P < 0.01, ***P < 0.001 and ns = not significant.

Ripening percentage

In avocado fruit, the ripening process occurs as a result of a decrease in membrane integrity and an increase in respiration rate and ethylene production within 3-4 days of shelf-life depending on temperature (Donetti and Terry2012). In this study, treatments had a significant effect (P < 0.05) on ripening percentage throughout the ripening period (Figure 3.6). There was an increase in ripening percentage with ripening days, irrespective of the treatments. These findings were consistent with the

finding of Sibuyi (2018), whereby, ripening percentage increased in 'Hass' avocado fruit, irrespective of treatments. However, control exhibited higher ripening percentage throughout the ripening period when compared with sucrose treatments (Figure 3.6). During ripening, fruit lose water through transpiration (Díaz-Pérez, 2019). Ethylene has been shown to be synthesised in response to water stress in various plant tissues including avocado fruit (Lallum *et al*, 2004). Supposedly, control fruit showed higher ripening percentage due to water stress-induced ethylene production. To substantiate this assumption, fruit pulsed with sucrose solution exhibited lower ripening percentage compared with control (Figure 3.6) confirming that pulsing treatment reduced fruit water loss as a result delayed fruit ripening.

In terms of sucrose treatments, Su (0.2 mM L^{-1}) and Su (0.5 mM L^{-1}) showed lower ripening percentage when compared with control throughout ripening days (Figure 3.6). Conversely, Su (0.5 mM L^{-1}) treated fruit showed increased ripening percentage when compared with Su (0.2 mM L^{-1}) throughout ripening (Figure 3.6). In 'Sweet Charlie' strawberry fruit, sucrose treatment improved ripening through accelerating fruit colouration change (Jia *et al.*, 2013). It was reported that sucrose promoted 'Benihoppe' strawberry fruit ripening by modulating the expression of ripening-related genes, ABA biosynthetic gene *FaNCED1* and through ABA-stress ripening transcription factor *ASR* (Luo *et al.*, 2019).

Figure 3.6: Effect of sucrose infusion through pedicel on the ripening percentage of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ±SE of means at $P \leq 0.05$

Chilling injury index

Chilling injury is a postharvest disorder that occurs after low temperature storage which can result in significant downgrade or rejection of fruit in the market (Erkan and Peckmezci, 2000). In this study, treatments had a significant effect ($P < 0.05$) on chilling injury of early season 'Hass' avocado fruit (Figure 3.7). However, control fruit showed the highest chilling injury index (CI) (2.83) compared with sucrose treated fruit. Whereas Su (0.2 mM L⁻¹) significantly reduced CI compared with control and Su (0.5 mM L⁻¹) (Figure 3.7). Henriod *et al.* (2005) found that exposure to low temperatures can gradually alter membrane permeability in chilling-sensitive species, possibly increasing ion leakage. The increase in chilling injury for control may be due to a loss of membrane integrity. According to Bower and Magwaza (2004), 'Hass' avocado fruit with lower moisture content are susceptible to chilling injury. Van Rooyen (2005) reported that passive water infusion through 'Pinkerton'

avocados fruit pedicel during cold storage at 5.5°C for 28 days reduced chilling injury symptoms. It is therefore assumed that treatment solution infusion reduced chilling injury in early season 'Hass' avocado fruit.

