EVALUATION OF HOT WATER AND METHYL JASMONATE TREATMENTS FOR MITIGATION OF CHILLING INJURY TO IMPROVE 'HASS' AVOCADO FRUIT SKIN COLOUR

BY

LETHABO SETAGANE

FULL-DISSERTATION

Submitted in fulfilment of the

requirement for the degree of

MASTER OF AGRICULTURAL MANAGEMENT

in

PLANT PRODUCTION

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: PROF TP MAFEO (UL)

CO-SUPERVISOR: DR N MATHABA (UMP)

APRIL 2020

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DECLARATION

I Setagane Lethabo [redacted] declare that the research report hereby submitted to the University of Limpopo, for the degree of Masters of Agricultural Management (Plant Production) has not been submitted previously by me or anybody for a degree at this or any other University. In addition, this is my work in design and in execution, and related materials contained herein had been duly acknowledged.

…….. ……

Signature Date

DEDICATION

I would like to dedicate this dissertation to my mother (Ms Mokgadi Gloria Setagane) and my late father (Mr Sedie Lebidike Edward ‘Stone’).

ACKNOWLEDGEMENTS

I would like to express my gratitude to the following people and organisations:

1) Prof TP Mafeo and Dr N Mathaba for their supervision, guidance and much appreciated patience during the duration of the research.
2) Agricultural Sector Education Training Authority (AgriSeta) and University of Limpopo for financial support and to attend Combine Congress Conference where oral paper was presented.
3) Agricultural Research Council-Institute for Tropical and Subtropical Crops for allowing me to conduct my project in their laboratory
4) Mr K Shikhwambana for his helpfulness in the laboratory and organisation of various chemicals and materials needed for the work to be carried out.
5) Mr J Mlimi for his assistance on the technical aspects of the project.
6) Nico Swart Estate (Kiepersol) for providing the fruit
7) My family and friends for their support and encouragement.

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ABSTRACT

Avocado fruit ‘Hass’ harvested during early-season and exposed to temperature at 5.5°C for 28 d are susceptible to chilling injury (CI); and therefore, develop poor skin colour during ripening. In ‘Hass’ avocado fruit, skin colour change during ripening is used by European market to indicate fruit ripeness and softness. Therefore, the aim of this study was to evaluate the use of hot water (HW) and methyl jasmonate (MJ) as postharvest treatment dips to mitigate CI; and thereby, enhance ‘Hass’ avocado fruit peel colour during ripening. Fruit were harvested randomly from 5 selected trees treated alike during early season (April 2018); and thereafter, transported to the laboratory. At the laboratory, experiments of this study were divided into 2: experiment (1) fruit were dipped into HW (38, 42 and 46°C for 30, 25 and 20 min, respectively); and experiment (2) fruit were dipped into MJ (10 and 100 µmol/L for 2 min) treatments. In both experiments after these treatments, fruit were allowed to dry for 60 minutes at ambient (±25°C) temperature and untreated fruit were used as control. Thereafter, fruit were stored at commercial shipping temperature (5.5°C) for up to 28 d. After removal from cold storage, fruit were ripened at ambient temperature (±25°C) and evaluated every after 2 d for weight loss, firmness loss, objective colour parameters (lightness-L^*, chroma-C^* and hue angle-h^*), subjective colour (eye colour) and ripening percentage. However, chilling injury (CI) and electrolyte leakage (EL) were evaluated immediately after removal from cold storage. The results showed that HW significantly ($P< 0.05$) increased weight and firmness loss during ripening. Furthermore, HW reduced EL and external chilling injury (ECI) of ‘Hass’ avocado fruit during cold storage. In addition, the results showed that HW had significant effect ($P< 0.05$) on colour parameter L^* and eye colour rating, but did not affect ($P> 0.05$) C^* and h^*. Avocado ‘Hass’ fruit subjected to HW at 42°C/25 and 46°C/20 min developed purple colour (eye colour rating 4.47 and 4.36, respectively) during ripening when compared with HW at 38°C/30 min and control fruit. Moreover, results showed that dipping fruit in 10 µmol/L had a significant effect ($P< 0.05$) on reducing weight loss during ripening. Methyl jasmonate (10 and 100 µmol/L) treatment reduced EL and alleviated external chilling injury (ECI) of ‘Hass’ fruit during cold storage. The results showed that MJ (10 and 100 µmol/L) treatments had significant effect ($P< 0.05$) on colour parameter L^*, h^* and eye colour rating, but did not affect ($P> 0.05$) C^*. Furthermore, ‘Hass’ fruit treated with 10 and 100 µmol/L MJ

reached the purple skin colour (eye rating 5.39 and 5.19, respectively) during ripening. Fruit dipped in MJ (10 µmol/L) had low weight loss when compared with fruit treated with MJ (100 µmol/L). In conclusion, the results of this study indicated that HW (42°C/25 minutes) and MJ (10 µmol/L) effectively alleviated external chilling injury; and therefore, improved ‘Hass’ skin colour development during ripening.

