EFFECT OF METHYL JASMONATE AND SALICYLIC ACID ON QUALITY PRESERVATION OF 'HASS' AVOCADO FRUIT DURING ULTRA-LOW COLD STORAGE

by

MONYELA NGOAKO FRANS

MINI-DISSERTATION

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in

HORTICULTURE

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agriculture and Environmental Science)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: DR N MATHABA (UMP)

CO-SUPERVISOR: PROF TP MAFEO (UL)

APRIL 2022

TABLE OF CONTENTS

DECLARATION

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

……. ……

Signature Date

DEDICATION

This study is dedicated to my family

ACKNOWLEDGEMENTS

I would like to express my sincere gratefulness towards the following people and organisations:

- My supervisory team Dr N Mathaba and Prof TP Mafeo for their guidance, patience, encouragement and constructive criticisms during the duration of the research.
- Mr K Shikhwambana for his helpfulness in the laboratory and constructive criticisms during the duration of the research.
- Dr TK Satekge and Dr NMD Buthelezi for their guidance and assistance during the duration of the research.
- Agricultural Sector Education Training Authority (AgriSeta) and National Research Foundation (NRF) for financial support.
- The University of Mpumalanga for allowing me to conduct my project in their laboratory.
- My family, friends and colleagues for their support and encouragement.
- Most importantly, I give all thanks to the almighty God for his grace, mercy and wisdom.

LIST OF FIGURES

ABSTRACT

The South African Avocado Industry has recently announced plans to expand exports into new markets, such as the United States (US). As a requirement for these markets, fruit of high quality must be stored at ultra-low temperature to mitigate phytosanitary risks. However, ‘Hass’ avocado fruit are susceptible to chilling injury when stored at temperatures below 3°C. Moreover, CI development resulted in uneven ripening and disease infestation due to damaged cell membranes. Therefore, the objective of this study was to evaluate the potential of methyl jasmonate (MeJA) and salicylic acid (SA) on quality maintenance of 'Hass' avocado fruit during ultra-low cold storage. Matured ‘Hass’ avocado fruit were harvested at commercial dry matter (22%). The experiment was conducted using a completely randomized design (CRD) with eight replications per treatment. Treatment concentrations for methyl jasmonate (MeJA) were 0 (control), 10 and 100 µmol•L^{-1}, while those for salicylic acid (SA) were 0 (control), 1.0, 2.0 and 3.0 mM. After treatments, fruit were stored at 2°C for 31 days and thereafter, ripened at ambient temperature (±25°C) until fully ripe. During ripening, fruit were evaluated for weight loss, exocarp colour, firmness, chilling injury, as well as physiological (vascular browning) and pathological disorders (fruit rot). In this study, dipping fruit in MeJA solution significantly ($P < 0.05$) reduced ‘Hass’ avocado fruit firmness loss. Moreover, MeJA showed a significant effect ($P < 0.05$) on hue angle (*h*°) but did not significantly affect ($P > 0.05$) visual colour rating, chroma (C*), lightness (L*) and weight loss. The results showed that ‘Hass’ avocado fruit treated with 10 µmol•L^{-1} MeJA reduced weight loss when compared with 100 µmol•L^{-1} MeJA from day 2 to day 8 of ripening. Overall results showed a visual change in ‘Hass’ avocado fruit exocarp colour, with eye colour changing from rating 1 (emerald-green) to 3 (olive-

green) for control and fruit treated with MeJA throughout the ripening days. Furthermore, MeJA reduced ‘Hass’ avocado fruit external chilling injury, physiological and pathological disorders. With respect to SA treatments, the result showed that dipping fruit at 1.0 and 2.0 mM SA had a significant effect ($P < 0.05$) on reducing firmness loss during ripening. Salicylic acid (1.0 mM) reduced and alleviated ‘Hass’ avocado fruit external chilling injury during ultra-low cold storage. Furthermore, result showed that 1.0 and 2.0 mM SA treatments had significant affect ($P < 0.05$) on firmness loss. Moreover, a significant effect was observed on visual colour and C* but did not affect ($P > 0.05$) L* and *h*°. Fruit treated with SA showed poor exocarp colour development with extended exposure to ultra-low cold storage, as a result, developed chilling symptoms. The treatment of ‘Hass’ avocado fruit with 1.0 mM SA inhibited the incidence of fruit rot and vascular browning when compared with control and fruit treated with 2.0 and 3.0 mM SA. In conclusion, 10 and 100 $\mu mol \bullet L^{-1}$ MeJA and 1.0, 2.0 and 3.0 mM SA effectively preserved ‘Hass’ avocado fruit quality during storage at ultra-low temperature.

Keywords: Chilling injury, fruit rot, exocarp colour, methyl jasmonate, salicylic acid, vascular browning

CHAPTER 1

INTRODUCTION

1.1. Background

Avocado (*Persea americana* Mill.) originated in Mexico, Central or South America and was first cultivated in Mexico as early as 500 BC (Muhammad, 2015). In South Africa, the avocado industry has rapidly grown in recent years; and currently, ranked eighth by world trade (DAFF, 2019). The South African Avocado Industry (SAAI) produces approximately 90 000 tons of fruits annually, in which approximately 98.5% is exported mainly to the European market (DAFF, 2019). The South African avocado production is dominated by 'Fuerte' (42%) and 'Hass' (33%) along with 'Ryan' (11%) and 'Pinkerton' (8.5%) cultivars (DAFF, 2019).

Currently, 'Hass' is the second leading avocado cultivar in terms of production, thereby, contributing about 70% to the global avocado trade (DAFF, 2019). The cultivar is known for its superior pulp quality, higher yield and later maturing than green skinned cultivars (Crane *et al.*, 2013). In addition to these attributes, 'Hass' avocado exocarp change from green to purplish-black as it ripens (Cox *et al.*, 2004). The SAAI is largely dependent on export, and most major export markets are distant and complex, which causes logistical challenges (Kremer-Köhne, 1998). It is extremely important to ensure that fruit does not undergo excessive ripening during shipping due to the climacteric nature of the fruit (Lütge, 2011). During export to the European markets, avocado fruit are usually stored for up to 30 days (Kok, 2011). Pesis *et al.* (2002) reported that the use of cold storage delays ripening by reducing fruit respiration and ethylene production.

Traditionally, South African avocados are shipped at 5.5°C, but early-season fruit are shipped at 7.5°C, with late-season fruit being shipped at 3.5°C (Sivankalyani *et al.*, 2015). Contrarily, avocado fruit are highly susceptible to chilling injury (CI) at temperatures below 10°C (Wills *et al.*, 2007). According to ElMasry *et al.* (2009), membrane damage due to chilling injury affects the normal ripening, resulting in uneven ripe fruits with rubbery texture. Furthermore, prolonged cold storage of 'Hass' avocado fruit resulted in poor exocarp colour development due to CI symptoms development (Mathaba *et al*., 2017).

Membrane damage and solute leakage during cold storage have been shown to influence the fruit weight loss and disease development (Carvajal *et al.*, 2018). Poor fruit quality and disease development are important factors that limits the storage life of avocados and results in appreciable losses at retailer and consumer levels (Pareek *et al*., 2015). The control of postharvest diseases of horticultural fruits mostly depends on fungicides and controlled environments (Ali *et al.,* 2021). However, there are consumer concerns about possible human health risks associated with the use of fungicides (Aghdam *et al.*, 2016).

Recent studies have shown that application of growth regulators known as resistance inducers to biotic or abiotic stresses can maintain the postharvest quality of horticultural fruits and vegetables (Zhou *et al.*, 2018; Rasouli *et al.*, 2019). In general, methyl jasmonate (MeJA) and salicylic acid (SA) are endogenous plant signalling molecules that modulate physiological processes in fruit to reduce chilling injury and maintain quality (Vlot *et al.*, 2009; Chen *et al.*, 2020). Sivankalyani *et al.* (2015) found that treatment with MeJA significantly reduced 'Hass' avocado fruit CI incidence by regulating stress-responsive enzyme lipoxygenase (LOX), fatty acid

desaturase (FAD) and heat shock proteins (HSPs). Whereas treatment with salicylic acid has been shown to reduce chilling injury in 'Eureka' lemon by activation of antioxidant enzymes system such as ascorbate peroxidase and glutathione reductase which scavenged cold-induced reactive oxygen species (ROS) (Siboza *et al.*, 2017). Furthermore, SA and MeJA have been reported to reduce the spoilage percentage in horticultural fruits by decreasing ethylene production and respiration and maintaining firmness in the fruit (Tasneem, 2004; Haider *et al.,* 2020).

1.2. Problem statement

In South Africa, most avocado producers choose to harvest early in order to sell to overseas markets at premium prices. To comply with phytosanitary standards to avoid the spread of undesirable insect pests in overseas markets (e.g., the USA), high-quality fruit must be stored at ultra-low temperature (Glowacz *et al.*, 2017b). However, chilling injury (CI) development in 'Hass' avocado fruit during storage at low temperatures is a major concern (Woolf *et al.*, 2003). Moreover, development of CI result in uneven ripening and disease infestation due to damaged cell membranes. Several postharvest methods such as hot air and water treatments, polyamines and 1-methylcyclopropene (1-MCP) treatment can be used to minimize avocado fruit CI and preserve fruit quality (Cao and Zheng, 2008c; Valero and Serrano, 2010). Although these treatments can effectively preserve quality during cold storage and at commercial application, they are challenging to adopt due to partial mitigation of CI and overall ripe quality. In recent research, plant growth regulators including methyl jasmonate (MeJA) and salicylic acid (SA) have been reported to effectively reduce CI symptoms in several horticultural crops (Siboza and Bertling, 2013). However, there is scarcity of information on the application of these hormones to control chilling and preserve the quality of 'Hass' avocado fruit.

Moreover, less is documented on shelf-life and postharvest quality of ‘Hass’ avocado fruit treated with the interaction of these hormones. Therefore, the purpose of this study was to investigate whether MeJA and SA will influence the CI control and preserve other ‘Hass’ avocado quality parameters during ultra-low storage.

1.3. Rationale

With the attention of improper risk due to use of chemicals at postharvest technology and consumers’ demand for healthy products. The use of plant hormones as postharvest treatments has been shown to be effective in maintaining the quality of horticultural produce (Shafiee *et al.*, 2010). Aghdam *et al.* (2014) and Asghari (2019) have reported that the postharvest application of SA and MeJA are effective in mitigating chilling injury in horticultural fruits and vegetables. Therefore, these treatments will play an important role in maintaining quality and controlling ‘Hass’ avocado fruit chilling injury during storage at ultra-low temperature. Furthermore, MeJA and SA application can assist to maintain avocado fruit that qualifies for a lucrative export market, allowing growth of South African avocado fruit sales into new markets while maintaining postharvest quality.

1.4. Aim and objectives of the study

1.4.1. Aim

Assessment of the effect of MeJA and SA on quality preservation of 'Hass' avocado during ultra-low cold storage.

1.4.2. Objectives

To determine the effect of methyl jasmonate on quality preservation of ‘Hass’ avocado during ultra-low cold storage.

To determine the effect of salicylic acid on quality preservation of ‘Hass’ avocado during ultra-low cold storage.

1.5. Hypotheses

Methyl jasmonate had an effect on quality preservation of ‘Hass’ avocado during ultra-low cold storage.

Salicylic acid had an effect on quality preservation of ‘Hass’ avocado during ultra-low cold storage.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

The avocado industry in South Africa is largely export-driven, making fruit quality an extremely important component (Kassim and Workneh, 2020). Previous research has indicated that approximately 40% of the South African avocado market is based on exports (Blakey, 2011). The most important export markets for South African avocados are the European Union (EU) and United Kingdom (UK) (Glowacz *et al.*, 2017b). According to the Department of Agriculture Forestry and Fisheries (DAFF) (2019), 54% of South African avocado was exported in 2018, while 12% was sold at fresh produce markets, and 34% was sold to retailers informally. Avocado cultivars exported include 'Fuerte' (42%), 'Hass' (33%), 'Ryan' (11%), and 'Pinkerton' (8.5%) (DAFF, 2019). In addition to its traditional major import countries, SAAI is planning to enter new markets, e.g., the USA (Glowacz *et al.*, 2017b).

However, maintaining and continuing to explore markets with such high returns can be challenging for the industry. There is constant competition from other exporters. In addition, new emerging high-paying markets (e.g., the USA) require fruit to be subjected to cold sterility to conform with phytosanitary regulations and avoid the spread of undesirable insect pests (Glowacz *et al.*, 2017b). In some studies, exposing fruits to temperatures between 1.1 and 2.2°C for 14 to 18 days has proven effective at controlling insect pests and could be used as a quarantine treatment (Sivankalyani *et al.*, 2015). The use of low temperatures to extend the storage life of fruits is a common postharvest practice. However, 'Hass' avocado fruit stored at a low temperature develops physiological disorders associated with chilling injury

(Woolf *et al.*, 2003). Furthermore, prolonged exposure to cold storage results in poor colour, weight loss, firmness and pathological disorders due to membrane damage and solute leakage (ElMasry *et al.,* 2009; Pareek *et al*., 2015).

2.2. Chilling injury physiology

Chilling injury is a physiological disorder that occurs in horticultural crops of tropical and subtropical origin, consequently, reducing fruit marketability (Woolf *et al.*, 2003). This is one of the major challenges of avocado trade growth on the world market (Glowacz *et al.*, 2017b). Chilling injury occurs as a result of cold damage disrupting the membrane lipids, thereby increasing its permeability and malondialdehyde (MDA) compound (Dorria *et al.*, 2010). According to Lütge *et al.* (2010), avocado fruit are susceptible to cold temperatures and develop chilling injury symptoms when stored below 5.5°C. The typical chilling injury symptoms on avocado fruit are pitting, surface darkening, mesocarp discolouration and uneven ripening (Yahia, 2011). Previous study has indicated that the susceptibility of horticultural products to chilling injury could be due to their low ability to modify membrane lipids under chilling stress (Lafuente *et al.*, 2005).

In the studies of Lyons (1973) and Razavi *et al.* (2018), it was found that plants under chilling stress undergo a physical cell membrane phase transition that changes from a normal flexible, liquid-crystalline structure to a solid gel structure. Therefore, as membranes solidify, membrane fluidity changes and membrane lipids undergo phase transitions, which result in the configuration of certain membrane-bound proteins and enzymes. As a result, the loss of activation energy results in reduced reaction times of enzymes (Wolfe, 1978; Aghdam *et al.*, 2016). It is believed that a prolonged chilling temperature causes lipids to solidify in the membrane,

causing membrane contraction , which results in cracks that increase cell permeability (Lyons, 1973). A large change in membrane bilayer associated with prolonged chilling is accompanied by increased ionic movement and water molecules through the membrane (Aghdam *et al.*, 2016). Changes in membrane bilayer leads to increased membrane permeability, resulting in solute leakage. The leakage results in irreversible secondary chilling due to cellular integrity loss (Razavi *et al.*, 2018).

2.3. Mitigation of chilling injury using methyl jasmonate (MeJA) and salicylic acid (SA) postharvest treatment

Researchers have tested several methods to reduce avocado fruit chilling injury, including hot water treatment (Blakey and Bower, 2007), low-temperature conditioning (LTC) (Woolf *et al.,* 2003), and 1-methylcyclopropene (1-MCP) (Pesis *et al.*, 2002). However, these treatments have proven to be modestly effective. Globally, environmental regulations and public health concerns have led to an increase in the use of generally recognised as safe (GRAS) compounds (Baswal *et al.*, 2020). GRAS compounds (e.g., MeJA, SA, MeSA, etc.) have been found to alleviate chilling and maintain horticultural products' quality. There has been evidence that the exogenous MeJA and SA treatment mitigate chilling injury and maintain quality and shelf-life of a wide range of fruit crops (Aghdam *et al.*, 2016; Asghari, 2019). Moreover, methyl jasmonate (MeJA) and salicylic acid (SA) are hormones found naturally in plants and can induce physiological processes in fruit that provide protection against chilling injury (González-Aguilar *et al.*, 2004; Tasneem, 2004). In ‘Beefstake’ tomato (Ding *et al.*, 2002) and ‘Mollar de Elche’ pomegranate fruits (Sayyari *et al.*, 2009), exogenous SA treatment has been found to reduce chilling injury and preserve fruit quality.

2.3.1. Effect of postharvest treatment with MeJA and SA on chilling injury

The composition of fatty acids in harvested fruits play a major role in their susceptibility to chilling damage (Asghari, 2019). Several factors such as heat shock proteins (HSPs) and soluble sugar content may also affect the chilling susceptibility of horticultural crops (Vasanthaiah, 2011). Eaks (1990) found that chilling-sensitive species have a high concentration of saturated fatty acids. Moreover, Rehman *et al.* (2018) found that cells with a higher ratio of unsaturated fatty acids to saturated fatty acids (unSFA/SFA) were more resistant to low temperatures.