Our results showed that treatment with Su (0.5 mM L^{-1}) resulted in higher chilling injury index (2.17) compared with Su (0.2 mM L^{-1}) (1.33) (Figure 3.7). According to Xu *et al.* (2016), sucrose application reduced chilling injury in 'Youxiu' broccoli by improving antioxidant activities. Furthermore, in *Arabidopsis thaliana*, treatment with sucrose reduced ROS stress during extended cold temperatures (Rosenwasser *et al.*, 2011). Therefore, lower chilling injury for Su (0.2 mM L^{-1}) treatment may be attributed to its role in antioxidants production which are known to mitigate chilling injury symptoms (Yang *et al.*, 2011). In contrast, higher chilling injury index with the postharvest sucrose (0.5 mM L^{-1}) treatment may be related to the effect of high sucrose content on increasing ethylene production (Fei *et al.*, 2020). In the study of Pesis *et al.* (2002), chilling injury symptoms expressed as mesocarp discoloration, resulted in ethylene synthesis in the tissue and a low temperature environment in 'Ettinger' avocado fruit.
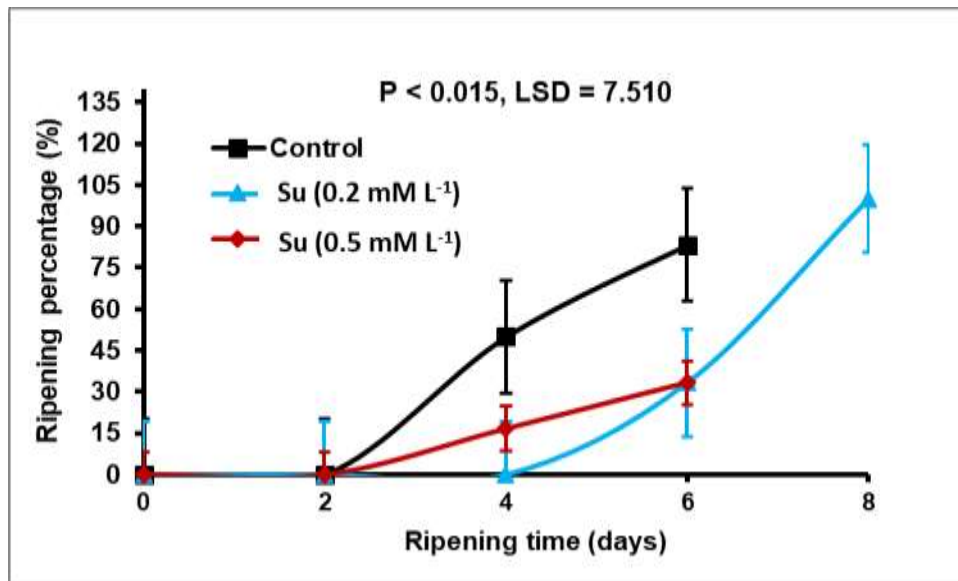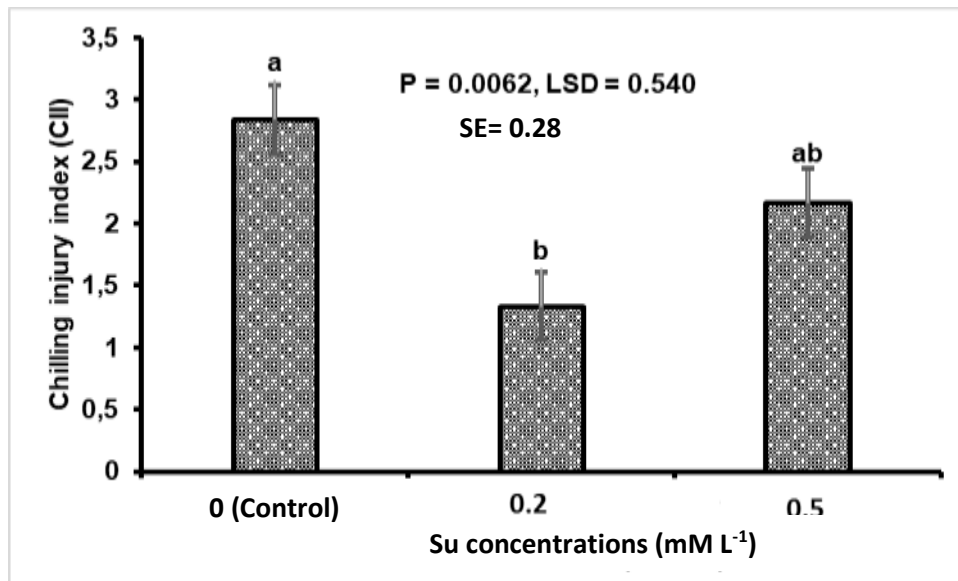
Figure 3.7: Effect of sucrose infusion through pedicel on the chilling injury index of 'Hass' avocado fruit after ripening. Values are means of 12 fruits. Error bars indicate ±SE of means at $P \leq 0.05$

CHAPTER 04

THE EFFECT OF POSTHARVEST CALCIUM-PULSING ON EARLY SEASON 'HASS' AVOCADO FRUIT EXOCARP COLOUR CHANGE DURING RIPENING

4.1. Introduction

Calcium is a secondary messenger that plays a pivotal role in regulating physiological functions in fruits and vegetables during postharvest (White and Broadley, 2003). Calcium and its mode of action have been shown to regulate postharvest physiology of fruits and vegetables at the cellular level (Aghdam *et al*., 2012). Several studies have highlighted the role of calcium in cell wall structure (Martins *et al*., 2018), ethylene biosynthesis/signalling (Ludwig *et al*., 2005) and enhanced anthocyanin accumulation in fruit, possibly, via anthocyanin structural genes upregulation (Xu *et al*., 2014). In 'Hass' avocado fruit, colour change is due to chlorophyll degradation concomitant with anthocyanin accumulation (Cox *et al*., 2004). Therefore, the purpose of this study was to determine the effect of calcium as postharvest treatment on early season 'Hass' avocado fruit exocarp colour change during ripening.