Keywords: ‘Hass’ avocado, methyl jasmonate, hot water treatment, chilling injury, skin colour

CHAPTER 01
GENERAL INTRODUCTION

1.1 Background

Avocadoes (Persea americana Mill.) are the most popular subtropical fruit crop produced; and extensively, consumed in the world (Nelson, 2010; Mathaba et al., 2015). Nurseries in South Africa produce an average of 110 000 trees annually with the replacement of old orchads (Donkin, 2007; DAFF, 2017). The cultivars produced from these nurseries include ‘Hass’ avocado contributing approximately 70% and the remaining 30% is comprise of Fuerte, Pinkertonand Ryan (DAFF, 2017). Most of the avocado fruit produced in South Africa are exported to the European market (Kassim et al., 2013). In terms of cultivars types and export volume, ‘Hass’ contributes approximately 55% of the total exports volume while 45% shared between green skinned cultivars (Bill et al., 2014). The European market continues to demand ‘Hass’ avocado fruit when compared to green skinned cultivar due to its good storage-life and nutty taste (Bill *et al.,* 2014). However, the South African Avocado Industry (SAAI) has been faced with a challenge of poor skin colour development of ‘Hass’ avocado fruit during ripening (Mathaba *et al*., 2015). According to Cox *et al*. (2004), ‘Hass’ avocado fruit supposed to change skin colour from green to purple then black during ripening. The challenge of poor colour development reduces the demand of fruit from South Africa by the lucrative European market. Countries competing with South Africa, such as Peru and Chile are supplying fruit that develop an acceptable skin colour during ripening (Nelson, 2010). The changes in skin colour of ‘Hass’ avocado fruit during ripening is used by European market to indicate fruit ripeness and softness (Donetti and Terry, 2012). To overcome this skin poor colour development, post-harvest technology that could improve skin colour development of ‘Hass’ avocado fruit is still need to be developed.

Studies on skin colour change of ‘Hass’ avocado fruit have highlighted that poor skin colouration during ripening is related to an array of factors such as; maturity (Mathaba *et al*., 2015), ripening temperature (Cox *et al*., 2004; Mathaba *et a*l., 2015), sugar accumulation (Mathaba *et al*., 2017) and external chilling damage development (Mathaba *et al*., 2015). Reduced synthesis of anthocyanin, specifically cyanidin-3-*O*-glucoside was also found to contribute to poor colouration of ‘Hass’ avocado fruit skin (Cox *et al.,* 2004; Ashton *et al*., 2006).

A study conducted by Mathaba *et al*. (2015) has shown that poor 'Hass' avocado fruit skin colouring is linked to storage duration and increases with chilling injury (CI) symptoms during storage and ripening. Therefore, oxidative stress due to prolonged exposure of fruit to low temperature may lead to development of CI symptoms on the fruit peel, leading to poor skin colour change during ripening. To mitigate against CI, various treatments have been studied. Among these treatments; hot water (HW) (Ding *et al*., 2001) and methyl jasmonate (MJ) (Gonzale-Aguilar *et al*., 2000b) have been applied to number fruit types (Yuan *et al*., 2013; Venkatachalam and Meenune, 2015). Methyl jasmonate is a plant growth regulator that occur naturally and responds to environmental factors through down regulation of reactive oxygen species (ROS) (Ding *et al*., 2001).

In 'Kent' mango fruit, MJ have been found to stimulate accumulation of colour pigments such as; anthocyanin and β-carotene during post-harvest (Gonza´lez-Aguilar *et al.*, 2000a). In 'Xiahui 5' peach fruit, hot water treatment was found to induce CI tolerance through reducing solute leakage and malondiadehyde content (Huan *et al*., 2017). Treatments that reduce CI (Mathaba *et al*., 2015) and enhance the accumulation of anthocyanin, specifically; cyanidin-3-O-glucoside (Cox *et al.,* 2004; Ashton *et al*., 2006) could improve skin colour of 'Hass' avocado fruit during ripening. Therefore, an overall aim of this study was to evaluate the potential use of HW and MJ as postharvest treatments to mitigate CI and enhance peel colour of 'Hass' avocado fruit harvested at early-season.