Methyl jasmonate (MeJA) and salicylic acid (SA) promote cold resistance in plants by inhibiting fatty acid desaturase (FAD) enzyme activity by directly interfering with fatty acid metabolism (Vasanthaiah, 2011; Aghdam *et al.*, 2015). Previously, studies have reported that the postharvest MeJA application on 'Mollar de Elche' pomegranate fruit retained high-unsaturated membrane-lipids that assisted the crop to adapt under oxidative stress (Sayyari *et al.*, 2011). Ding *et al*. (2002) and Luo *et al*. (2011) demonstrated that postharvest salicylic acid (SA) application increased production of unsaturated fatty acids such as linoleic acid during cold stress in order to increase the membrane fluidity of ‘Beefstake’ tomato and Qingnai’ plum fruits. As membrane fluidity increases, the tendency for membranes to transition from flexible liquid crystal to rigid sol-gel is reduced, which result in improved CI resistance (Los and Murata, 2004; Aghdam *et al.*, 2016). The increased membrane integrity loss was observed by Aghdam *et al.* (2014) in ‘Newton’ tomato fruit as the symptoms of CI developed, resulting in increased phospholipase D (PLD) and lipoxygenase (LOX) activities. However, exogenous salicylic acid (SA) treatment was able to reduce

these enzymatic activities in ‘Fuyang’ loquat fruit during storage at chilling temperatures and resulted in preservation of membrane integrity (Cao *et al*., 2008a).

Creelman and Mullet (1997) observed that MeJA was produced by the enzymatic oxidation of unsaturated fatty acids mediated by the enzyme LOX in *Arabidopsis thaliana*. The results of González-Aguilar *et al.* (2004) showed that exogenous 10^{-5} M MeJA reduced electrolyte leakage and CI in ‘Kothrud’ and ‘Karela’ guava fruit as well as the activity of both phenylalanine ammonia lyase (PAL) and LOX enzymes. According to Aghdam *et al.* (2014), SA causes fruit to be more tolerant to CI by reducing the enzymes that catalyse membrane oxidation, such as PLD and LOX, decreasing lipid peroxidation and preserving membrane semi-permeability. Moreover, Meir *et al.* (1996) and Glowacz *et al.* (2017b) both found that dipping ‘Hass’ avocado fruit in MeJA concentration at 2.5 μmol•L^{-1} reduced CI symptom severity by alteration of the fatty acids content and prevented fruit weight loss during cold storage. In addition, salicylic acid (SA) has been found to alleviate CI in ‘Cardenal’ tomato by increasing the expression of HSPs, antioxidant activity, the arginine pathway that changes polyamines, nitric oxide (NO) and proline production and polyphenol oxidase (PPO) enzyme activity (Polenta *et al*., 2007).

There are also other mechanisms in which MeJA can enhance crop resistance to low temperatures. In one study, it was found that MeJA regulate the C-repeat binding factor pathways during cold stress of ‘Orin’ apple (Asghari, 2019). In 'Orin' apple fruit, postharvest 10^{-3} mol•L^{-1} MeJA application strengthen resistance to cold by increasing expression of genes involved in chilling responses including malus-domestica-basic-helix-loop-helix (MdCIbHLH1) and C-repeat binding factor1 (MdCBF1) (Wang *et al.*, 2018). Additionally, experiments on protein degradation

showed that MeJA associates positively with Malus domestica-jasmonate-ZIM-domains 1/4 (MdJAZ1/4) proteins responsible for chilling susceptibility (Wang *et al.*, 2018; Asghari, 2019). Therefore, JAs are capable of modulating freeze tolerance through CBF expressions-C-repeat pathways.

2.3.2. Effects of MeJA and SA on fruit weight loss

In fruits, water loss is a major challenge because it has a major effect on quality and storage potential of the fruit (Mendieta *et al.*, 2016). Lallu *et al.* (2004) found that water loss in avocado results in poor fruit quality manifested as weight loss and shrivelling. It has also been demonstrated that factors such as peroxidation, transpiration, respiration, membrane breakdown, and cellular breakdown at low temperatures affect fruit weight (Habibi and Ramezanian, 2017).

In various studies, MeJA and salicylic acid (SA) were shown to reduce fruit weight and water loss in different plants. Baswal *et al.* (2020) found that treatment with 0.001 µmol•L^{-1} MeJA and 0.002 µmol•L^{-1} SA reduced 'Kinnow' mandarin fruit weight loss through the delay of senescence and maintenance of cellular integrity. Furthermore, salicylic acid (SA) and MeJA have been reported to inhibit the activities of different senescence enzymes such as 1-aminocyclopropane-1-carbxylic acid synthase (ACS), 1-aminocyclopropane-1-carbxylic acid oxidase (ACO), cellulase, polygalacturonase (PG), pectin methyl esterase (PME) and catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) in 'Newton' tomato (Aghdam *et al.*, 2014). Previous studies showed that inhibition of ACS, ACO and PME lowered the rate of respiration in 'Kinnow' mandarin fruits (Haider *et al.,* 2020). Meir *et al.* (1996) found that dipping 'Hass' avocado fruit in 2.5 µmol•L^{-1} MeJA reduced the severity of CI symptoms and prevented fruit weight loss during cold storage. In addition, high

levels of exogenous 100 µM MeJA have been reported to reduce dehydration in 'Moro' blood oranges (Habibi *et al.*, 2019).

2.3.3. Effects of MeJA and SA on fruit exocarp colour

of Avocado fruit 'Hass' colour development contributes to quality and subsequent market value (Kayesh *et al.*, 2013). According to Cox *et al.* (2004), 'Hass' avocado fruit colour changes from green to purple-black due to chlorophyll degradation and anthocyanin (cyanidin 3-*O*-glucoside) synthesis and accumulation during ripening. Anthocyanins are one of the major secondary metabolites responsible for the distinctive tissue colouration in several fruit crops (Gu *et al.*, 2019). According to Stintzing and Carle, (2004), anthocyanins are important antioxidants that plants use to withstand low temperatures and radiation stress. Similar to these antioxidants, MeJA and SA are the naturally occurring compounds used by plants to defend against biotic and abiotic stresses (Creelman and Mullet, 1997; Wang *et al.*, 2021). In different fruit species, the relationship between MeJA, SA and anthocyanin synthesis and accumulation has been demonstrated (Shan *et al.*, 2009).

In fruit crops, anthocyanin accumulation can be induced by JAs and SA treatment (Martínez-Esplá *et al.*, 2017; Shen and Yang, 2017). Jasmonic acid (JAs) have been shown to upregulate anthocyanin biosynthetic genes required for anthocyanin synthesis and accumulation (Loreti *et al.*, 2008; Shan *et al.*, 2009). The JASMONATE-ZIM-DOMAIN (JAZ) repression mediated by JASMONATE is removed by JAs to allow the anthocyanin complexes to activate transcriptional function. It has been shown that exogenous MeJA can induce degradation of the JAZ proteins via the SCF^{COI1}-26S (Skp1-Cullin-F-box CORONATINE INSESITIVE1) proteasome pathway (Chung *et al.*, 2009; Qi *et al.*, 2011). Therefore, JAs activate downstream

transcriptional cascades, such as WD-repeat/bHLH/MYB complexes, resulting in anthocyanin biosynthesis (Thines *et al.*, 2007; Qi *et al.*, 2011).

Anthocyanin production is regulated by enzyme activities such as PAL, chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose: flavonoid 3-*O*-glucosyltransferase (UFGT) (Mori *et al.*, 2005; Gu *et al.*, 2019). These enzymes are classified as 'early' anthocyanin biosynthetic enzymes (PAL, CHS, CHI, F3H) and 'late' biosynthetic enzymes (DFR, LDOX and UFGT) (Mori *et al.*, 2005; Shan *et al.*, 2009). There is evidence that postharvest application of MeJA and SA have a direct impact on anthocyanin biosynthesis by increasing enzyme activity involved in the pathway. In *Arabidopsis thaliana*, application of 25 µM MeJA combined with 1% sucrose was shown to induce anthocyanin accumulation through upregulating DFR, LDOX, and UFGT enzymes (Shan *et al.*, 2009).

In a study conducted by Oraei *et al.* (2019), postharvest 100.0 mM SA and 100 µmol•L^{-1} MeJA application increased the anthocyanin concentration in 'Sahebi' grapes and 'Willamette' red raspberry fruits, thereby, increasing PAL enzyme activity which catalyse the formation of naringenin. Furthermore, it was reported that SA increased the expression of genes codifying by the enzymes including chalcone synthase (CHS) and chalcone isomerase (CHI), responsible for the flavonoid pathway (Oraei *et al.,* 2019). Previous research suggests that SA increased the activities of both PAL and chalcone flavanone isomerase (CHFI) in grape cell cultures when applied at low concentrations (Obinatai *et al.*, 2003). Thus, the results

confirmed that both JAs and salicylic acid modulate the biosynthesis of anthocyanins for fruit crops.

2.3.4. Effects of MeJA and SA on fruit firmness

In avocado fruits, firmness is an important characteristic and the most reliable method of determining whether fruit is ripe to eat (Arzate-Vazquez *et al.*, 2011). Firmness is determined by cell density, compactness, cell wall structure, lignin and cuticle thickness (Wills *et al.*, 1998). Fruit firmness comprises a wide attribute including tissue, water content and cell wall composition (Valero and Serrano, 2010). Softening occurs concurrently with the increase in ripening enzyme activity after fruit harvest (Kassim *et al.*, 2013). Fruit softening is closely related to changes in the cell wall, which result from molecular, biochemical and physiological changes (Wang *et al.*, 2015a).

Studies have shown that the changes in cell wall materials are always accompanied by activation of cell wall degrading enzymes, which are consistent with reduced firmness during ripening (Wang *et al.*, 2021). The degradation and depolymerization of cell wall materials, such as the cellulose, hemicellulose and pectic substances are facilitated by the coordination of cell wall degrading enzymes such as, carboxymethyl cellulase (CMCase), polygalacturonase (PG), pectin methyl esterase (PME) and *β*-galactosidase (*β*-GAL) (Haider *et al.*, 2020; Wang *et al.*, 2021). Ezzat *et al.* (2017) suggested that MeJA efficacy to maintain fruit firmness is attributed with MeJA induced inhibition of lipoxygenase (LOX), PG and PME activities.

In ‘Kinnow’ mandarins, treatment with 0.001 μmol•L^{-1} MeJA was also shown to inhibit the activity of degrading enzymes, such as PME and cellulose (Baswal *et al.*,

2020). The treatment with MeJA and SA reduced cell wall degradation by modulating the activity of wall-modifying enzymes as well as calcium within the cell wall in ‘Jiubao’ peach (Meng *et al.*, 2009)and ‘Kinnow’ mandarin (Baswal *et al*., 2020). Previous study has reported that JAs and SA play an important role in maintaining fruit firmness by enhancing gene expression and PAL enzyme activity and other enzymes associated with the phenylpropanoid pathway in ‘Grand Nain’ bananas (Alali *et al*., 2021). Chen *et al.* (2019) found that 10 μmol•L^{-1} MeJA enhanced cell wall integrity and decreases electrolyte leakage through the stimulation of phospholipid remodelling in ’Xiazhimeng’ peach fruit.

2.4. Physiological and pathological disorders

Diseases and disorders contribute to fruit damage at the cosmetic, organoleptic and nutritional levels, resulting in a reduction in fruit quality (Ramírez-Gil *et al.*, 2021). Furthermore, diseases may be of biotic nature (such as fruit rot caused by microbial pathogens) or abiotic (such as disorders caused by physiological problems) (Burdon *et al.*, 2013; Sharma *et al.*, 2017). Most abiotic disorders of avocado fruit are caused by rapid temperature change and storage at low temperatures (White *et al.*, 2007; Burdon *et al.*, 2013). Whereas diseases that develop after harvest usually begin in a quiescent state before harvest. In addition, this condition creates challenges for their management because symptoms typically appear at the market stage, when the disease could not be controlled (Hartill and Everett, 2002; Sharma *et al.*, 2017). The exogenous application of plant growth regulators, such as MeJA and SA is one of the most effective strategies for alleviating plant physiological and pathological disorders. According to Aghdam *et al.* (2016) and Asghari (2019), jasmonate and salicylic acid enhance plant resistance to diseases and pathogen attacks by stimulating the synthesis of antioxidant enzymes.

2.4.1. Vascular browning

Vascular browning can be described as a physiological disorder caused by poor storage temperature management and notable by browning of vascular strands that run longitudinally through the fruit tissue (Arpaia *et al.*, 2004). Mhlophe and Kruger (2012) found that the incidence of vascular browning for ‘Maluma’ avocado fruit decreases with maturity. Furthermore, vascular browning was found to increase with temperature and storage duration. A study by Leclereq (1990) observed that browning was most prevalent in avocado fruits stored at 5.5°C for 30 days. According to previous research, browning is caused by the oxidation of *O*-diphenols to *O*-quinones by the enzyme polyphenol oxidase (PPO) (Ramírez-Gil *et al.*, 2021).

2.4.2. Fruit rot

Fruit rot is an economically important fruit disease associated with the microorganism Rhizopus stolonifer (Ramírez-Gil *et al.*, 2021). Johnson and Kotzé (1994) found that avocado fruit infected with fruit rot showed shrivelled skin and a water pulp covered with fungal mycelium. A previous study showed that ‘Hass’ avocado fruit stored at either 1.0, 5.0 or 12.0°C for 4 days showed less fruit rot than those stored for a longer period of 14 days (Arpaia *et al.*, 2015). In addition, a study on ‘Maluma’ and ‘Ryan’ avocado showed higher levels of fruit rot during storage at 8°C when compared with ‘Hass’ avocado fruit (Kruger and Lemmer, 2012).

2.4.3. Effect of MeJA and SA on physiological and pathological disorders

Salicylic acid and methyl jasmonate play an important role in the development, growth, and tolerance of plants to pathogens (Aghdam *et al.*, 2016). According to Stintzi *et al.* (1993), MeJA and SA stimulate chitinase and *β*-1,3-glucanase enzymes

that hydrolyse polymers of the fungal cell wall, resulting in weakened cell walls and cell lysis. Furthermore, chitinase and *β*-1,3-glucanase enzymes have been found to delay the growth of fungi and reduce fruit decay (Theis and Stahl, 2004). In 'Superchief' tomato plants, MeJA and SA increased the activities of peroxidase, superoxide dismutase (SOD) and PAL enzymes and decreased anthracnose rot severity (Asghari, 2019). Glowacz *et al.* (2017a) found that exogenous 100 µmol•L^{-1} MeJA and 10 µmol•L^{-1} MeSA delayed anthracnose development in 'Hass' avocado fruit. Similar results were reported in 'Jiefang Zhong' loquat fruit (Cao *et al.*, 2008a, b) treated with MeJA at 10 µmol•L^{-1} and 'Matisu' mango fruit treated with SA solution at 1000 µmol•L^{-1} (Zeng *et al.*, 2006), which delayed fruit infection. The application of 0.2 µmol•L^{-1} MeJA and 2.0 mM SA at postharvest reduced brown rot occurrence in 'Hongdeng' sweet cherries during cold storage (Yao and Tian, 2005).

2.5. Research Gap

The SAAI recently announced plans to expand exports into new lucrative markets, such as the USA (Glowacz *et al.*, 2017b). A requirement for these markets is that fruit be subjected to ultra-low temperature to comply with phytosanitary requirements (Sivankalyani *et al.*, 2015). However, 'Hass' avocado fruit are susceptible to chilling injury when stored at temperature below 3°C (Woolf *et al.*, 2003). Several studies have examined different methods for alleviating chilling injury of 'Hass' avocado fruit stored at low temperatures chilling injury persists (Woolf *et al.*, 2003; Sivankalyani *et al.*, 2015; Glowacz *et al.*, 2017b). The use of methyl jasmonate and SA has been found to mitigate CI in various horticultural crops (Aghdam *et al.*, 2016). There is no definitive evidence regarding MeJA and SA treatments' effects on 'Hass' avocado chilling injury and quality preservation.

CHAPTER 3

EFFECT OF METHYL JASMONATE ON QUALITY PRESERVATION OF 'HASS' AVOCADO FRUIT DURING ULTRA-LOW COLD STORAGE

3.1. Introduction

Ultra-low cold storage has been found to be an ideal phytosanitary measure when shipping avocado fruits from areas harbouring undesired insect pests (Sivankalyani *et al.*, 2015). Previously, cold storage resulted in CI, particularly in 'Hass' avocado fruit which are very susceptible to chilling injury (Blakey *et al*., 2014). Furthermore, extended exposure of 'Hass' avocado fruit to low temperatures resulted in poor exocarp colour development due to CI symptoms (Mathaba *et al*., 2017). The collapse of fruit cell membranes during chilling has been reported to inhibit weight loss and development of pathological disorders (Carvajal *et al*., 2018). These authors reported that the increased loss of weight was attributed to loss of water and solute leakage through the damaged membranes.