4.2. Materials and Methods

4.2.1. Experimental procedures, sites, and design

The experiment was conducted as a completely randomised design with three replications. The treatments were two concentrations of calcium chloride namely, 0.2. Harvested 'Hass' avocado fruit were pulsed with 2 and 3 mM L^{-1} calcium chloride solution, while control fruit were not pulsed. The experimental site, experimental design, storage, and ripening temperature were similar to those explained in Chapter 03.

4.2.2. Data collection

All the data collected for fruit firmness, subjective and objective colour, ripening percentage and external chilling injury were done as explained in Chapter 03.

4.2.3. Data analysis

Data was analysed using a windows software GenStat® version 16th (VSN International, Hemel Hempstead, UK) as explained in Chapter 03

4.3. Results and Discussion

Fruit firmness

Firmness loss is an important ripeness indicator and directly affects shelf-life. A highly significant ($P < 0.001$) decreasing trend in fruit firmness was found amongst all the treatments (Figure 4.1). There was a gradual decrease in fruit firmness with days to ripening for all treatments (Figure 4.1). However, postharvest $CaCl_2$ treatment delayed firmness loss during ripening when compared with control fruit. Moreover, there was no significant difference in firmness decline between $CaCl_2$ treatments (2 and 3 mM L^{-1})

throughout the ripening days (Figure 4.1). Similar results have been reported by Wickramasinghe *et al.* (2013), wherein, no significant differences in firmness were observed between four $CaCl_2$ (0, 2, 4 and 6%) concentrations for 'Pollock' avocado fruit. Similarly, Jain *et al.* (2019) reported that Jujube fruit (*Ziziphus mauritiana*) treated with $CaCl_2$ had significantly higher firmness as compared to the control fruits of both varieties ('Kaithali' and 'Umran') and there was no significant effect of $CaCl_2$ concentration on fruit firmness.

In agreement with the findings of Lara *et al.* (2004), cell wall components were found to be significantly maintained by $CaCl_2$ pre-treatment in 'Pájaro' strawberry. Similarly, calcium application maintained fruit firmness during storage in 'O'Neal' and 'Bluecrop' blueberries (Angeletti *et al.*, 2010). According to White and Broadley (2003), calcium protects cells from cell wall degrading enzymes, thereby delaying softening. In 'Vinhão' grape berry fruit, $CaCl_2$ treatment maintained fruit firmness by promoting calcium cross-link with pectin and inhibited pectin degradation (Martins *et al.*, 2021). Postharvest calcium treatment seems to increase cell wall pectin characteristics and maintain cell wall integrity by reducing uronic acid solubilization in the pectin (Jain *et al.*, 2019) and disassembly of cellulose-hemicellulose network. Therefore, delayed firmness loss with $CaCl_2$ treatment in this study could be attributed to the calcium role in stabilising the cell wall.
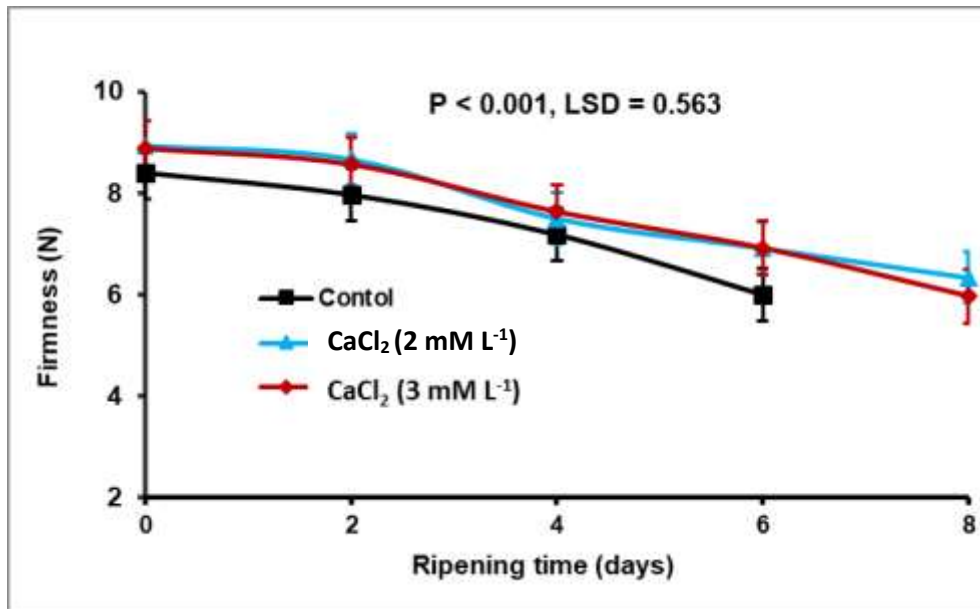
Figure 4.1: Effect of calcium chloride infusion through pedicel on the firmness of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ± SE of means at $P \leq 0.05$