1.2 Problem statement

The South African Avocado Industry (SAAI) has been plagued with revenue loss due to poor 'Hass' avocado fruit skin colouration during ripening (Nelson, 2010; Mathaba *et al*., 2015). In general, 'Hass' avocado fruit skin colour is expected to change from green to purple then black during ripening. However, South African 'Hass' avocado fruits are not changing skin colour to purple then black when ripe as compared with fruit from Spain and Peru (Donetti and Terry, 2012). To date, the progress made on 'Hass' avocado fruit skin colour change unravelled that poor skin colouring during ripening relates to harvest time (early season) and external chilling injury (Mathaba *et al*., 2015). In order to mitigate against poor 'Hass' skin colouring, there is a need to develop postharvest practice that will reduce CI; and thereby, improving skin

colour during ripening and revenue while maintaining the industry credibility. The proposed study would evaluate the use of hot water and methyl jasmonate as postharvest treatments to enhance CI tolerance, assumedly, improving 'Hass' skin colour during ripening.

1.3 Motivation of the study

Avocado fruit 'Hass' skin colour change is an important quality attribute for lucrative export markets. In the fruit peel, colour change is associated with degradation of chlorophyll and anthocyanin (cyanidin -3-*O*-glucoside) accumulation during ripening (Cox *et al*., 2004). An array of pre-harvest factors such as; production site, maturity, branch girdling, orchard topography, fruit canopy position and storage duration were investigated on 'Hass' avocado fruit during ripening (Mathaba *et al*., 2016). However, this postharvest physiological challenge persist to exist. According to Mathaba *et al*. (2015), the development of CI symptoms due to extended storage duration at low temperature reduces skin colour change of 'Hass' avocado fruit during ripening. While postharvest hot water (HW) and methyl jasmonate (MJ) treatments have been applied to number of fruit crops to mitigate against CI development (Gonzale-Aguilar *et al*., 2000b; Ding *et al*., 2001). In 'Beskid' raspberry fruit, MJ treatments have been found to maintain high total anthocyanin content during post-harvest (Ghasemnezhad and Javaherdashti, 2008).

Therefore, the current study would evaluate the potential use of postharvest HW and MJ treatment to enhance CI tolerance, assumable; improve 'Hass' avocado fruit skin colour change of during ripening. The results would be beneficial to the avocado industry, distributors and ripeners by generating new scientific information and new innovative methods to mitigate poor skin colour development of 'Hass' avocado fruit during ripening.

1.4 Aim

The study aimed to mitigate CI on 'Hass' avocado using HW and MJ treatments as post-harvest treatment.

1.5 Objective

Hot water and MJ treatments alleviated chilling injury and improved skin colour change of ‘Hass’ avocado fruit during ripening.

1.6 Hypothesis

i) Hot water and MJ treatments alleviated chilling injury and improved skin colour change of ‘Hass’ avocado fruit during ripening.

CHAPTER 02
LITERATURE REVIEW

2.1 Introduction

In South Africa, large volume of avocado fruit produced are exported to European market, particularly; 'Hass' cultivar (Kassim *et al*., 2013). The demand of 'Hass' avocado fruit by European market keep on increasing due to their good storage-life and nutty taste (Bill *et al.,* 2014). The change in 'Hass' avocado fruit skin colour is used to determine ripeness, therefore, plays an important role in consumer perception (Nelson, 2010). The South African 'Hass' avocado fruit show heterogeneity in skin colour change during ripening (Mathaba *et al*., 2016). Postharvest treatments for improving skin colour change of 'Hass' avocado fruit are essential. Therefore, this review would discuss work done and not yet done on research problem.