Currently, there are several techniques employed to maintain the quality of 'Hass' avocado. Methyl jasmonate treatments have been found to preserve quality in 'Hass' avocado (Meir *et al.*, 1997). Preliminary results have shown MeJA treatments to reduce the spoilage percentage in horticultural fruits by decreasing ethylene production, respiration and maintaining fruit firmness (Tasneem, 2004). Therefore, the present study was conducted to determine the effect of MeJA on quality preservation of 'Hass' avocado fruit during ultra-low cold storage.

3.2. Materials and Methods

3.2.1. .Experimental sites

Matured ‘Hass’ avocado fruit were randomly harvested at their commercial dry matter (22%) from Halls and Sons commercial farm in Nelspruit (25° 27’ 07.18” S, 30° 56’ 29.17” E), Mpumalanga, South Africa. Thereafter, fruit were packed in boxes and carefully transported to the University of Mpumalanga (25° 26' 13.6" S, 30° 58' 54.5" E) postharvest laboratory, thereafter, sorted, treated, stored, ripened and analysed.

3.2.2. Experimental design, treatments and procedures

The experiment was carried out using a completely randomized design (CRD) with eight replications per treatment. The concentrations of methyl jasmonate (MeJA) for the treatment were 0 (control), 10 and 100 µmol•L^{-1}. Eighteen fruit were dipped for 30 minutes in MeJA solution containing the above concentrations. Treated fruit were allowed to dry at room temperature (25°C) for 1 hour. Thereafter, treated fruit were weighed, analysed for colour parameters and firmness, and then packed in commercial carton boxes and subsequently, cold stored at 2°C for 31 days. Fruit were removed from cold storage after 31 days and transferred to room temperature at 25°C to ripen.

3.3. Data collection

3.3.1. *Determination of weight loss*

Fruit weight was measured using an electronic scale (AP Series, Shimadzu Scientific Instruments, Columbia, Maryland) (Figure 3.1). Each fruit was weighed at the beginning of the experiment (i.e., day 0) and after removal from cold storage and thereafter every two days until fully ripe. The difference between the initial and final

weight of the fruit was considered the total weight loss (WL), and the results were expressed as a percentage of the initial weight as follows:

WL (%) = [(initial weight – final weight)/initial weight] × 100

Figure 3.1 Measuring ‘Hass’ avocado fruit weight during ripening

3.3.2. *Determination of exocarp colour*

Subjective colour was determined using the following eye colour rating 1- emerald green; 2 - forest green; 3 - approximately 25% coloured; 4 - approximately 75% coloured; 5 - purple and 6 – black (White *et al.,* 2007). Furthermore, objective colour change assessments were carried out after withdrawal from cold storage and on every two days during ripening using a Minolta Chromameter (CR-400, Konica Minolta, Japan, Osaka) (Figure 3.2). Colour parameters were measured by taking three readings on the equatorial region of each individual fruit to determine the L* value (lightness or brightness), a* value (redness or greenness) and b* value

(yellowness or blueness). From these parameters, chroma (C*) and hue° angle (*h*°) were determined as explained by McGuire (1992) using the formula:

Chroma (C*) = (a*^2 + b*^2)1/2

Hue angle (h°) = tan-1 (a*/b*)

Figure 3.2 Measuring objective exocarp 'Hass' avocado fruit colour parameters during ripening

3.3.3. *Determination of fruit firmness*

Fruit firmness was determined by a non-destructive method according to Köhne *et al.* (1998) using a hand-held densimeter (HP-series, Bareisis, Oberdischingen Germany) with a 5 mm tip on a scale of 85-90 (unripe) to 60 (soft or ready to eat) densimeter units (Figure 3.3). Three readings were taken around the circumference of each fruit and the average reading recorded in newtons (N).

Figure 3.3 Measuring firmness of ‘Hass’ avocado fruit during ripening

3.3.4. *Determination of external chilling injury*

External chilling injury was assessed on exocarp lesions using a scale of 0 to 3 and was expressed in percentage where by 0 = 0% (no chilling); 0.5 = 5% chilling; 1 = 10% chilling; 1.5 = 15% chilling; 2 = 25% chilling; 2.5 = 33% chilling and 3 = 50% chilling (White *et al.*, 2007). The following formula was used to calculate the chilling injury index (CII):

CII = [(sum of CI level) × (number of fruit at CI level)]/total number of fruit in the treatment

3.3.5. *Determination of pathological and physiological disorders*

Upon reaching full ripeness, fruit were cut and assessed for vascular browning and fruit rot anthracnose. Vascular browning was evident by browning of the vascular bundles running longitudinally through the fruit tissues. Fruit rot was evident by rots through the fruit exocarp. Disorders were assessed on rots and browning using a

scale o to 3 where by 0 = 0% (rots or browning); 1 = 10% (rots or browning); 2 = 25% (rots or browning) and 3 = 50% (rots or browning).

3.4. Data analysis

Data was subjected to analysis of variance (ANOVA) using GenStat 20th edition statistical software (VSN International, Hemel Hempstead, UK). The Least Significant Difference (LSD) at 5% was used to compare the mean difference of the treatment.

3.5. RESULTS

3.3.3. Fruit weight loss

In this study, there was no significant difference (P = 0.557) in 'Hass' avocado fruit weight loss during ripening (Appendix 3.1). In general, 'Hass' avocado fruit weight loss increased with ripening days at 25°C, regardless of treatment (Figure 3.4). However, the control fruit lost significantly higher weight during ripening when compared with fruit treated with MeJA. Furthermore, fruit treated with 100 µmol•L^{-1} MeJA showed the higher weight loss from day 2 to day 6 of ripening when compared with fruit treated with 10 µmol•L^{-1} MeJA. Overall, treatment of avocado fruit with 10 µmol•L^{-1} MeJA showed the reduced weight loss throughout the ripening period.

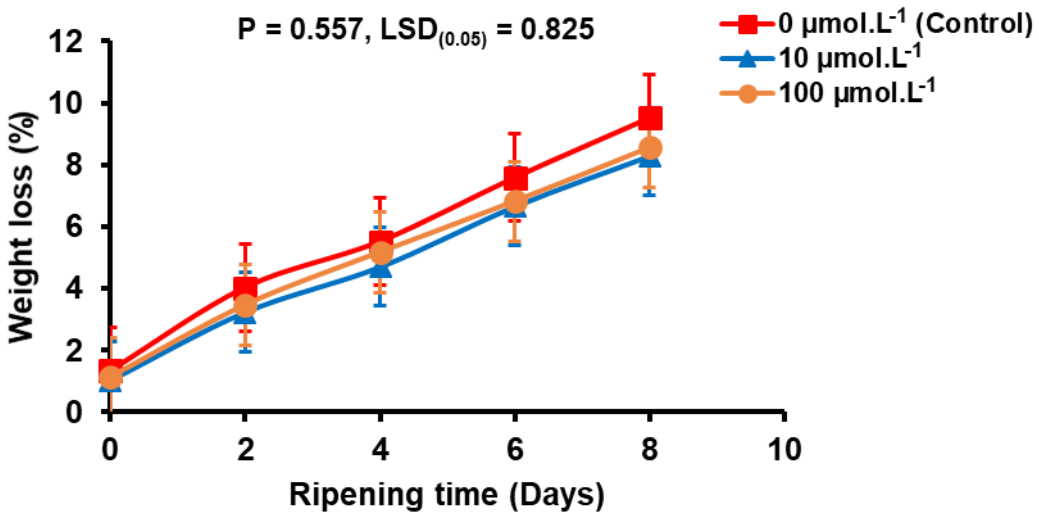

Figure 3.4. Weight loss of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.3.4. Fruit colour (visual and objective parameters)

In the current study, there was no significant difference (P = 0.065) in 'Hass' avocado fruit visual colour between studied treatments (Appendix 3.2). The visual colour of 'Hass' avocado fruit treated or untreated with MeJA changed from rating 1-emerald green to 3-olive green during ripening at 25°C (Figure 3.5a). Furthermore, control fruit showed the higher visual colour values from day 4 to day 6 of ripening when compared with 10 and 100 µmol•L^{-1} MeJA treated fruit. In overall, fruit treated with 10 and 100 µmol•L^{-1} MeJA showed the similar trend of lower visual colour values throughout the ripening period.

A significant difference was not found between MeJA-treated and control fruit in terms of objective exocarp colour L* (P = 0.095) and C* (P = 0.263) during ripening (Appendices 3.3 and 3.4). In contrast, there was a significant difference (P = 0.029)

between the studied treatments on hue angle (*h*°) during ripening (Appendix 3.5). The objective colour parameters (L*, C* and *h*°) showed a decreasing trend for all treatments during ripening (Figure 3.5b - d). Fruit treated with 10 and 100 µmol•L^{-1} MeJA showed the consistent trend of higher L* values from day 0 to day 4 of ripening when compared with control fruit. Moreover, fruit treated with 100 µmol•L^{-1} MeJA showed the lighter colour when compared with 10 µmol•L^{-1} MeJA treated and control fruit.

In this study, the C* values were for 10 µmol•L^{-1} MeJA treated and control fruit from day 2 to day 4 of ripening when compared with fruit treated with 100 µmol•L^{-1} MeJA. However, the control fruit showed the lower C* values from day 6 to day 8 of ripening when compared with fruit treated with 10 and 100 µmol•L^{-1} MeJA. Similarly, the control fruit showed the lower *h*° values from day 2 to day 8 of ripening when compared with fruit treated with 10 and 100 µmol•L^{-1} MeJA. A similar trend of higher *h*° values was observed in fruits treated with 10 and 100 µmol•L^{-1} MeJA for day 0 to day 4 of ripening. However, fruit treated with 10 µmol•L^{-1} MeJA showed the decreased *h*° values from day 6 to day 8 of ripening when compared with 100 µmol•L^{-1} MeJA treated fruit.

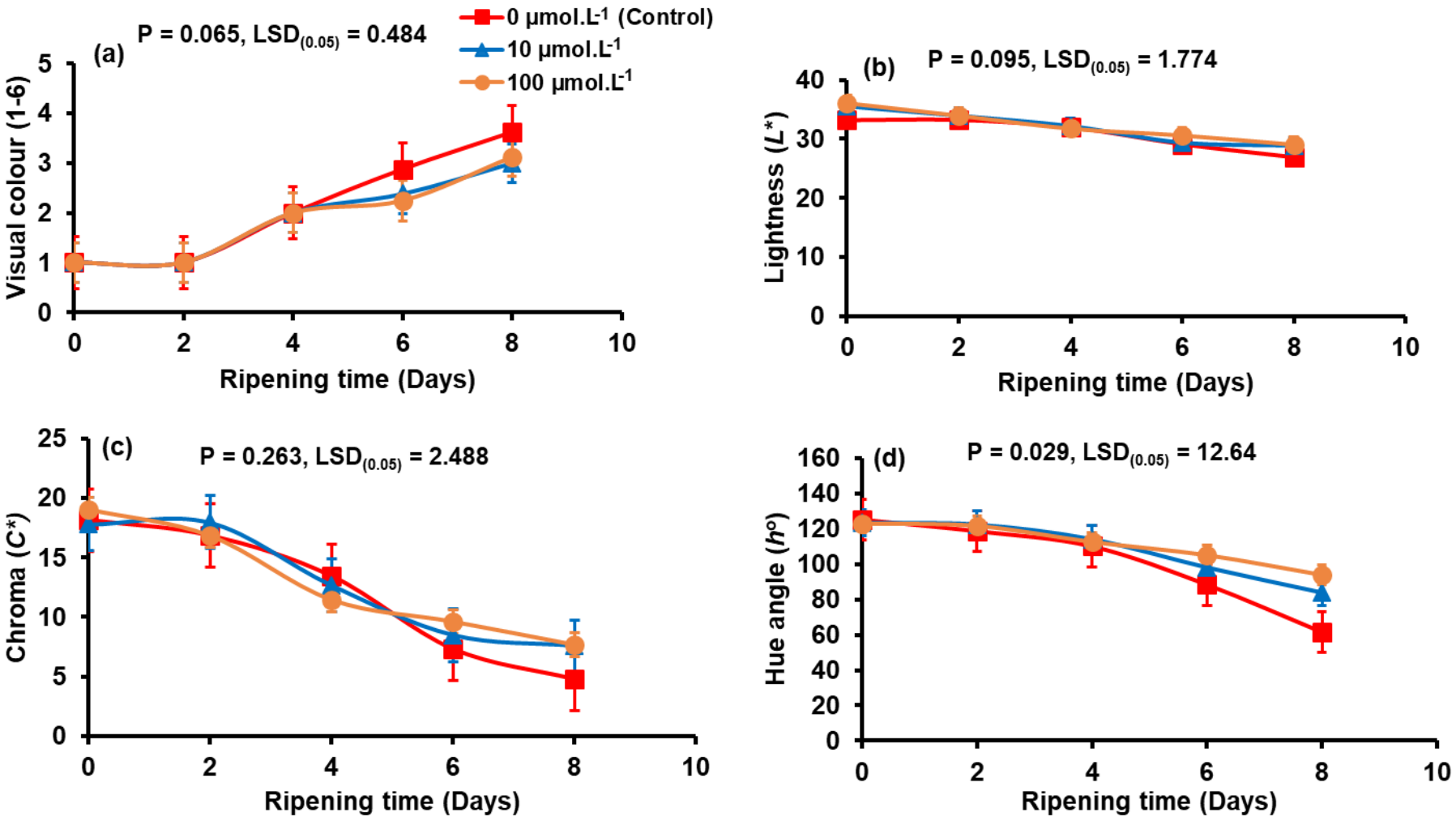

Figure 3.5. Fruit (**a**) visual colour; (**b**) L* values; (**c**) C* values; (**d**) *h*° values of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.3.5. Fruit firmness

There was a significant difference (P = 0.021) on the studied treatments on 'Hass' avocado fruit firmness loss during ripening (Appendix 3.6). In general, 'Hass' avocado fruit softened with ripening days, regardless of treatment (Figure 3.6). However, fruit treated with 100 µmol•L^{-1} MeJA showed the reduced firmness loss when compared with the control fruit from day 4 to day 8 of ripening at ambient temperature. In overall, treatment of 'Hass' avocado fruit with 10 and 100 µmol•L^{-1} MeJA maintained reduced firmness loss when compared with control fruit.

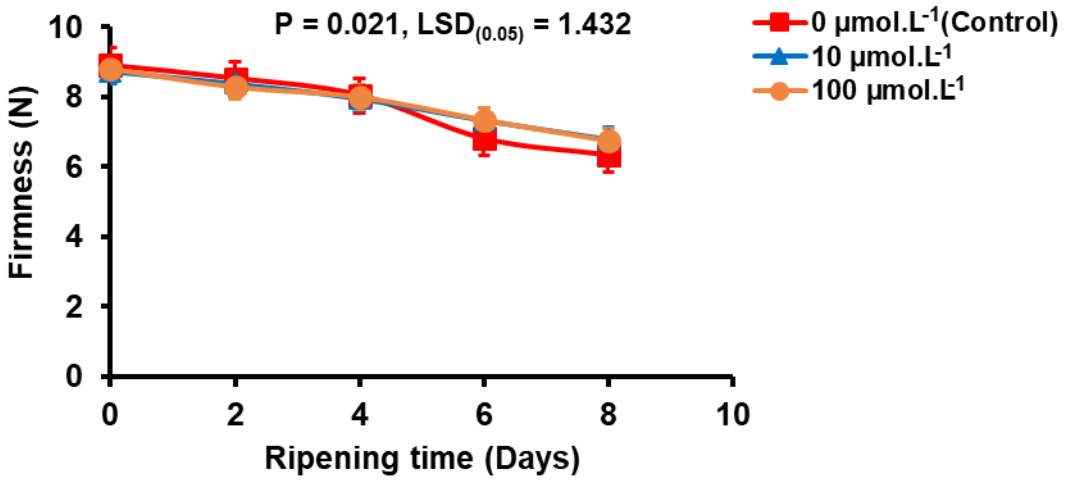

Figure 3.6. Fruit firmness of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.3.6. External chilling injury

In the current study, treatment had no significant (P = 0.285) effect on 'Hass' avocado fruit external chilling symptoms after cold storage (Appendix 3.7). The control fruit showed significantly higher chilling injury symptoms when compared with

100 µmol•L^{-1} MeJA treated fruit (Figure 3.7). Furthermore, the overall results showed that fruit treated with 10 and 100 µmol•L^{-1} MeJA had the reduced chilling injury symptoms after storage at ultra-low cold storage.