Subjective and objective colour change

In avocado 'Hass', exocarp colour changes from green to purple then black during fruit ripening (Cox *et al.*, 2004; Villa-Rodriguez *et al.*, 2011). In this study, treatments had a significant effect ($P < 0.001$) on visual colour during ripening (Figure 4.2). In general, there was an increase in exocarp visual colour with ripening days, irrespective of the treatments (Figure 4.2). However, calcium chloride treatments showed a modest improvement in colour development during ripening when compared with control (Plate 4.1). Moreover, $CaCl_2$ treatments exhibited no significant difference in terms of visual colour change during ripening from day 0-6 except on day 8, whereby, $CaCl_2$ (3 mM L^{-1}) treatment had higher visual colour rating (Figure 4.2). Furthermore, treated with $CaCl_2$ treatments (2 and 3 mM L^{-1}) softened with visual colour changing from emerald green

($\approx$ 1 colour rating) to purple ($\approx$4 colour rating) during 8 ripening days (Figure 4.3). Furthermore, the correlation between visual colour and firmness for $CaCl_2$ (2 mM L^{-1}) and $CaCl_2$ (3 mM L^{-1}) was negative and significant (P < 0.001). Moreover, the correlation between visual colour and firmness for $CaCl_2$ (2 mM L^{-1}) and $CaCl_2$ (3 mM L^{-1}) correlation was slightly different, with correlation coefficients of r = -0.907 and r = -0.865, respectively (Table 4.1). These findings suggested that firmness declined with increase in exocarp colour change in $CaCl_2$ treatment.

In terms of objective exocarp colour change, $CaCl_2$ treated, and control fruit showed no significant difference for *L*, C** and *h°* (P < 0.001) during ripening (Figures 4.2b-d). The objective exocarp colour parameters (*L*, C*,* and *h°*) in this study decreased for all treatments during ripening. Similarly, Shikwambana *et al.* (2021) also found objective exocarp colour parameters (*L*, C*,* and *h°*) values to decrease as 'Hass' avocado fruit ripened. The current study also found that control fruit showed lower *L** values than $CaCl_2$ treated fruit from day 0-6, which also correlated with the maintenance of green colour. The *C** values did not differ between $CaCl_2$ treated throughout the ripening period and were higher than control. Moreover, the *h°* values for control were lower when compared with $CaCl_2$ treated fruit on day 4 and 6. In addition, the correlations obtained between visual colour and objective colour parameters were negative and significant (P < 0.001), which could mean that exocarp colour change was dependent on decreased objective colour parameters (*L*, C** and *h°*) (Table 4.1).

However, $CaCl_2$ treatments took longer to ripen and achieved the purple colouration as compared with control which changed to olive green ($\approx$ 3 colour rating) on final day of ripening. Zhi *et al.* (2017) found that immersing 'Dongzao' jujube fruits with 10 g L^{-1}