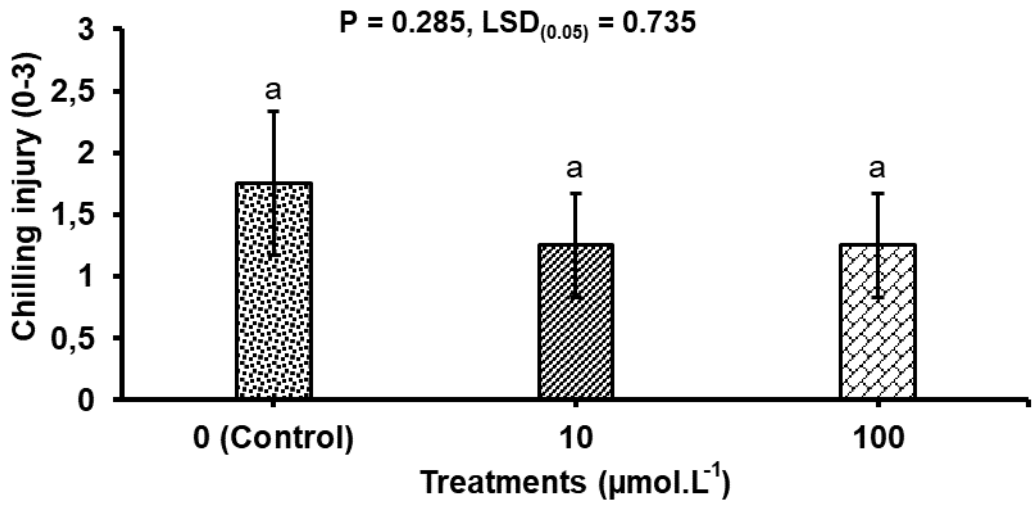

Figure 3.7. External chilling injury of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.3.7. Fruit rot

The significant difference was not observed (P = 0.350) between the treatments on fruit rot incidence of avocado fruit during ripening (Appendix 3.9). However, the control fruit exhibited the pronounced fruit rot incidence when compared with 10 and 100 µmol•L^{-1} MeJA treated fruit (Figure 3.9). Furthermore, fruit treated with 10 µmol•L^{-1} MeJA showed the higher incidence of rot when compared with 100 µmol•L^{-1} MeJA treated fruit. Moreover, treatment with 100 µmol•L^{-1} MeJA inhibited the development of fruit rot in 'Hass' avocado fruit during fruit ripening.

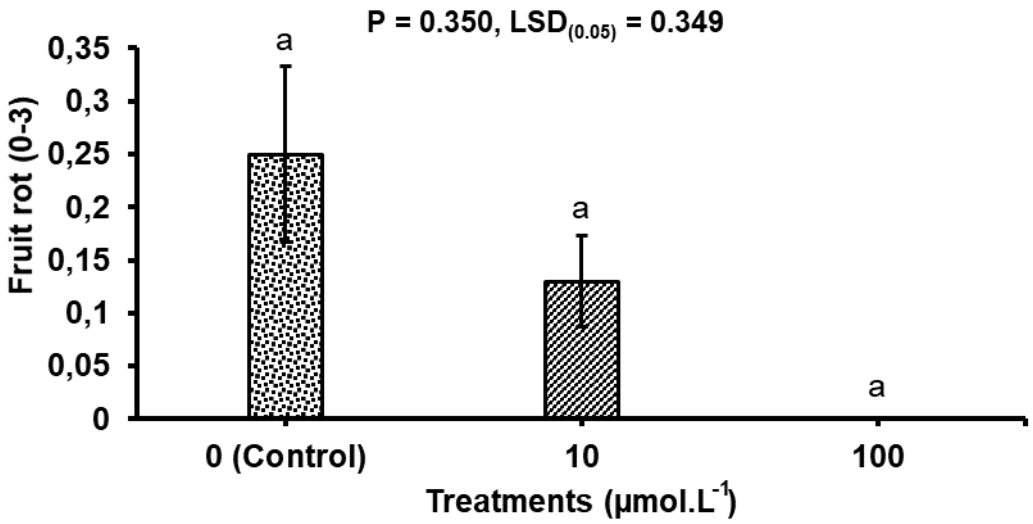

Figure 3.9. Fruit rot of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.3.8. Vascular browning

There was no significant difference (P = 0.142) between the treatments on the incidence of vascular browning of 'Hass' avocado fruit after withdrawal from ultra-low cold storage (Appendix 3.10). However, control fruit showed the higher incidence of vascular browning when compared with 10 and 100 µmol•L^{-1} MeJA treated fruit (Figure 3.10). Furthermore, treatment 10 µmol•L^{-1} MeJA showed the higher incidence of vascular browning when compared with 100 µmol•L^{-1} MeJA treated fruit. Conversely, fruit treated with 100 µmol•L^{-1} MeJA inhibited the development of vascular browning.

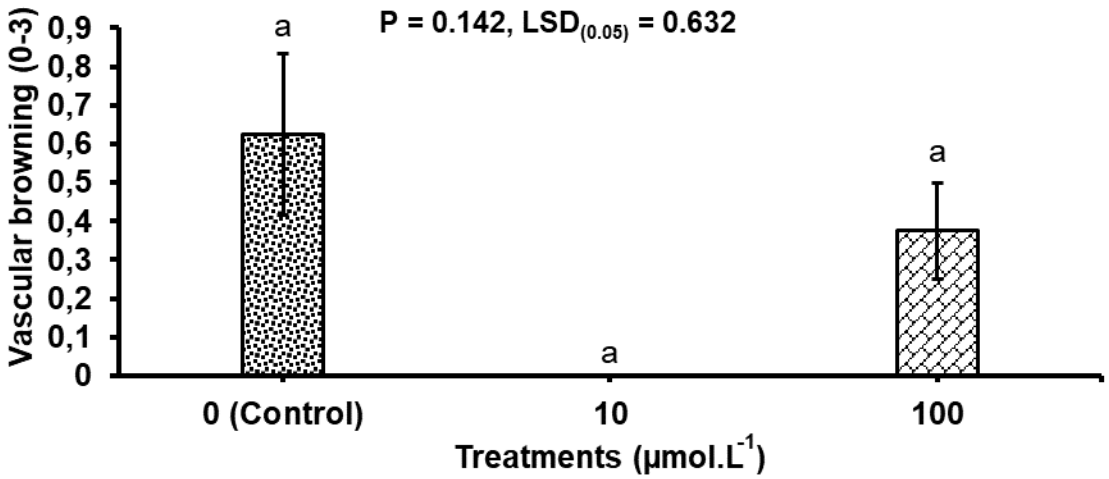

Figure 3.10. Vascular browning of 0 (control), 10 and 100 µmol•L^{-1} MeJA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

3.4. DISCUSSION

3.4.3. Fruit weight loss

In avocado fruit, weight loss is a critical factor that affects postharvest quality and shelf-life (Ramírez-Gil *et al.*, 2021). Bower and Jackson (2003) stated that 'Hass' avocado weight loss is related to loss of water. In consequence, fruit that are stored at lower temperatures loses less water than when transferred to room temperature. In our study, 'Hass' avocado fruit weight loss increased with ripening days, regardless of treatment (Figure 4.1). Our results were in agreement with Zolfagharinasab and Hadian (2007) and González-Aguilar *et al.* (2001) who found that weight loss in 'Malas Save' pomegranate and 'Kent' mango increased when fruits were transferred from cold storage to room temperature.

With respect to hormone treatments, fruit treated with 10 µmol•L^{-1} MeJA showed a reduced weight loss when compared with the control and 100 µmol•L^{-1} MeJA treated fruit. This was attributed to the decrease in water loss being induced by the treatments, consequently, reducing respiration and transpiration of the fruit. Previous study reported that exogenous 0.001 µmol•L^{-1} MeJA treatment reduced 'Kinnow' mandarin fruits weight loss through the delay of ripening and senescence and maintenance of cellular integrity (Baswal *et al.*, 2020).

In this study, fruit treated with 100 µmol•L^{-1} MeJA lost higher weight from day 2 to day 6 of ripening when compared with fruit treated with 10 µmol•L^{-1} MeJA (Figure 3.4). This may be due to the effect of methyl jasmonate on ethylene production when applied at high concentration. Fan *et al.* (1997) reported that exogenous 10^{-3} M MeJA application resulted in enhanced ethylene production through the increased expression and activity of ethylene biosynthetic enzymes. González-Aguilar *et al.* (2003) also reported similar results in 'Sunrise' papaya fruit treated with 100 µmol•L^{-1} MeJA.

3.4.4. Fruit colour (visual and objective parameters)

According to Cox *et al.* (2004), 'Hass' avocado fruit exocarp colour is an important ripening indicator used by growers, exporters and consumers. In the present study, 'Hass' avocado fruit visual colour changed from rating 1 (emerald-green) to 3 (olive green) during ripening, regardless of treatment. Similar findings were reported by Mathaba *et al.* (2015), whereby, early harvest 'Hass' avocado fruit colour development only reached 3 (olive green).

In the current study, the control fruit obtained an olive-green colour from day 4 to day 6 of ripening while fruit treated with 10 and 100 µmol•L^{-1} MeJA reached forest-green colour (Figure 3.5a). This was attributed to failure of colour development due to low anthocyanin production and development of chilling injury symptoms. According to Fung *et al.* (2006), exogenous application of 100 µmol•L^{-1} MeJA at the breaker stage failed to stimulate the red colour development in 'Newton' tomato fruit. These authors reported that colour development failure was due to the effect of high concentration of MeJA on ethylene production which enhanced the fruit to become more susceptible to chilling injury, thus, more symptoms development. According to Glowacz and Rees (2016), MeJA application at high concentration accelerates fruit ripening by increasing ethylene production. These results were an agreement with the current study, whereby, purple exocarp colour development was inhibited in all treatments throughout the ripening period as a result of chilling symptom development.

In the current study, the exocarp of all fruit untreated and treated with either MeJA showed a decrease in exocarp lightness (L*), chroma (C*) and hue angle (*h*˚) (Figure 4.2b - d). Similar results have been reported by Cox *et al.* (2004) and Donetti and Terry (2011), who observed that lightness, chroma and hue angle of 'Hass' avocado fruit decreased with ripening. These authors reported that the decrease in colour parameters L*, C* and *h*˚ was due to the development of a darker exocarp colour. Moreover, Öztürk *et al.* (2015) reported the decreased C* in 'Fortune' and 'Friar' Japanese plums treated with 2240 mg•L^{-1} MeJA. These authors reported that these effects were due to the role of MeJA in chlorophyll degradation and carotenoid biosynthesis.

Rudell *et al.* (2002) reported that exogenous 2.24 g•L^{-1} MeJA decreased the h° of 'Fuji' apple fruit stored at 21°C for 7 days as a result of the increased peel anthocyanin content. In the current study, MeJA failed to induce 'Hass' avocado fruit exocarp anthocyanin synthesis and accumulation of. This was attributed to chilling injury as a result of the extended exposure of fruit to ultra-low cold storage Moreover, this was associated with failure of the treatment to enhance the activities of enzymes responsible for anthocyanin biosynthesis. However, previous research reported that exogenous application of 100 µM MeJA enhance the activity of the PAL enzyme involved in anthocyanin synthesis in 'Autumn Bliss' raspberries (Moro *et al.*, 2017). Additionally, 50 µM MeJA was shown to upregulate the expression of the anthocyanin structural genes and regulatory genes in 'Red Delicious' apple fruit (An *et al.*, 2015). These authors found that methyl jasmonate promoted accumulation of anthocyanins by the induction of structural genes (MdMYB9 and MdMYB11) in 'Red Delicious' apple fruit.

3.4.5. Fruit firmness

Avocado fruit firmness is an important ripeness indicator and directly affects shelf-life (Kassim *et al.*, 2013). The firmness of 'Hass' avocado fruit decreased with ripening time, regardless of treatment (Figure 3.6). These findings were in line with those reported by Shikwambana *et al.* (2021), who observed a decrease in 'Hass' avocado fruit firmness, regardless of treatment. In this study, MeJA application was effective in maintaining fruit firmness when compared with control. Öztürk *et al* (2015) found that postharvest application of 2240 mg•L^{-1} MeJA preserved the fruit firmness of 'Fortune' plums. According to González-Aguilar *et al.* (2001), fruit firmness is dependent on MeJA concentration. However, in the current study MeJA applied at 10 and 100 µmol•L^{-1} showed similar effectiveness in maintaining fruit firmness

during ripening. This was attributed to the effect of MeJA treatment on cell wall degrading enzymes. Similar results were also reported by González-Aguilar *et al.* (2003), who found similar firmness values in 'Sunrise' papaya fruit treated with 10 and 100 μmol•L^{-1} MeJA.

3.4.6. External chilling injury

Avocado fruit 'Hass' are susceptible to cold damage when stored at temperatures below 5.5°C (Bill *et al.*, 2014). In the current study, chilling injury symptoms were observed to be severe in control fruit, whereas, substantially reduced in fruit treated with MeJA (Figure 3.7). These results were similar to those of Glowacz *et al.* (2017b), who observed the increased 'Hass' avocado fruit CI symptoms during storage at 2°C. The control and fruit treated with MeJA showed the poor exocarp colour development. This was attributed to chilling injury as a result of the extended exposure of fruit to ultra-low storage temperature. Mathaba *et al.* (2015) reported that the extended 'Hass' avocado fruit chilling injury symptoms may lead to poor colour change during ripening.

The incidence of chilling injury was high in the fruit treated with higher concentrations of 100 μmol•L^{-1} MeJA compared with the lower concentrations of 10 μmol•L^{-1} MeJA (Figure 3.7). Similar results have been reported in 'Mollar de Elche' pomegranate fruits, in which CI alleviation was achieved at lower concentration 5.0 mM MeJA (García-Pastor *et al.*, 2020). Previous studies have reported that MeJA reduced CI symptoms in horticultural fruits through inhibition of membrane peroxidation, preservation of membrane integrity, increases in heat shock proteins (HSPs), enhanced antioxidant systems and suppressed activities of browning-related enzymes (Khademi *et al.*, 2019). Moreover, studies have reported that HSPs

alleviate CI through stabilization of cell membranes, thus, maintaining the fluidity and integrity of cell membranes in fruits and vegetables (Horvath *et al.*, 2008; Aghdam and Bodbodak, 2013).

3.4.7. Fruit rot

In general, storage conditions are critical to the onset of postharvest diseases and disorders (Ramírez-Gil *et al.*, 2021). In the current study, 'Hass' avocado fruit developed the fruit rot after withdrawal from ultra-low cold storage. Moreover, the control fruit exhibited the pronounced fruit rot incidence when compared with 10 and 100 µmol•L^{-1} MeJA treated fruit (Figure 3.9). These observations were not surprising, since fruit with chilling injury are often highly susceptible to fungal infections (Sevillano *et al.*, 2009). Previous research has shown that treatment of 'Mollar de Elche' pomegranate fruit with 10 and 100 µmol•L^{-1} MeJA reduced the development of fruit rot incidence during storage at 2°C (Sayyari *et al.*, 2011). These results were in agreement with the current study, whereby, fruit treated with 10 µmol•L^{-1} MeJA were observed to be less susceptible to fruit rot. Furthermore, Glowacz and Rees (2016), reported that the exogenous application of 10 and 100 µmol•L^{-1} MeJA on 'Sunrise' papaya and 'Hari Chhal' banana during storage at low temperatures effectively reduced the postharvest rot incidence of the fruits and preserved quality. The results of the current study showed that treatment 'with 100 µmol•L^{-1} MeJA inhibited the occurrence of Hass' avocado fruit rot incidence (Figure 3.9). This was attributed to the effect of MeJA on membrane integrity. These results were confirmed by Glowacz *et al.* (2017a), whereby, 'Hass' avocado fruit treated with 100 µmol•L^{-1} MeJA showed the reduced incidence rot after withdrawal from cold storage. The observed reduction in fruit rot incidence may be due to the effect of MeJA on improved membrane stability and increased activity of chitinase and *β*-1,3-

glucanase enzymes (Cao *et al.*, 2008b; Luo *et al.*, 2011). Based on these results, it can be concluded that MeJA treatment effectively control the fruit rot in 'Hass' avocado fruit.

3.4.8. Vascular browning

According to Yahia (2011), 'Hass' avocado fruit exposure to storage temperature between 3.0 - 5.0°C for 2 weeks resulted in internal flesh browning, failure to ripen and increased susceptibility to pathogen attack. In the current study, fruit treated with MeJA showed the reduced incidence of vascular browning when compared with control fruit (Figure 3.10). Previous study has reported that treatment of 'Qingzhong' loquat fruit with 10 μmol•L^{-1} MeJA effectively reduced the browning incidence after withdrawal from cold storage (Coa *et al.*, 2008a). In addition, the present study showed that treatment with 10 μmol•L^{-1} MeJA inhibited the incidence of 'Hass' avocado fruit vascular browning . In agreement with these results, Cao *et al.* (2009) observed that 'Fuyang' loquat fruit did not brown when treated with 10 μM MeJA during storage at 1°C. Based on these results, MeJA treatments are capable of alleviating chilling injury and vascular browning in 'Hass' avocado fruit.

CHAPTER 4

EFFECT OF SALICYLIC ACID ON QUALITY PRESERVATION OF 'HASS' AVOCADO FRUIT DURING ULTRA-LOW COLD STORAGE

4.1. Introduction

The South African Avocado Industry face challenges of the competitive export market. Supplying high quality fruit and compliant with phytosanitary standards of importing countries require cold sterilization of avocado fruit in order to eradicate various insect pest (Glowacz *et al.*, 2017b). Such a treatment is a well-established phytosanitary standard for most high paying and competitive markets (Sivankalyani *et al.*, 2015). However, the prolonged exposure of fruits to low temperatures results in failure of normal ripening, development of rots, and CI, thus affecting fruit quality. The loss of avocado quality results in enormous economic losses, as fruit marketability is reduced (Yahia, 2011). Previously, several methods have been employed to preserve the quality of 'Hass' avocado fruit.

In the last decade, SA treatments have received much attention as a potential technique to preserve fruit quality in 'Mollar de Elche' pomegranate (Sayyari *et al.*, 2011). Salicylic acid has been reported to inhibit the activities of different senescence and ethylene production enzymes during postharvest storage (ElMasry *et al.* 2009; Pareek *et al*., 2015). Moreover, exogenous SA has been reported to reduce CI through expression of HSPs, antioxidant activity, the arginine pathway that change polyamines, nitric oxide (NO) and proline production and polyphenol oxidase (PPO) enzyme activity (Aghdam and Bodbodak, 2014). However, there is scarcity of information on the application of SA to preserve the quality of 'Hass' avocado fruit.

Therefore, objective of the present study was to determine the effect of salicylic acid on quality preservation of ‘Hass’ avocado fruit under ultra-low cold storage.

4.2. Materials and Methods

4.2.1. Experimental sites

Same as explained in section 3.2.1

4.2.2. Experimental procedure and design

The experiment was carried as a completely randomized design (CRD) with eight replications per treatment. The concentrations of salicylic acid (SA) treatment were 0 (control), 1.0, 2.0 and 3.0 mM. Eighteen fruit were dipped for 30 minutes in SA solution containing the above concentrations. Treated fruit were allowed to dry at room temperature (25°C) for 1 hour. Thereafter, treated fruit were weighed, analysed for colour parameters and firmness, and then packed in commercial carton boxes, subsequently, cold stored at 2°C for 31 days. Fruit were removed from cold storage after 31 days and transferred to room temperature at 25°C to ripen.

4.3. Data collection

All the data collected for weight loss, objective colour, subjective colour, firmness, physiological and pathological disorders were collected as explained in section 3.3.

4.4. Data analysis

The data was analysed using GenStat 20th edition statistical software as explained in section 3.4.

4.5. RESULTS

4.5.1. Fruit weight loss

In this study, the treatment had no significant (P = 0.663) effect on Hass' avocado fruit weight loss 'during ripening (Appendix 4.1). On overall, 'Hass' avocado fruit weight loss increased with days of ripening at ambient temperature (Figure 4.1). However, the control fruit exhibited more weight loss when compared with fruit treated with SA treatment. Furthermore, fruit treated with 3.0 mM SA showed a higher weight loss similar to control fruit from day 4 to day 6 when compared with 1.0 and 2.0 mM treated fruit. overall, fruit treated with 1.0 and 2.0 mM showed the similar trend of reduced weight loss throughout the ripening period.

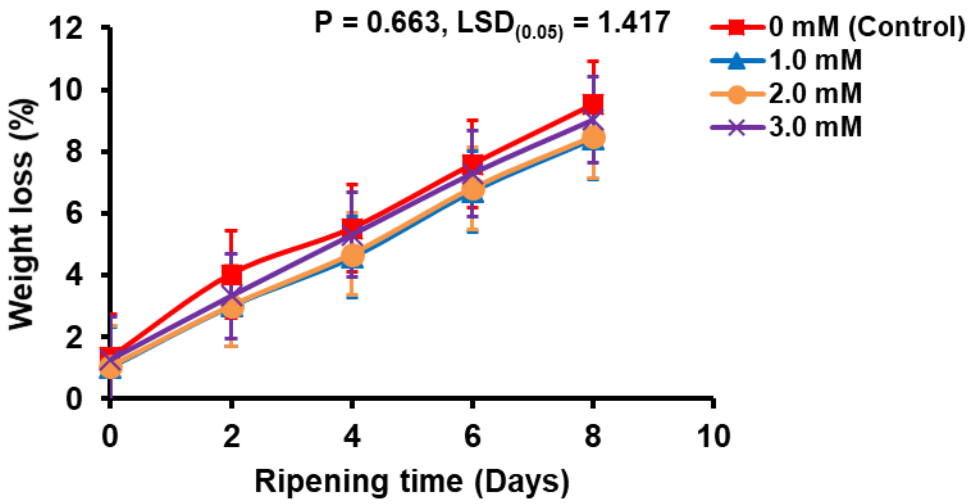

Figure 4.1. Weight loss of 0 (control), 1.0, 2.0 and 3.0 mM SA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

4.5.2. Fruit colour (visual and objective parameters)

There was a significant difference (P = 0.047) in visual colour between the studied treatments during ripening (Appendix 4.2). The 'Hass' avocado exocarp colour was observed to change from emerald-green to olive green during ripening ambient temperature, regardless of treatment (Figure 4.2a). Furthermore, both treated and untreated fruit showed the similar trend of visual colour values from day 0 to day 4 of ripening. Moreover, the control and 2.0 mM SA treated fruit showed similar trend of higher visual colour values from day 4 to day 6 of ripening when compared with 2.0 and 3.0 mM SA treated fruit. Further, fruit treated with 1.0 and 3.0 mM SA showed a similar trend of visual colour throughout the ripening period.

There was no significant difference between SA and control fruit in terms of objective colour L* (P = 0.220) and *h*° (P = 0.066) during ripening (Appendices 4.3 and 4.4). Conversely, there was a significant difference (P = 0.002) between the studied treatment on hue angle (*h*°) during ripening (Appendix 4.5). The objective colour parameters (L*, C* and *h*°) showed a decreasing trend for all treatments during ripening (Figure 4.2b - d). Treatment with 1.0 mM SA showed higher L* values from day 2 to day 6 of ripening when compared with 2.0 and 3.0 mM SA treated and control fruit. Moreover, fruit treated with 3.0 mM SA showed the lowest L* values throughout the ripening period as compared to 1.0 and 2.0 mM SA.

In this study, C* values were higher for 1.0 mM SA treated fruit when compared with 2.0 and 3.0 mM SA treated and control fruit (Figure 4.2c). However, fruit treated with 3.0 mM SA showed the lowest C* values throughout the ripening period. Similarly, *h*° values for control fruit were lower when compared with 1.0, 2.0 and 3.0 mM SA

treatments throughout ripening days at 25°C. Further, 2.0 mM SA treated fruit showed a similar trend with control fruit from day 4 to day 6 of ripening.

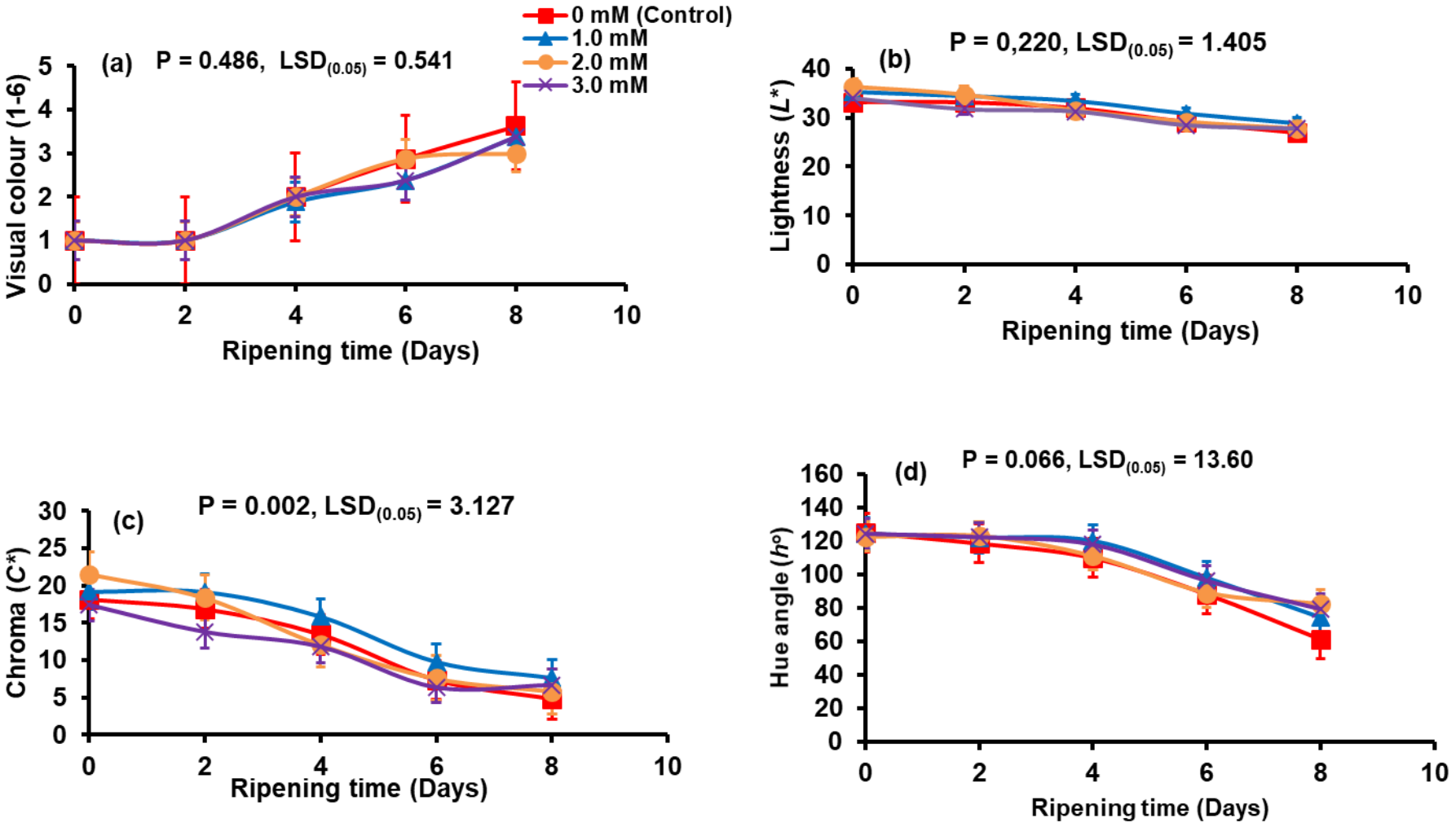

Figure 4.2. Fruit (**a**) visual colour; (**b**) L* values; (**c**) C* values; (**d**) *h*° values of 0 (control), 1.0, 2.0 and 3.0 mM SA 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

4.5.3. Fruit firmness

There was a significant difference ($P < 0.001$) on the studied treatments on 'Hass' avocado fruit firmness loss during ripening (Appendix 4.6). In general, fruit firmness decreased with days of ripening regardless of treatment (Figure 4.3). The control and fruit treated with 1.0, 2.0 and 3.0 mM SA showed the similar trend of firmness loss from day 0 to day 4 of fruit ripening. However, fruit treated with 1.0, 2.0 and 3.0 mM SA showed the reduced firmness loss from day 4 to day 8 when compared with the control fruit.

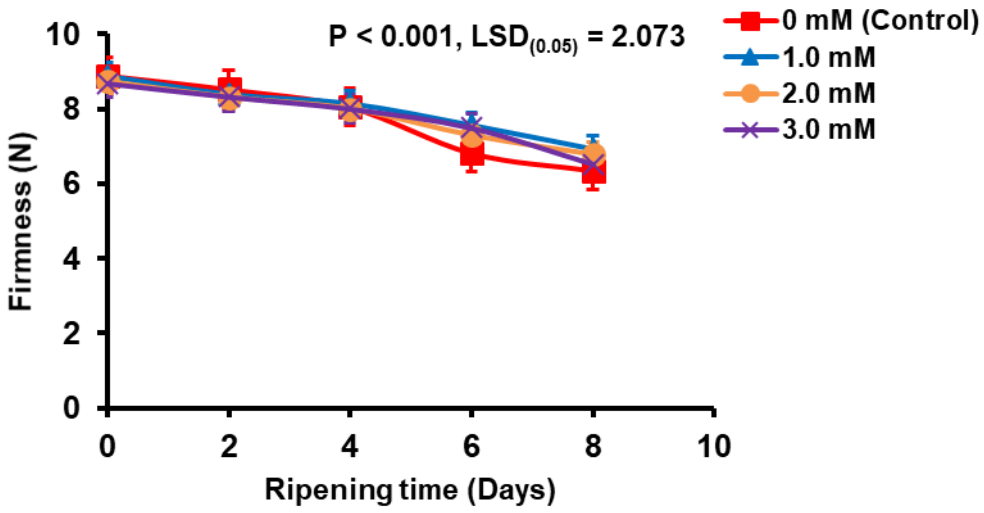

Figure 4.3. Fruit firmness of 0 (control), 1.0, 2.0 and 3.0 mM SA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at $P \leq 0.05$

4.5.4. External chilling injury

In this study, the treatment had the significant ($P = 0.020$) effect on 'Hass' avocado fruit external chilling injury symptoms after the ultra-low cold storage (Appendix 4.7). The control fruit showed the significantly higher chilling symptoms when compared

with SA treated fruit (Figure 4.4). Furthermore, fruit treated with 3.0 mM SA showed the higher chilling injury symptoms when compared with fruit treated with 1.0 and 2.0 mM SA. Overall, treatment of fruit with 1.0 mM SA was observed to be effective in reducing the chilling symptoms when compared with fruit treated with 2.0 and 3.0 mM SA and control fruit

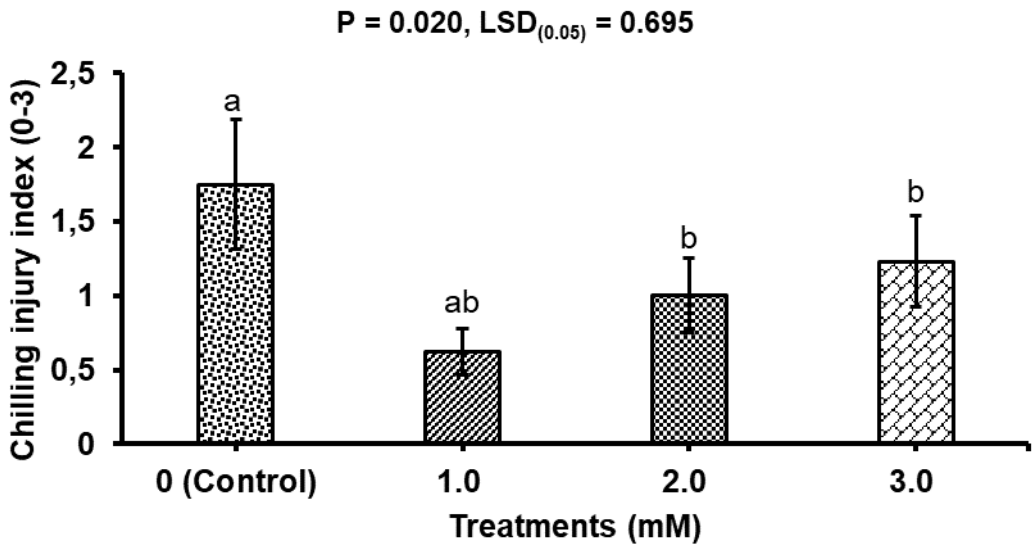

Figure 4.4. External chilling injury of 0 (control), 1.0, 2.0 and 3.0 mM SA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

4.5.5. Fruit rot

A significant difference was not observed (P = 0.541) between the treatments on avocado fruit rot incidence during ripening (Appendix 4.9). The control fruit showed the highest rot incidence when compare with fruit treated with 1.0, 2.0 and 3.0 mM SA (Figure 4.6). Furthermore, treatment of fruit with 1.0, 2.0 and 3.0 mM SA was observed to be effective in inducing resistance of 'Hass' avocado fruit to rot during ripening.