CaCl$_2$ increased their colour by stimulating anthocyanin accumulation. In 'Vinhao' grape cultivar, anthocyanin biosynthetic gene expression, DFR and UFGT were enhanced by calcium treatment (10 mM CaCl$_2$) (Martins *et al.*, 2018). The expression anthocyanin biosynthetic genes, DFR, ANS and UFGT were stimulated by calcium treatment in 'Ruegen F7-4' red fruit bearing strawberry inbred line (Xu *et al.*, 2014). In CaCl$_2$ treatments, higher visual colour rating may be attributed to the role of calcium in anthocyanin biosynthetic pathway.
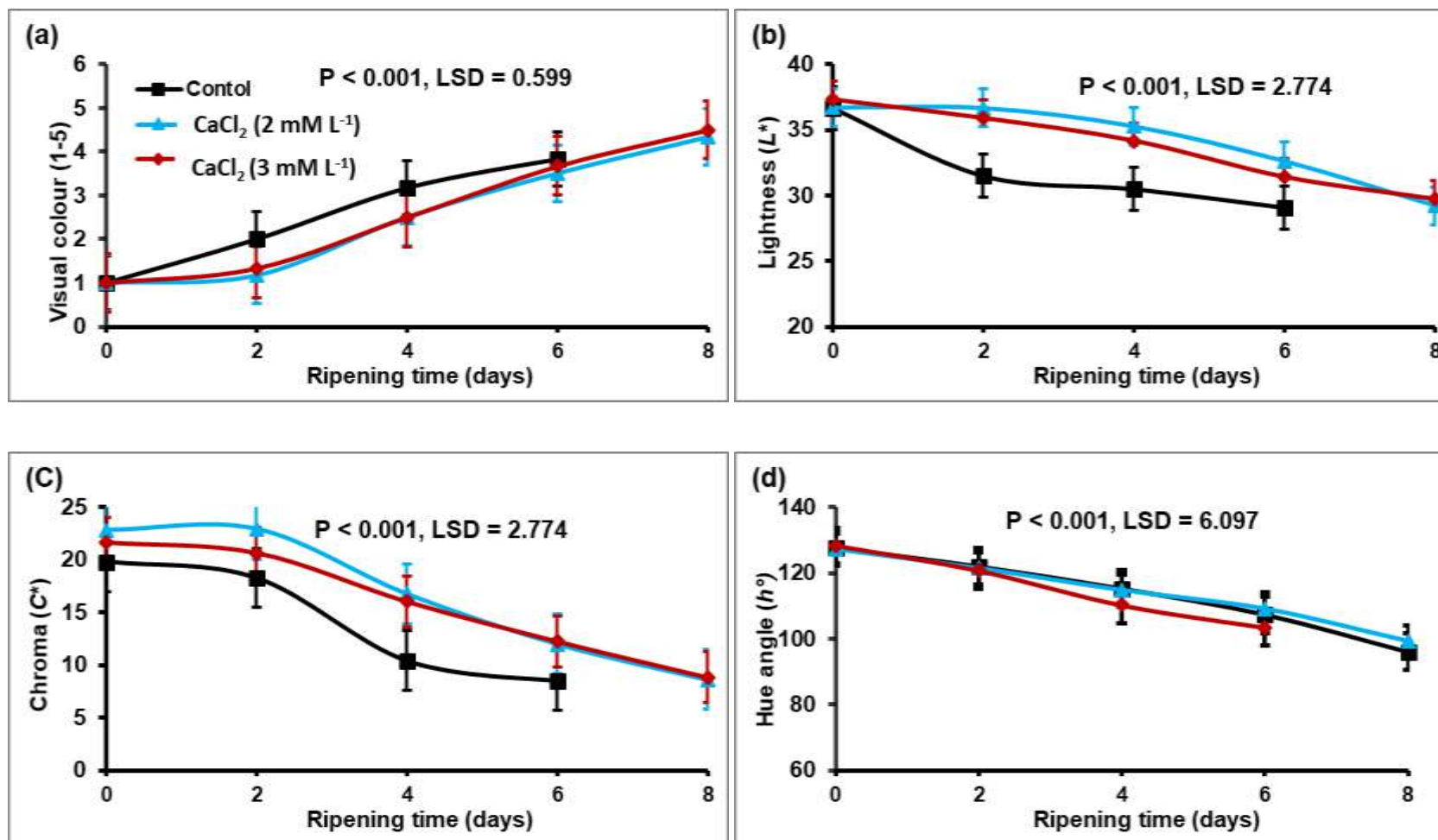
Figure 4.2: Effect of calcium chloride infusion through pedicel on subjective (visual colour) (**a**) and chromaticity parameters (*L**-*lightness, C**-chroma and *h°*-*hue angle*)(**b**), (**c**), and (**d**), respectively) of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ± SE of means at P ≤ 0.05