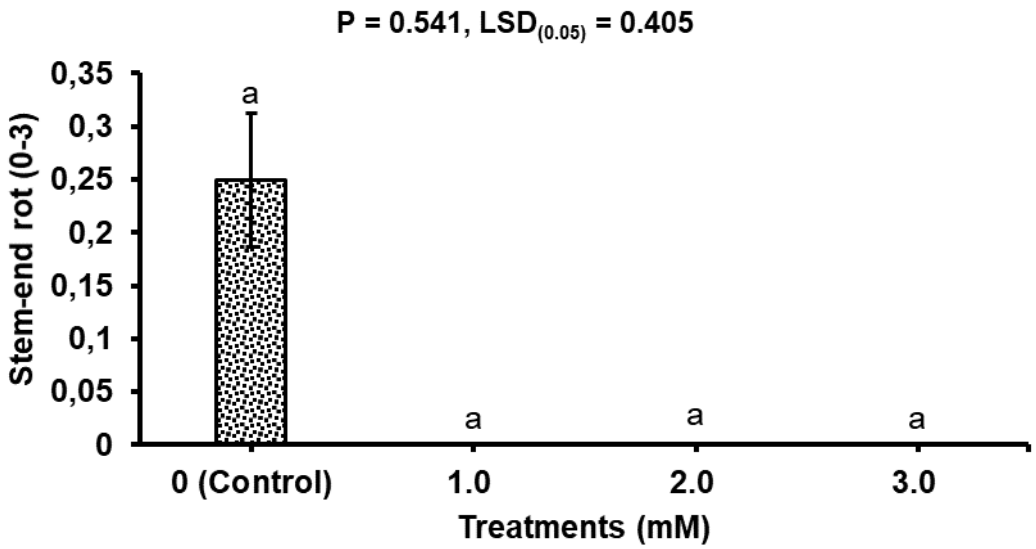

Figure 4.6. Fruit rot of 0 (control), 1.0, 2.0 and 3.0 mM SA 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

4.5.6. Vascular browning

There was no significant difference (P = 0.550) between the studied treatments on 'Hass' avocado fruit vascular browning incidence after withdrawal from ultra-low cold storage (Appendix 4.10). However, the control fruit showed the highest incidence of vascular browning when compared with fruit treated with salicylic acid (Figure 4.7). Furthermore, fruit treated with 2.0 and 3.0 mM SA showed the similar results of higher vascular browning incidence when compared with fruit treated with 1.0 mM SA treated fruit. Overall, treatment of 'Hass' avocado fruit with 1.0 mM SA was observed to be effective in inhibiting the development of vascular browning incidence during ripening.

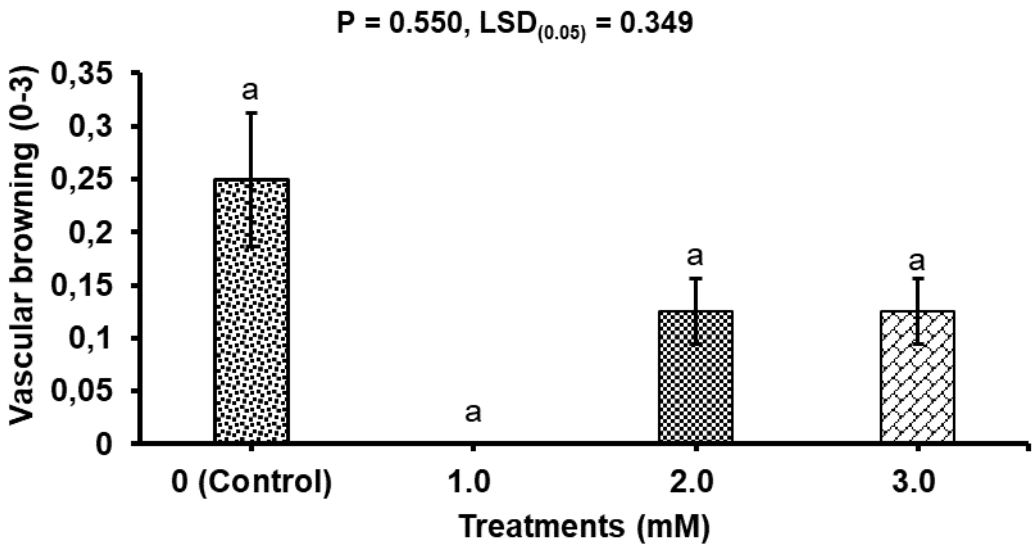

Figure 4.7. Vascular browning of 0 (control), 1.0, 2.0 and 3.0 mM SA treated 'Hass' avocado fruit during ripening at ambient temperature. Values are means of 8 replicates (n = 8) and ±SE of means at P ≤ 0.05

4.6. DISCUSSION

4.6.1. Fruit weight loss

The activation of resistance mechanisms against water stress conditions is very important for the reduction of postharvest losses, since water loss is one of the major causes of postharvest losses during handling and storage of fruit crops (Asghari, 2019). In this study, the results showed that treatment of with 1.0, 2.0 and 3.0 mM SA was effective in reducing 'Hass' avocado fruit weight loss when compared control fruit. This was attributed to the potential of SA on reduction of water loss in 'Hass' avocado fruit. These results were in agreement with Shen and Yang (2017) who observed the reduced weight loss in 'Punjab Beauty' pear fruit treated with 2.0 mM SA. These authors reported that this was due to the effect of SA on ripening and

senescence delay. Furthermore, exogenous 2.0 mM SA application combined with chitosan was reported to reduce the water loss and respiration rate of ‘Punjab Beauty’ pear through blockage of fruit lenticels (Shen and Yang, 2017).

In the current study, treatment of ‘Hass’ avocado fruit with 1.0 and 2.0 mM SA reduced the weight loss when compared with 3.0 mM SA treated and control fruit. This was attributed to the effect of SA on reduced metabolic activities of the fruit. In agreement with these results, Haider *et al.* (2020) reported that SA inhibit the activities of senescence enzymes such as 1-aminocyclopropane-1-carboxylic acid synthase (ACS), 1-aminocyclopropane-1-carboxylic acid oxidase (ACO), polygalacturonase (PG) and pectin methyl esterase (PME) in ‘Kinnow’ mandarin fruit. In horticultural crops, enzyme inhibition decreases respiration rate (Aghdam *et al.*, 2014; Haider *et al.*, 2020). Srivastava and Dwivedi (2000) reported that 1.0 mM SA treatment decreased the respiration rate, ethylene production and cell wall degrading enzyme activities in ‘Hari Chhal’ banana fruit.

Fruit treated with high concentrations of SA lost higher weight from day 2 to day 6 of ripening when compared with fruit treated with lower concentrations (Figure 4.1). This was attributed to the effect of SA on ethylene production when applied at higher concentration. In agreement with these results, Van de Poel *et al.* (2012) reported the increased respiration rate and ethylene production in ‘Bonaparte’ tomato fruit treated with 10 μmol•L^{-1} MeSA where the dose was too low to limit ethylene production. However, Alali *et al.* (2021) found that SA reduced weight loss in ‘Grand Nain’ banana fruit when applied at a higher concentration (2.0 mM).

4.6.2. Fruit colour (visual and objective parameters)

Visual quality is an important aspect for the food supply chain, since fresh produce with good appearance is preferred by customers and retailers (Glowacz and Rees, 2016). During the current study, visual colour change from emerald green to olive green was observed in fruit treated and untreated with SA (Figure 4.2a). According to Mathaba *et al.* (2015), chilling damage development in 'Hass' avocado fruit result in poor exocarp colour development. These results were an agreement with the current study whereby, the development of purple exocarp colour was inhibited in all treatments throughout the ripening period as a result of chilling symptom development.

In the current study, a decrease in exocarp colour parameters; lightness (L*), chroma (C*) and hue angle (h°) (Figure 4.2b - d) for all fruit treated and untreated with salicylic acid was observed. Similarly, Valero *et al.* (2011) observed the decreased exocarp L* in 'Cristalina' and 'Prime Giant' sweet cherries treated with 1000 µmol•L^{-1} SA. These authors reported that the decrease in fruit chromaticity was due to the degradation of chlorophyll and flavonoids accumulation. According to Wills *et al.* (2007), the loss of green colour during fruit senescence has been associated with degradation of chlorophyll structure as a result of the chlorophyllase enzyme activity. The results obtained in this study showed the lower L*, C* and h° values in 2.0 and 3.0 mM SA treated fruit when compared with fruit treated with 1.0 mM SA (Figure 4.2b – d). Studies have shown that exogenous 2.0 mM SA enhance chlorophyll degradation by increasing the activity of chlorophyllase (Champa and Gamage, 2020; Cavusoglu *et al.*, 2021). These results were in agreement with the current study, whereby, a change from emerald green to olive green colour was observed.

Anthocyanin biosynthetic pathway is regulated by enzyme activities such as phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), dihydroflavonol 4-reductase (DFR), leucoanthocyanidin dioxygenase (LDOX) and UDP-glucose: flavonoid 3-O-glucosyltransferase (UFGT) (Mori *et al.*, 2005; Obinatai *et al.*, 2003). Results from previous studies have reported that the exogenous application of 10^{-3} and 10^{-5} M SA enhanced the anthocyanin biosynthesis in 'Halia Bara' ginger through the activation of the chalcone synthase (CHS) enzyme activity (Ghasemzadeh *et al.*, 2012). However, in the current study treatment of 'Hass' avocado fruit with 1.0, 2.0 and 3.0 mM SA failed to induce the development of purple/black colour during fruit ripening. This was attributed to effect of SA treatment on activation of anthocyanin biosynthetic enzymes which failed to induce the accumulation of anthocyanin in the fruit exocarp.

4.6.3. Fruit firmness

Texture loss during storage is a serious problem for fresh produce industry because it reduces marketability of the product (Glowacz and Rees, 2016). In the current study. fruit firmness of 'Hass' avocado was observed to decrease with ripening time regardless of treatment (Figure 4.3). Furthermore, firmness values of fruit treated with 1.0 and 2.0 mM SA were higher throughout the ripening compared with control and fruit treated with 3.0 mM SA. The high firmness may be due to the effect of SA on decreased activities of fruit softening enzymes, mainly polygalacturonase (PG), cellulase and pectin methyl esterase (PME), which account for hydrolysing glycosidic linkage in the cell wall integrity which is directly related to ripening (Marin-Rodriguez *et al.*, 2002; Valero and Serrano, 2010). Baswal *et al.* (2020) found that application of 0.002 $\mu mol \bullet L^{-1}$ SA maintained fruit quality by decreasing weight loss, firmness and

retarded the activities of enzymes mainly cellulase and PME. Moreover, Srivastava and Dwivedi (2000) reported that 1.0 mM SA treatment decreased the respiration rate, ethylene production and cell wall degrading enzyme activities in 'Hari Chhal' bananas.

4.6.4. External chilling injury

Chilling injury is one of the major challenges of avocado trade growth on the world market (Glowacz *et al.*, 2017b). The present study showed that fruit treated with salicylic acid had the reduced chilling injury symptoms when compared with the control fruit (Figure 4.4). Furthermore, treatment of fruit with 1.0 mM SA was observed to be effective in reducing the chilling symptoms when compared with fruit treated with 2.0 and 3.0 mM SA. These results were consistent with Khademi *et al.* (2019), whereby, CI was effectively reduced in 'Cavendish' banana fruit treated with 1.0 mM SA. Rasouli *et al.* (2019) reported that 2.0 mM SA treatment was effective in alleviating chilling injury in 'Navel' orange fruit during cold storage at 1°C. This was in agreement with the current study in which 2.0 mM SA substantially reduced the development of CI symptoms compared with 3.0 mM SA treated and control fruit.

The reduction in CI development in the current study was associated to the effect of SA on fatty acids, fruit membrane integrity and chilling stress responsive enzymes. According to Razavi *et al.* (2018), plant tissue subjected to chilling stress undergoes physical cell membrane phase transition from a normal flexible, liquid-crystalline to a solid gel structure. When membranes solidify, membrane fluidity changes and membrane lipids undergo phase transition which results in configuration of certain essential membrane-bound proteins and enzymes. Glowacz *et al.* (2017b) and Aghdam *et al.* (2016) reported that SA can alleviate CI by alteration of fatty acid

content, reduction of cell membrane damage, and LOX enzymatic activities, thus, decreasing lipid peroxidation. These results were in agreement with the results of the current study whereby chilling injury was substantially reduced in fruit treated with 1.0 and 2.0 mM SA (Figure 4.4).

4.6.5. Fruit rot

Postharvest fruit rot is one of the main problems affecting avocado quality during storage and leads to important economic losses (Ramírez-Gil *et al.*, 2021). During the current study, fruit rot was more prevalent in control fruit when compared with fruit treated with SA (Figure 4.6). According to Tareen *et al.* (2012), exogenous treatment of 'Flordaking' peach fruit with 500 µmol•L^{-1} SA reduced the incidence of rot and decay during storage at low temperatures. These authors reported that this was associated with the effect of SA on maintenance of fruit cellular and membrane integrity. Similar to their study, the current study found that treatment with 1.0, 2.0 and 3.0 mM SA effectively inhibited the development of fruit rot incidence in 'Hass' avocado during storage and ripening. According to Glowacz *et al.* (2017a), 100 µmol•L^{-1} MeSA treatment effectively reduced the rot incidence in 'Hass' avocado fruit during storage at 2°C. These authors reported this was due to the increased activities of chitinase and *β*-1,3-glucanase enzymes. Previous studies demonstrated that chitinase and *β*-1,3-glucanase enzymes are responsible for hydrolysing polymers of fungal cell wall leading to weakened cell wall and cell lysis, thus, delaying fungal growth (Stintzi *et al.*, 1993; Theis and Stahl, 2004).

4.6.6. Vascular browning

Vascular browning is one of the important economic disorders which affects the avocado trade growth on the world market (Arpaia *et al.*, 2004). The current study

showed that the incidence of vascular browning was less pronounced in fruit treated with SA when compared with control. Furthermore, fruit treated with 1.0 mM SA inhibited the development of vascular browning when compared with 2.0 and 3.0 mM SA treated and control fruit. Previous studies have shown that 1000 μmol•L^{-1} SA treatments were effective in reducing the incidence of internal browning in 'Luoyangqing' loquat during storage at low temperatures (Cai *et al.*, 2006). Moreover, a study by Zhao *et al.* (2021) showed that 1 μmol•L^{-1} SA delayed the internal browning of 'Jinqiuhongmi' peach fruit stored at 4°C. These authors reported that this was due to the effect of SA on CI alleviation and activity of enzymes responsible for oxidation. Based on these results, it can be concluded that SA treatments are capable of alleviating chilling injury and vascular browning in 'Hass' avocado fruit.

CHAPTER 5

SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

5.1. Summary

The purpose of this study was to investigate the effect that MeJA and SA have on quality preservation of ‘Hass’ avocado during the ultra-low cold storage. Methyl jasmonate treatment had no significant impact on fruit weight, visual and objective colour, chilling injury and pathological and physiological disorders. However, the significant effects were recorded on fruit firmness. In comparison with 10 µmol•L^{-1} MeJA, treatment with 100 µmol•L^{-1} MeJA caused high weight loss coupled with severe chilling injury symptoms. The results indicated that 10 µmol•L^{-1} MeJA was the effective treatment with regard to reducing weight loss and chilling injury symptoms. Moreover, treatment with 10 µmol•L^{-1} MeJA maintained a higher unit of firmness throughout the ripening period compared with the control and 100 µmol•L^{-1} MeJA. In general, postharvest application of 10 µmol•L^{-1} MeJA induced tolerance to CI and preserved quality of ‘Hass’ avocado fruit. However, this treatment failed to enhance the purple/black colour development of the fruit but only showed colour change from emerald-green to olive-green.

Salicylic treatment had the significant effect on Chilling injury, firmness and visual colour of ‘Hass’ avocado fruit. However, the treatment had no significant impact on weight loss and pathological and physiological disorders. Treatment of SA at high concentration (3.0 mM) showed the higher fruit weight loss. Further, treatment with 1.0 and 2.0 mM SA failed to enhance the development of black colour in fruit during ripening. Moreover, SA (1.0 mM) treatment was observed to effectively maintain fruit firmness and inhibit the development of pathological and physiological disorders. It

can therefore be concluded that expression of antioxidants involved in metabolic pathway although not quantified in the current study was possibly not highly affected when fruit were treated with 100 µmol•L^{-1} MeJA and 3.0 mM SA compared with 10 µmol•L^{-1} MeJA and 1.0 and 2.0 mM SA

5.2. Recommended future research

The current study did not include the antioxidant study, analysis of enzymatic antioxidants and the impact of the treatments on membrane integrity and solute leakage. Therefore, future research should aim at developing assays to quantify the potency of enzymatic antioxidants (e.g., PPO, SOD, CAT, POD) and develop antioxidant capacity that also includes bioactive compounds and enzymatic antioxidants. Membrane composition can be affected by lipid composition. Cellular membranes which contain highly saturated, long-chained lipids are more chilling than those containing unsaturated long-chained lipids. Fruit sourced from chilling resistant micro-climates might have an altered membrane lipid composition compared with those from chilling-susceptible climates. Therefore, the role of micro-climate and orchard practises on membrane lipid composition must be investigated before application of any post-harvest treatments such as methyl jasmonate or salicylic acid is commercially recommended.