	Day 0	Day 2	Day 4	Day 6	Day 8
Control					
CaCl$_2$ (2 mM L^{-1})					
CaCl$_2$ (3 mM L^{-1})					
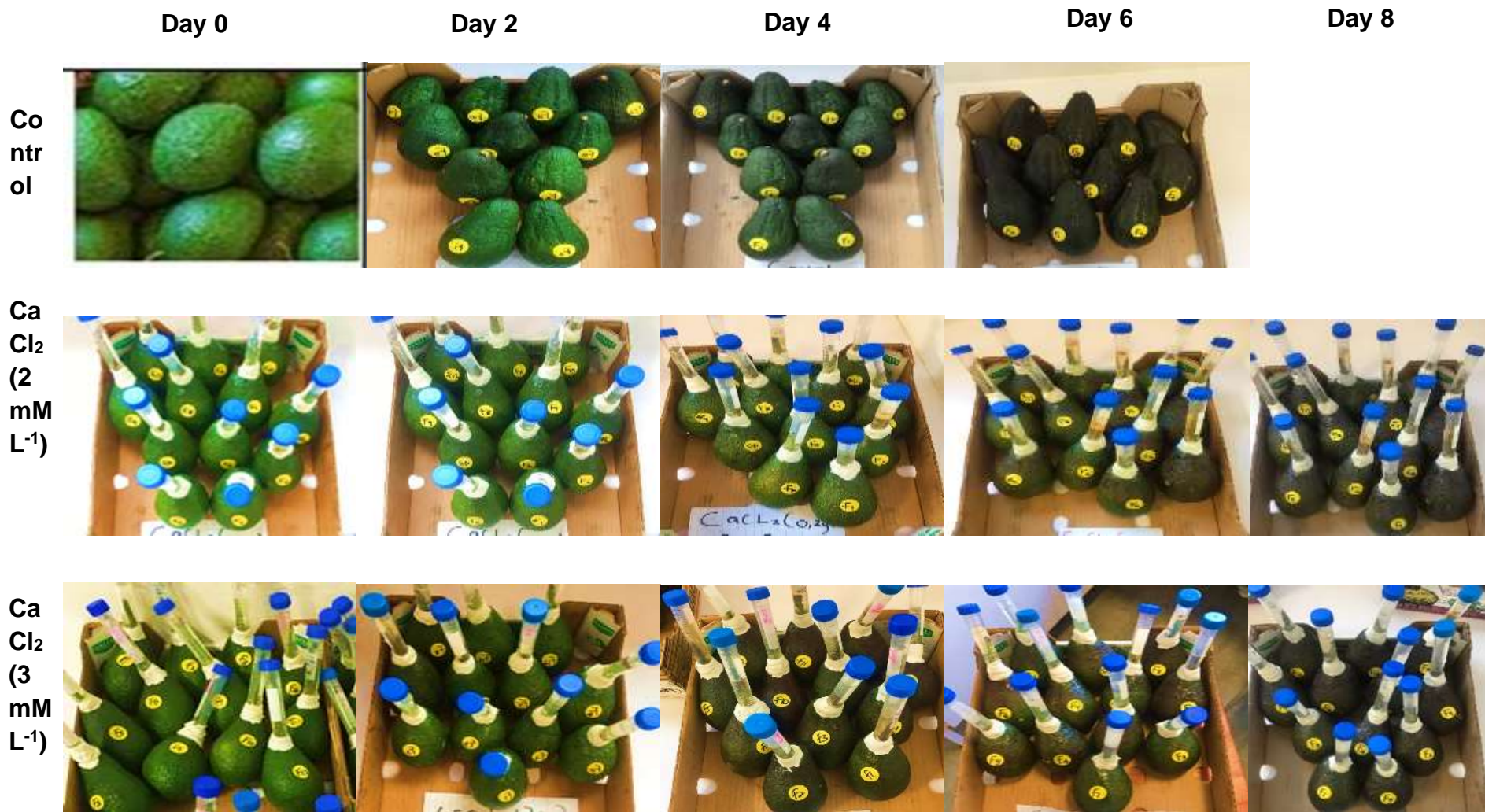
Plate 4.1: Visual colour change for control and calcium chloride treatments during ripening

Table 4.1: Pearson correlation coefficient between objective colour parameters (L^*, C^*, h^o) and subjective (visual colour) of 'Hass' avocado fruit exocarp colour measurement/firmness during ripening as influenced by control, $CaCl_2$ (2 mM L^{-1}) and $CaCl_2$ (3 mM L^{-1}) treatment.

	Control	$CaCl_2$ (2 mM L^{-1})	$CaCl_2$ (3 mM L^{-1})
Correlations		R^2	
Firmness × visual colour	-0,682***	-0,917***	-0,938**
Firmness × L^*	0,722^{ns}	0,820^{ns}	0,734^{ns}
Firmness × C^*	0,663^{ns}	0,911^{ns}	0,864^{ns}
Firmness × h^o	0,732^{ns}	0,880^{ns}	0,887^{ns}
Visual colour × L^*	-0,588***	-0,868***	-0,819***
Visual colour × C^*	-0,744***	-0,962***	-0,931***
Visual colour × h^o	-0,903***	-0,919***	-0,917***

*Represent significant difference at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ns = not significant.