5.3. Conclusion

The present study showed that postharvest application of MeJA and SA had profound effect on quality of ‘Hass’ avocado. In conclusion, MeJA and SA can be used as postharvest treatments on chilling injury and avocado quality during ultra-low cold storage. The best treatments for 'Hass' avocado fruit was salicylic acid (1.0

mM) as quality parameters such as weight loss and firmness were maintained. In addition to providing good external and internal quality, this treatment also reduced the development of postharvest diseases and disorders when compared with 10 and 100 µmol•L^{-1} MeJA and 2.0 and 3.0 mM SA. To prevent CI of 'Hass' avocado fruit and poor exocarp colour development during ripening, further investigations are required regarding the combination of MeJA and SA and fruit maturity.

REFERENCES

Aghdam, M.S. and Bodbodak, S. 2013. Physiological and biochemical mechanisms regulating chilling tolerance in fruit and vegetables under postharvest salicylates and jasmonates treatments. *Scientia Horticulturae*, 156: 73-85.

Aghdam, M.S. and Bodbodak, S. 2014. Postharvest heat treatment for mitigation of chilling injury in fruit and vegetables. *Food Bioprocessing Technology*, 7: 37-53.

Aghdam, M.S., Asghari, M., Babalar, M. and Sarcheshmeh, M.A.A. 2016. Impact of salicylic acid on postharvest physiology of fruits and vegetables. *Eco-Friendly Technology for Postharvest Produce Quality*, 8: 243-268.

Aghdam, M.S., Asghari, M., Khorsandi, O. and Mohayeji, M. 2014. Alleviation of postharvest chilling injury of tomato fruit by salicylic acid treatment. *Journal of Food Science Technology*, 51: 2815-2820.

Alali, A.A., Awad, M.A., Al-Qurashi, A.D. and Mohamed, S.A. 2021. Postharvest gum Arabic and salicylic acid dipping affect quality and biochemical changes of 'Grand Nain' bananas during shelf life. *Scientia Horticulturae*, 237: 51-58.

Ali, I., Wang, X., Tareen, M.J., Wattoo, F.M., Qayyum, A., Hussain, M., Shafique, M., Liaquat, M., Asghar, S., Hussain, T., Faiz, S. and Ahmed, W. 2021. Foliar application of salicylic acid at different phenological stages of peach fruit 'Flordaking' improves harvest quality and reduces chilling injury during low temperature storage. *Plants*, 10: 1-20.

An, X.H., Tian, Y., Chen, K.Q., Liu, X.J., Liu, D.D., Xie, X.B., Cheng, C.G., Cong, P.H. and Hao, Y.J. 2015. MdMYB9 and MdMYB11 are involved in the

regulation of the JA-induced biosynthesis of anthocyanin and pro-anthocyanidin in apples. *Plant Cell Physiology*, 56: 650-662.

Arpaia, M.L., Collin, S., Sievert, J. and Obenland, D. 2015. Influence of cold storage prior to and after ripening on quality factors and sensory attributes of 'Hass' avocado. *Postharvest Biology and Technology*, 110: 149-157.

Arpaia, M.L., Van Rooyen, Z., Bower, J.P., Hofman, P.J. and Woolf, A.B. 2004. Grower practices will influence postharvest fruit quality, II International Avocado Conference Quillota, Chile: 1-9.

Arzate-Vazquez, I., Chanona-Perez, J.J., De Jesus Perea-Flore, M., Calderón-Domínguez, G., Moreno-Armendάriz, M.A., Calvo, H., Godoy-Calderón, S., Quevedo, R. and Gutiérrez-López, G. 2011. Image processing applied to classification of avocado variety 'Hass' *(Persea americana* Mill.) during ripening process. *Food and Bioprocess Technology*, 4: 1307-1313.

Asghari, M. 2019. Impact of jasmonates on safety, productivity and physiology of food crops. *Trends in Food Science and Technology*, 91: 169-183.

Baswal, A., Dhaliwal, H., Singh, Z., Mahajan, B. and Gill, K. 2020. Postharvest application of methyl jasmonate, 1-methylcyclopropene and salicylic acid extends the cold storage life and maintain the quality of "Kinnow" mandarin (*Citrus nobilis* L. X C. deliciosa L.) fruit. *Postharvest Biology and Technology*, 161: 1-8.

Bill, M., Sivakumar, D., Thompson, K. and Korsten, L. 2014. Avocado fruit quality management during the postharvest supply chain. *Food Reviews International,* 30: 169-202.

Blakey, R.J. 2011. Management of avocado postharvest physiology. PhD. thesis, School of Agricultural Sciences and Agribusiness. University of KwaZulu-Natal, Pietermaritzburg, South Africa

Blakey, R.J. and Bower, J.P. 2007. The feasibility of a hot water treatment for South African avocados (*Persea americana* [Mill.] cv Hass). *South African Avocado Growers' Association Yearbook*, 30: 66-68.

Blakey, R.J., Tesfay, S.Z., Bertling, I. and Bower, J.P. 2014. Ripening physiology and quality of 'Hass' avocado (*Persea americana* Mill.) after cold storage at 1°C. *Journal of Horticultural Science and Biotechnology*, 89(6): 655-662.

Bower, J.P. and Jackson, J. 2003. Effect of fruit coating and packaging on external and internal quality. *South African Avocado Growers' Association Yearbook,* 26: 15-19.

Burdon, J., Connolly, P., de Silva, N., Lallu, N., Dixon, J. and Pak, H. 2013. A meta-analysis using a logit non-linear mixed effects model for 'Hass' avocado postharvest performance data. *Postharvest Biology and Technology,* 86: 134-140.

Cai, C., Li, X. and Chen, K. 2006. Acetylsalicylic acid alleviates chilling injury of postharvest loquat (*Eriobotrya japonica* Lindl.) fruit. *European Food Research Technology*, 223: 533-539.

Cao, S., Zheng, Y., Yang, Z., Tang, S. and Jin, P. 2008a. Control of anthracnose rot and quality deterioration in loquat fruit with methyl jasmonate. *Journal of the Science of Food and Agriculture*, 88: 1598-1602.

Cao, S., Zheng, Y., Yang, Z., Tang, S., Jin, P., Wang, K. and Wang, X. 2008b. Effect of methyl jasmonate on inhibition of *Colletotrichum acutatum* infection in loquat fruit and the possible mechanisms. *Postharvest Biology and Technology*, 49: 301-307.

Cao, S.F. and Zheng, Y.H. 2008c. Post-harvest biology and handling of loquat fruit. *Stewart Post-harvest Review*, 4: 1-5.

Cao, S.F., Hu, Z.C. and Wang, H.O. 2009. Effect of salicylic acid on the activities of antioxidant enzymes and phenylalanine ammonia-lyase in cucumber fruit in relation to chilling injury. *Journal Horticultural Science and Biotechnology*, 84: 125-130.

Carvajal, F., Rosales, R., Palma, F., Manzane, S., Canizares, J., Jamilena, M. and Garrido, D. 2018. Transcriptomic changes in Cucurbita pepo fruit after cold storage; differential response between two cultivars contrasting in chilling sensitivity. *BMC Genomics*, 19: 1-16.

Cavusoglu, S., Yilmaz, N., Islek, F., Tekin, O., Sagbas, H.I., Ercisli, S., Rampácková, E and Necas, T. 2021. Effect of methyl jasmonate, cytokinin, and lavender oil on antioxidant enzyme system of apricot fruit (*Prunus armeniaca* L.). *Sustainability*, 13: 1-10.

Champa, W.A.H. and Gamage, K.G.N.M. 2020. Postharvest dip application of putrescine and salicylic acid delayed postharvest quality deterioration and extended the storage life of Lime (*Citrus Aurantifolia* Swingle) Fruit. *International Journal of Fruit Science*, 3: 1629-1638.

Chen, L., Zhao, X., Wu, J. He, Y. and Yang, H. 2020. Metabolic analysis of salicylic acid-induced chilling tolerance of banana using NMR. *Food Research International*, 128: 1-10.

Chen, M., Guo, H., Chen, S., Li, T., Li, M., Rashid, A. and Wang, K. 2019. Methyl jasmonate promotes phospholipid remodelling and jasmonic acid signalling to alleviate chilling injury in peach fruit. *Journal of Agricultural and Food Chemistry*, 67: 9958-9966.

Chung, H.S., Niu, Y., Browse, J. and Howe, G.A. 2009. Top hits in contemporary JAZ: An update on jasmonate signalling. *Phytochemistry*, 70: 1547-1559.

Cox, K.A., Mcghie, T.K., White, A. and Woolf A.B. 2004. Skin colour and pigment changes during ripening of 'Hass' avocado fruit. *Postharvest Biology and Technology*, 31: 287-294.

Crane, J.H., Douhan, G., Faber, B.A., Arpaia, M.L., Bender, G.S., Balerdi, C.F. and Barrientos-Priego, A.F. 2013. Cultivars and rootstocks, In: Schaffer, B., Wolstenholme, B.N. and Whiley, A.W. (Eds.), The avocado: botany, production and uses, (2nd ed.). CAB International, Nosworthy Way, Wallingford, UK.

Creelman, R.A. and Mullet, J.E. 1997. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, 48: 355-381.

Department of Agriculture, Forestry and Fisheries (DAFF). 2019. A profile of South African avocado market value chain, national department of agriculture. Pretoria, South Africa.

Ding, C. K., Wang, C.Y., Gross, K.C. and Smith, D.L. 2002. Jasmonate and salicylate induce the expression of pathogenesis-related-protein genes and increase resistance to chilling injury in tomato fruit. *Planta*, 214: 895-901.

Donetti, M. and Terry, L.A. 2011. Investigation of skin colour changes as non-destructive parameter of fruit ripeness of imported 'Hass' avocado fruit. *Acta Horticulturae*, 945: 189-193.

Dorria, M., Ahmed, A. M. L., Yousef, R. M. and Hassan, H. S. A. 2010. Relationship between electrical conductivity, softening and colour of 'Fuerte' avocado fruit during ripening. *Agriculture and Biology Journal of North America,* 1: 878-885.

Eaks, I.L. 1990. Change in the fatty acid composition of avocado fruit during ontogeny, cold storage and ripening. *Acta Horticulturae*, 269: 141-152.

ElMasry, G., Wang, N. and Vigneault, C. 2009. Detecting chilling injury in Red Delicious apple using hyperspectral imaging and neural networks. *Postharvest Biology and Technology*, 52: 1-8.

Ezzat, A., Ammar, A., Szabo, Z., Nyeki, J. and Holb, I.J. 2017. Postharvest treatments with methyl jasmonate and salicylic acid for maintaining physico-chemical characteristics and sensory quality properties of apricot fruit during cold storage and shelf-Life. *Polish Journal of Food and Nutrition Sciences*, 67: 159-166.

Fan, X., Fellman, J.K. and Patterson, M.E. 1997. Effect of methyl jasmonate on ethylene and volatile production by summerred apples depends on fruit developmental stage. *Journal of Agricultural and Food Chemistry,* 45: 208-211.

Fung, R.W., Wang, C.Y., Smith, D.L., Gross, K.C., Tao, Y and Tian, M. 2006 Characterization of alternative oxidase (AOX) gene expression in response to methyl salicylate and methyl jasmonate pre-treatment and low temperature in tomatoes. *Journal of Plant Physiology*, 163: 1049-1060

García-Pastor, M. E., Serrano, M., Guillén, F., Giménez, M. J., Martínez-Romero, D., Valero, D. and Zapata, P. J. 2020. Preharvest application of methyl jasmonate increases crop yield, fruit quality and bioactive compounds in pomegranate 'Mollar de Elche' at harvest and during postharvest storage. *Journal of Food Science and Agriculture*, 100: 145-153.

Ghasemzadeh, Ali., Hawa, Z., Jaafar, E. and Karimi, E. 2012. Involvement of salicylic acid on antioxidant and anticancer properties, anthocyanin production and chalcone synthase activity in ginger (*Zingiber officinale* Roscoe) varieties. *International Journal of Molecular Sciences*, 13: 14828-14844.

Glowacz, M. and Rees, D. 2016. Using jasmonates and salicylates to reduce losses within the fruit supply chain. *European Food Research Technology*, 242: 143-156.

Glowacz, M., Bill, M., Tinyane, P.P. and Sivakumar, D. 2017b. Maintaining postharvest quality of cold stored ’Hass’ avocados by altering the fatty acids content and composition with the use of natural volatile compounds - methyl jasmonate and methyl salicylate. *Journal of the Science of Food and Agriculture*, 97: 5186-5193.

Glowacz, M., Roets, N. and Sivakumar, D. 2017a. Control of anthracnose disease via increased activity of defence related enzymes in ‘Hass’ avocado fruit

treated with methyl jasmonate and methyl salicylate. *Food Chemistry*, 234: 163-167.

González-Aguilar, G., Tiznado-Hernandez, M.E. and Martinez-Tellez, M.A. 2004. Methyl jasmonate treatment reduce chilling injury and activate the defence of guava fruits. *Biochemical and Biophysical Research Communications*, 313: 694-701.

González-Aguilar, G.A., Buta, J.G. and Wang, C.Y. 2001. Methyl jasmonate reduces chilling injury symptoms and enhances colour development of 'Kent' mangoes. *Journal of the Science of Food and Agriculture*, 81: 1244-1249.

González-Aguilar, G.A., Buta, J.G. and Wang, C.Y. 2003. Methyl jasmonate and modified atmosphere packaging (MAP) reduce decay and maintain postharvest quality of papaya 'Sunrise'. *Postharvest Biology and Technology*, 28: 361-370.

Gu, K., Wang, C., Hu, D. and Hao, Y. 2019. How do anthocyanins paint our horticultural products? *Scientia Horticulturae*, 249: 257-262.

Habibi, F. and Ramezanian, A. 2017. Vacuum infiltration of putrescine enhances bioactive compounds and maintains quality of blood orange during cold storage. *Food Chemistry*, 227: 1-8.

Habibi, F., Ramezanian, A., Rahemi, M., Eshghi, S., Guillén, F., Serrano, M. and Valero, D. 2019. Postharvest treatments with γ-aminobutyric acid, methyl jasmonate or methyl salicylate enhance chilling tolerance of blood orange fruit at prolonged cold storage. *Journal of the Science of Food and Agriculture*, 99: 6408-6417.

Haider, S.T., Ahmad, S., Khan, A.S., Anjum, M.A., Nasir, M. and Naz, S. 2020. Effect of salicylic acid on postharvest quality of “Kinnow” mandarin under cold storage. *Scientia Horticulturae*, 259: 1-13.

Hartill, W.F.T. and Everett, K.R. 2002. Inoculum sources and infection pathways of pathogens causing fruit rots of ‘Hass’ avocado (*Persea Americana). New Zealand Journal of Crop and Horticultural Science*, 30: 249-260.

Horvath, I., Multhoff, G., Sonnleitner, A. and Vígh, L. 2008. Membrane-associated stress proteins: more than simply chaperones. *Biochimica Biophysica Acta.* 1778: 1653-1664.

Johnson, G.I. and J.M. Kotze. 1994. Avocado stem end rot. In: Ploetz, R.C., Zentmyer, G.A., Nishijima, W.T, Rohrbach, K.G. and OHR, H.D. (eds.) Compendium of Tropical Fruit Diseases. St Paul, Minnesota.

Kassim, A. and Workneh, T.S. 2020. Influence of postharvest treatments and storage conditions on the quality of ‘Hass’ avocados. *Heliyon*, 6: 1-9.

Kassim, A., Workneh, T.S. and Bezuidenhout, C.N. 2013. A review on postharvest handling of avocado fruit. *African Journal of Agriculture Research*, 8: 2385-2402

Kayesh, E., Shangguan, L., Korir, N.K., Sun, X., Bilkish, B., Zhang, Y., Han, J., Song, J., Cheng, Z. and Fang, J. 2013. Fruit skin colour and the role of anthocyanin. *Acta Physiologiae Plantarum*, 35: 2879-2890.

Khademi, O., Ashtari, M. and Razavi, F. 2019. Effects of salicylic acid and ultrasound treatments on chilling injury control and quality preservation in banana fruit during cold storage. *Scientia Horticulturae*, 249: 334-339.

Köhne, J.S., Kremer-Köhne, S. and Gay, S.H. 1998. Non-destructive avocado fruit firmness measurement. *South African Avocado Growers' Association Yearbook*, 21: 19-21.

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

Kruger, F.J. and Lemmer, D. 2012. Respiration and softening rates of 'Maluma' and 'Ryan' avocados and the effect that cold chain breaks have on the ripening and quality of these cultivars. *South African Avocado Growers' Association Yearbook*, 35: 12-20.

Lafuente, M. T., Zacarias, L., Sala, J.M., Sánchez-Ballesta, M.T., Gosalbes, M.J., Marcos, J.F. and González-Candelas, L. 2005. Understanding the basis of chilling injury in citrus fruit. *Acta Horticulturae*, 682: 831-842.

Lallu, N., Punter, M., Haynes, G., Pidakala, P. and Burdon, J. 2004. Role of water loss in ripening of 'Hass' avocados. *New Zealand Avocado Growers' Association Annual Research Report,* 4: 70-79.

Leclereq, H. 1990. Observations on overseas markets during the 1989 avocado season. *South African Avocado Growers' Association Yearbook*, 13: 11-13.

Loreti, E., Povero, G., Novi, G., Solfanelli, C., Alpi, A. and Perata, P. 2008. Gibberellins, jasmonate and abscisic acid modulate the sucrose- induced expression of anthocyanin biosynthetic genes in Arabidopsis. *New Phytologist*, 179: 1004-1016.

Los, D.A. and Murata, N. 2004. Membrane fluidity and its roles in the perception of environmental signals. *Biochimica Biophysica Acta*, 1666: 142-157.

Luo, Z., Chen, C. and Xei, J. 2011. Effect of salicylic acid treatment on alleviating postharvest chilling injury of 'Qingnai' plum fruit. *Postharvest Biology and Technology*, 62: 115-120.

Lütge, A. 2011. Ultra-low temperature shipping and cold chain management of 'Fuerte' avocados (*Persea americana* Mill.) grown in the KwaZulu-Natal midlands. School of Agricultural, Earth and Environmental Science. MSc (space) English dissertation, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

Lütge, A., Bower, J.P. and Bertling, I. 2010. Low temperature shipping and cold chain management of 'Fuerte' avocados: An opportunity to reduce shipping costs. *South African Avocado Growers' Association Yearbook*, 33: 39-43.

Lyons, M.J. 1973. Chilling injury in plants, Annual. *Reviews of Plant Physiology*, 24: 445-466.

Marin-Rodriguez, M.C., Orchard, J. and Seymour, G.B. 2002. Pectate lyases, cell wall degradation and fruit softening. *Journal of Experimental Botany,* 53: 2115-2119.

Martínez-Esplá, A., Serrano, M., Valero, D., Martínez-Romero, D., Castillo, S. and Zapata, P.J. 2017. Enhancement of antioxidant systems and storability of two plum cultivars by preharvest treatments with salicylates. *International Journal of Molecular Science*, 18: 1-14.

Mathaba, N., Mafeo, T.P. and Kruger, F.J. 2015. The skin colouring problem of 'Hass' avocado fruit during ripening. *South African Avocado Growers' Association Yearbook,* 38: 51-57.

Mathaba, N., Mathe, S., Mafeo, T.P., Tesfay, S.Z. and Mlimi, J. 2017. Complexities of 'Hass' avocado skin colour change during ripening. *South African Avocado Growers' Association Yearbook*, 40: 129-132.

McGuire, R.G. 1992. Reporting of objective colour measurements. *Horticultural Science,* 27: 1254-1255.

Meir, S., Philosoph-Hadas, S., Lurie, S., Droby, S., Akerman, M. and Zauberman, G. 1996. Reduction of chilling injury in stored avocado, grapefruit, and bell pepper by methyl jasmonate. *Canadian Journal of Botany*, 74: 870-874.

Mendieta, B., Olaeta, J. A., Pedreschi, R. and Undurraga, P. 2016. Reduction of cold damage during cold storage of 'Hass' avocado by a combined use of pre-conditioning and waxing. *Scientia Horticulturae*, 200: 119-124.

Meng, X., Han, J., Wang, Q. and Tian, S. 2009. Changes in physiology and quality of peach fruits treated by MeJA under low temperature stress. *Food Chemistry*, 114: 1028-1035.

Mhlophe S.D. and Kruger, F.J. 2012. Effects of harvest maturity, storage temperature, controlled atmosphere and Smart Fresh on the post-harvest vascular staining disorder of 'Maluma' avocado fruit. *South African Avocado Growers' Association Yearbook*, 35: 8-12.

Mori, K., Sugaya, S. and Gemma, H. 2005. Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. *Scientia Horticulturae*, 105: 319-330.

Moro, L., Hassimotto, N.M.A. and Purgatto, E. 2017. Postharvest auxin and methyl jasmonate effect on anthocyanin biosynthesis in red raspberry (*Rubus idaeus* L.). *Journal of Plant Growth Regulation*, 36: 773-782.

Muhammad, S.M. 2015. Bruising in Avocado (*Persea americana* M.) Cv. 'Hass' Supply. *Postharvest Biology and Technology*, 100: 33-40.

Obinatai, N., Yamakawa, T., Takamiya, M., Tanaka, N., Ishimaru K. and Kodama, T. 2003. Effects of salicylic acid on the production of procyanidin and anthocyanin in cultured grape cells. *Plant Biotechnology*, 20: 105-111.

Oraei, M., Panahirad, S., Zaare-Nahandi, F. and Gohari, G. 2019. Pre-véraison treatment of salicylic acid to enhance anthocyanin content of grape (*Vitis vinifera* L.) berries. *Journal of the Science of Food and Agriculture*, 99: 5946-5952.

Öztürk, B., Yildiz, K., & Kucuker, E. (2015). Effect of pre-harvest methyl jasmonate treatments on ethylene production, water-soluble phenolic compounds and fruit quality of Japanese plums. *Journal of the Science of Food and Agriculture*, 95: 583-591.

Pareek, S., Valero, D. and Serrano, M. 2015. Postharvest biology and technology of pomegranate. *Journal of Science of Food and Agriculture*, 95: 2360-2379.

Pesis, E., Ackerman, M., Ben-Arie, R., Feygenberg, O., Feng, X., Apelbaum, A., Goren, R. and Prusky, D. 2002. Ethylene involvement in chilling injury

symptoms of avocado during cold storage. *Postharvest Biology and Technology*, 24: 171-181.

Polenta, G.A., Calvete, J.J. and Gonzalez, C.B. 2007. Isolation and characterization of the main small heat shock proteins induced in tomato pericarp by thermal treatment. *FEBS Journal*, 274: 6447-6455.

Qi, T., Song, S., Ren, Q., Wu, D., Huang, H., Chen, Y., Fan, M., Peng, W., Ren, C. and Xie, D. 2011. The Jasmonate-ZIM-domain proteins interact with the WD-Repeat/bHLH/MYB complexes to regulate Jasmonate-mediated anthocyanin accumulation and trichome initiation in *Arabidopsis thaliana*. *Plant Cell*, 23: 1795-1814.

Ramírez-Gil, J.G., Henao-Rojas, J.C. and Morales-Osorio, J.G. 2021. Postharvest diseases and disorders in avocado cv. Hass and their relationship to preharvest management practices. *Heliyon*, 7: 1-12.

Rasouli, M., Saba, M.K. and Ramezanian, A. 2019. Inhibitory effect of salicylic acid and Aloe vera gel edible coating on microbial load and chilling injury of orange fruit. *Scientia Horticulturae*, 247: 27-34.

Razavi, F., Hajilou, J. and Aghdam, M.S. 2018. Salicylic acid treatment of peach trees maintains nutritional quality of fruits during cold storage. *Advances in Horticultural Science*, 32: 33-40.

Rehman, M., Singh, Z. and Khurshid, T. 2018. Methyl jasmonate alleviates chilling injury and regulate fruit quality in ‘Midknight’ Valencia sweet orange. *Postharvest Biology and Technology*, 141: 58-62.

Rudell, D., Mattheis, J., Fan, X. and Fellman, J. 2002. Methyl Jasmonate enhances anthocyanin accumulation and modifies production of phenolics and pigments in ‘Fuji’ Apples. *Journal of American Society for Horticultural Science*, 127: 435-441.

Sayyari, M., Babalar, M., Kalantari, S., Martínez-Romero, D., Guillén, F. and Serrano, M. 2011. Vapour treatments with methyl salicylate or methyl jasmonate alleviated chilling injury and enhanced antioxidant potential during postharvest storage of pomegranates. *Food Chemistry*, 124: 964-970.

Sayyari, M., Babalar, M., Kalantari, S., Serrano, M. and Valero, D. 2009. Effect of salicylic acid treatment on reducing chilling injury in stored pomegranates. *Postharvest Biology and Technology*, 53: 152-154.

Sevillano, L., Sanchez-Ballesta, M.T., Romojaro, F., Flores, F.B., 2009. Physiological, hormonal and molecular mechanisms regulating chilling injury in horticultural species. *Journal of the Science of Food and Agriculture,* 89: 555-573.

Shafiee, M., Taghavi, T.S. and Babalar, M. 2010. Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. *Scientia Horticulturae,* 124: 40-45.

Shan, X., Zhang, Y., Peng, W., Wang, Z. and Xie, D. 2009. Molecular mechanism for jasmonate-induction of anthocyanin accumulation in *Arabidopsis. Journal of Experimental Botany*, 13: 3849-3860.

Sharma, G., Maymon, M. and Freeman, S. 2017. Epidemiology, pathology and identification of Colletotrichum including a novel species associated with

avocado (*Persea americana*) anthracnose in Israel. *Scientific Reports*, 7: 1-16.

Shen, Y. and Yang, H. 2017. Effect of preharvest chitosan-*g*-salicylic acid treatment on postharvest table grape quality, shelf life, and resistance to *Botrytis cinerea*-induced spoilage. *Scientia Horticulturae*, 224: 367-373.

Shikwambana, K., Mafeo, T.P. and Mathaba, N. 2021. Effect of postharvest methyl jasmonate treatment on early matured “Hass” avocado fruit exocarp colour development during ripening. *Agricultural Science*, 12: 875-887.

Siboza, X.I. and Bertling, I. 2013.The Effects of methyl jasmonate and salicylic acid on suppressing the production of reactive oxygen species and increasing chilling tolerance in ‘Eureka’ lemon [*Citrus limon* (L.) Burm. F]. *Horticulturae Scientia*, 88: 269-276.

Siboza, X.I., Bertling, I. and Odindo, A.O. 2017. Enzymatic antioxidant in response to methyl jasmonate and salicylic acid and their effect on chilling tolerance in lemon fruit [*Citrus limon* (L.) Burm. F.]. *Scientia Horticulturae*, 225: 659-667.

Sivankalyani, V., Feygenberg, O., Maorer, D., Zaaroor, M., Fallik, E. and Alkan, N. 2015. Combined treatments reduce chilling injury and maintain fruit quality in avocado fruit during cold quarantine. *PLoS ONE*, 10: 1-16.

Srivastava, M.K. and Dwivedi, U.N. 2000. Delayed ripening of banana fruit by salicylic acid. *Plant Science*, 158: 87-96.

Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Goeffroy, P., Legrand, M. and Fritig, B. 1993. Plant ‘pathogenesis-related’ proteins and their role in defence against pathogens. *Biochimie*, 75: 687-706.

Stintzing, F.C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends in Food Science and Technology,* 15: 19-38.

Tareen, M.J., Abbasi, N.A.and Hafiz, I.A. 2012. Postharvest application of salicylic acid enhanced antioxidant enzyme activity and maintained quality of peach cv. “Flordaking” fruit during storage. *Scientia Horticulturae*, 142: 221-228.

Tasneem, A. 2004. Postharvest treatments to reduce chilling injury symptoms in stored mangoes. Master of Science thesis. Department of Bioresource Engineering. Macdonald campus of McGill University Ste-Anne-de-Bellevue, QC, H9X3 V9, Canada.

Theis, T. and Stahl, U. 2004. Antifungal proteins: targets, mechanisms and prospective applications. *Cellular and Molecular Life Sciences*, 61: 443-455.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G., Nomura, K., He, S.Y., Howe, G.A. and Browse, J. 2007. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature*, 448: 661-665.

Valero, D, Diaz-Mula, H.M., Zapata, P.J., Castillo, S., Guillen, F., Martinez-Romero, D. and Serrano, M. 2011. Postharvest treatments with salicylic acid, acetylsalicylic acid or oxalic acid delayed ripening and enhanced bioactive compounds and antioxidant capacity in sweet cherry. *Journal of Agricultural and Food Chemistry*, 59: 5483-5489.

Valero, D. and Serrano, M. 2010. Postharvest biology and technology for preserving fruit quality. CRC Press, Taylor and Francis group, New York, NY.

Van de Poel, B., Bulens, I., Hertog, M., Van Gastel, L., De Proft, M.P., Nicolai, B.M. and Geeraerd, A.H. 2012. Model-based classification of tomato fruit development and ripening related to physiological maturity. *Postharvest Biology and Technology,* 67: 59-67.

Vasanthaiah, H. 2011. Drought tolerance and stress hormones: from model organisms to forage crops. InTech publishing, UK, London.

Vlot, A.C., Dempsey, D.M.A. and Klessig, D.F. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, 47: 177-206.

Wang, H., Cheng, X., Wu, C., Fan, G., Li, T. and Dong, C. 2021. Retardation of postharvest softening of blueberry fruit by methyl jasmonate is correlated with altered cell wall modification and energy metabolism. *Scientia Horticulturae*, 276: 1-9.

Wang, L., Jin, P., Wang, J., Jiang, L., Shan, T. and Zheng, Y. 2015a. Effect of beta aminobutyric acid on cell wall modification and senescence in sweet cherry during storage at 20°C. *Food Chemistry*, 175: 471-477.

Wang, Y., Xu, H., Liu, W., Wang, N., Qu, C. and Jiang, S. 2018. Methyl jasmonate enhances apple' cold tolerance through the JAZ-MYC2 pathway. *Plant Cell, Tissue and Organ Culture,* 136: 75-84.

White, A., Woolf, A., Hofman, P. and Arpaia, M.L. 2007. The international avocado quality manual. *Plant and Food research, New Zealand*.

Wills, R. B. H., McGlasson, W.B., Graham, D. and Joyce, D.C. 2007. Postharvest, An Introduction to physiology and handling of fruit, vegetables and ornamentals. 5th ed. University of New South Wales Press, Sydney.

Wills, R., McGlasson, B., Graham, D., and Joyce, D. 1998. Postharvest, an introduction to the physiology and handling of fruit and vegetables and ornamentals (4th ed.). Sydney 2052, Australia: University of New South Wales Press Ltd.\University of New South Wales.

Wolfe, J. 1978. Chilling injury in plants the role of membrane lipid fluidity. *Plant, Cell and Environment*, 1: 241-247.

Woolf, A.B., Cox, K.A., White, A. and Ferguson, I.B. 2003. Low temperature conditioning treatments reduce external chilling injury of 'Hass' avocados. *Postharvest Biology and Technology*, 28: 113-122.

Yahia, E.M. 2011. Avocado, Chapter 8 in Rees, D., Farrell, G. and Orchard, J.E. (eds), Crop Postharvest: Science and Technology, Volume 3, Perishables, Wiley-Blackwell Publishing, Oxford, UK. In press.

Yao, H.J. and Tian, S.P. 2005. Effects of pre- and postharvest application of SA or MeJA on inducing disease resistance of sweet cherry fruit in storage. *Postharvest Biology and Technology*, 35: 253-262.

Zeng, K., Cao, J. and Jiang, W. 2006. Enhancing disease resistance in harvested mango (*Mangifera indica* L. cv. 'Matisu') fruit by salicylic acid. *Journal of the Science of Food and Agriculture*, 86: 694-698.

Zhao, Y., Song, C., Brummell, D.A., Qi, S., Lin, Q., Bi, J. and Duan, Y. 2021. Salicylic acid treatment mitigates chilling injury in peach fruit by regulation of sucrose metabolism and soluble sugar content. *Food Chemistry*, 358: 1-7.

Zhou, Y., Ma, J., Xie, J., Deng, L., Yao, S. and Zeng, K. 2018. Transcriptomic and biochemical analysis of highlighted induction of phenylpropanoid pathway

metabolism of citrus fruit response to salicylic acid, *Pichia membranaefeciens* and *oligochitosan. Postharvest Biology and Technology*, 142: 81-92.

Zolfagharinasab, R. and Hadian, J. 2007. Influence of methyl jasmonate on inducing chilling tolerance in pomegranate fruits *(Malas Save*). *Pakistan Journal of Biological Sciences*, 10: 612-616.