Ripening percentage

Fruit softening usually decreases with increased ethylene production that occurs during avocado fruit ripening (Donetti and Terry, 2012). Results showed that treatments had a significant effect ($P = 0.052$) on ripening percentage of 'Hass' avocado (Figure 4.3). In general, there was an increase in early season 'Hass' avocado fruit ripening percentage during ripening days, irrespective of treatments (Figure 4.3). However, control exhibited higher ripening percentage from day 0 to day 6 when compared with $CaCl_2$ treatments (Figure 4.3). Calcium chloride

treatments showed a lower ripening percentage throughout the ripening period in contrast to control (Figure 4.3). In many fruits, delayed ripening has been observed with calcium treatment including 'Ruegen F7-4' strawberry (Xu *et al.*, 2014), and 'Vinhao' grape (Yu *et al.*, 2020). It has been established that calcium delayed ripening in 'Golden smoothee' apples by reducing fruit respiration and ethylene production; and thereby, preventing softness and starch molecule breakdown (Recasens *et al.*, 2004). Moreover, Gao *et al.* (2019) found that $CaCl_2$ treatment delayed 'Huanong 1' papaya fruits ripening and softening by inhibiting enzymes and gene expression related to cell wall degradation and ethylene signal transduction.

Figure 4.3: Effect of calcium chloride infusion through pedicel on the ripening percentage of 'Hass' avocado fruit during ripening. Values are means of 12 fruits. Error bars indicate ±SE of means at P ≤ 0.05

Chilling injury index

Cold storage of 'Hass' avocado fruit for a prolonged period below 6°C results in chilling injury (CI) development, as a consequence, poor colour development during ripening (Mathaba *et al.*, 2015). In this study, treatments had a significant effect (P <

45

0.05) on early season 'Hass' avocado fruit chilling injury index (CII) (Figure 4.4). However, there was no significant difference between CaCl₂ treatments in terms of CII in early season 'Hass' avocado. The treatments exhibited lower CII when compared with control, suggesting that CaCl₂ play a role in reducing fruit chilling injury during storage. Hou *et al.* (2021) reported that CaCl₂ application can trigger antioxidant system activity and maintain ROS homeostasis to increase cold tolerance of postharvest vegetables and fruits. For instance, in 'Nanguo' pear fruit CaCl₂ application effectively alleviated peel browning caused by chilling injury on account of membrane lipid peroxidation inhibition and a higher activity and expression of superoxide dismutase (SOD) (Zhang *et al.*, 2019).
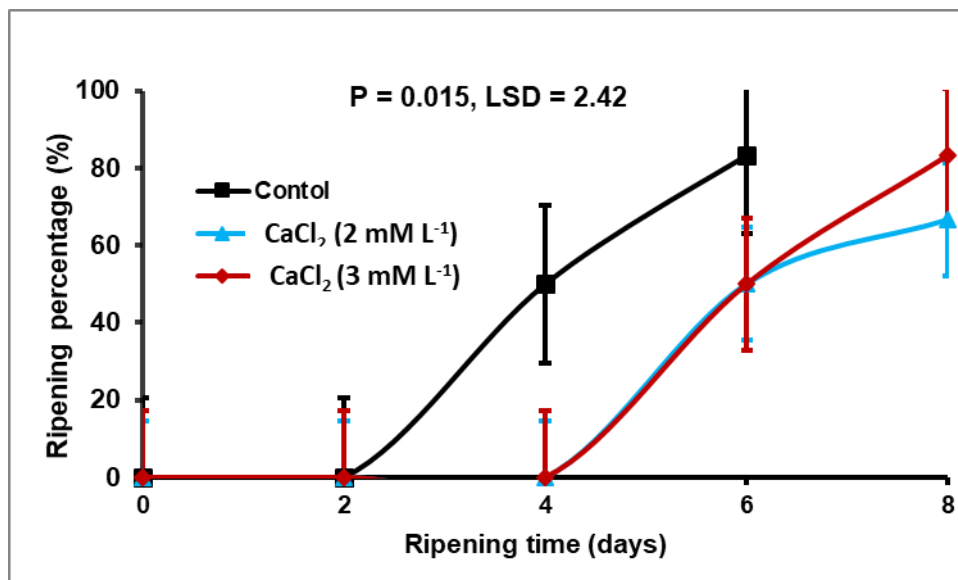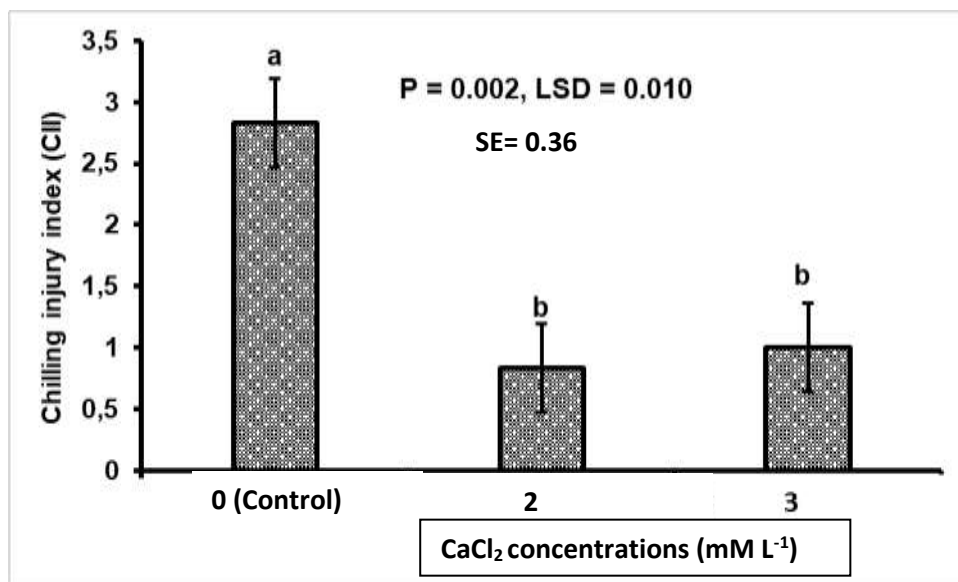

Figure 4.4: Effect of calcium chloride infusion through pedicel on the chilling injury index of 'Hass' avocado fruit after ripening. Values are means of 12 fruits. Error bars indicate ±SE of means at P ≤ 0.05

CHAPTER 05

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary and conclusion

This study investigated the effect of postharvest sucrose and calcium pulsing on exocarp colour development of early season 'Hass' avocado during ripening. The 'Hass' avocado exocarp develops poor colour due to insufficient cyanidin 3-*O*-glucoside accumulation during ripening. The results of this study showed that continuous pulsing with $CaCl_2$ at 2 and 3 mM L^{-1} through the fruit pedicel resulted in an improved exocarp attributes such as colour chromaticity (*L*, C** and *h°*) and visual colour (purple). Moreover, $CaCl_2$ treatments reduced fruit chilling injury after storage at 5.5°C for 28 days. Fruit firmness was also maintained with calcium treatment concurrent with increased visual colour development.

Similarly, sucrose treatments effectively reduced chilling injury and enhanced exocarp colour development of 'Hass' avocado fruit during ripening. With Su (0.5 mM L^{-1}) being the most effective treatment to improve 'Hass' avocado fruit exocarp colour. Generally, Su (0.5 mM L^{-1}) improved colour but quality parameters such as firmness decline and chilling injury were high. This concentration cannot be recommended due to its effect on other parameters., Low concentration (0.2 mM L^{-1} Su) worked best, since it showed lower chilling injury, and maintained fruit firmness. Therefore, it can be concluded that calcium or sugar accumulation in avocado fruit exocarp contribute to colour development during ripening.

5.2. Recommendations and future research

This study revealed that calcium and sucrose play a role in regulating colour development in early season 'Hass' avocado fruit. Therefore, it may be recommended that, in order to control postharvest poor exocarp colouration in 'Hass' avocado, production practices that increase sucrose and calcium accumulation in the exocarp should be considered.

Moreover, sugar and calcium applied as postharvest treatments may contribute to avocado colour development by increasing anthocyanin accumulation. However, the study did not focus on the physiology of how calcium and sucrose influence anthocyanin biosynthesis and accumulation in 'Hass' avocado fruit. Future research should investigate whether these treatments affect anthocyanin biosynthesis at the gene level.

The benefits of calcium chloride lie in its ability to extend storage time recommended for export marketing while maintaining fruit quality. Further research is needed to understand how calcium chloride can reduce chilling injury, firmness loss, and improve exocarp colour early in the season.

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