2.2 Work done on the research problem

2.2.1 Chilling injury in avocado: An overview

Chilling injury is a physiological dysfunction that occurs when tropical and subtropical crops are exposed to low or non freezing temperature below critical level (Woolf *et al.,* 2003). In an avocado fruit, symptoms appear as mesocarp discolouration, surface darkening and pitting (Woolf *et al*., 1995). However, symptoms severity has been shown to depend on fruit maturity, cultivar susceptibility and cold storage duration (Dixon *et al*., 2008). Mathaba *et al*., (2015) found that early season 'Hass' avocado fruit (fruit maturity) were highly susceptible to CI when compared with late season. In addition, Dixon *et al*. (2008) reported that 'Hass' avocado fruit harvested during February were highly susceptible to CI when compared with fruit harvested during October or December after storage at 2°C for 6 weeks. Furthermore, Woolf *et al.* (2003) found that storage at 3°C or below induced CI in 'Hass' avocado fruit. Lutge *et al*. (2010) investigated ultra-low temperature shipping and cold chain management of 'Fuerte' avocado grown in the KwaZulu-Natal midlands. The study revealed that exposing 'Fuerte' avocado fruit to 2 and 5.5°C storage temperature for 28 d resulted in development of internal and external CI.

Moreover, the development of CI symptoms resulted in variable skin colouration of ‘Hass’ avocado fruit during ripening (Mathaba *et al*., 2015). Mathaba *et al*. (2015) further showed that ‘Hass’ avocado fruit harvested at early season had higher CI symptoms, thereby, exhibiting poor skin colour development during ripening.

2.2.2 Hot water as post-harvest treatment

The use of many chemical fumigation and dips as disinfestation treatments or for quality maintenance have been restricted due to their residual effect on agricultural products (Woolf *et al*., 1995). However, hot water dips have been used as alternative methods or treatments for reducing CI and maintaining quality of many horticultural crops (Gonza´lez-Aguilar *et al*., 2000b; Erkan *et al*., 2005). According to Zakariya and Alhassan (2014), the efficacy of hot water treatment (HWT) depends primarily on treatment duration, temperature and fruit maturity. In general, fruit should be exposed to higher temperatures for shorter period (Boonkorn, 2016). The effect of HWT on CI have been reported in Keitt’ and ‘Nam doc mai’ mango (Zakariya and Alhassan, 2014), ‘Hass’ avocado (Blakey and Bower, 2007), ‘Osbeck’ valencia citrus fruit (Erkan *et al*., 2005), ‘Seeda’ tomato and bell pepper fruit (Gonza´lez-Aguilar *et al*., 2000b).

Effect of HWT on CI and fruit crops membrane

Hot water treatment at 45°C for 15 min increased the incidence of CI in ‘Cerasiform Alef’ cherry tomato fruit stored at 5°C for 19 days followed by 6 d shelf-life at 20°C (Yang *et al*., 2009). In ‘Carmello’ tomato fruit, HWT ranged from 30 to 45°C for 30 min have been found to reduce CI after cold storage at 4°C for 10 d to 20°C (El-Assi, 2004). In another study, HWT at 50°C/10 or 20 min and 55°C/10 min reduced the incidence of CI on ‘Fuyu’ persimmon fruit after storage at 0°C for 5 months when compared with control fruit. Furthermore, HWT have been found to reduce CI due to reduced solute leakage and malondialdehyde in ‘Xiuhui’ peach fruit (Huan *et al*., 2017), and enhanced antioxidant activity in ‘Frangi’ papaya fruit (Shadmanin *et al*., 2015).

Antioxidants protect plants against reactive oxygen species (ROS), and are classified into enzymatic (superioxide (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), catalase (CAT), dehydroscorbate reductase and

glutathione reductase) and non-enzymatic (carotenoids, phenolics, ascorbate, glutathione and tocopherols). In 'Frangi' papaya fruit, the use of HWT (42°C for 30 min) increased APX activity which led to reduced CI during storage at 6 and 12°C for 3 weeks (Shadmanin *et al*., 2015). In 'Navel' and 'Valencia' late orange fruit stored at 10°C for 20 d, HWT (41°C for 20 min) increased activity of CAT and enhanced peroxidase level which also resulted in reduced CI (Bassal and El-Hamahmy, 2011). Moreover, HWT at 50°C/30 min increased total carotenoids content in 'Keitt' mango fruit after 3 d at 20°C when compared with control. However, in 'Valencia' orange fruit, HWT at 66°C for 60 seconds resulted in higher electrolyte leakage and lower peroxidase activity during storage at 10°C for 7 d (John-Karuppiah, *et al*., 2004).

According to Woolf *et al*. (1995), HWTs reduce CI by inducing the synthesis and accumulation of specific heat-shock proteins (HSPs) that increase chilling tolerance of the fruit. Heat-shock proteins are part of a group of proteins induced by abiotic and biotic stresses; and play an important role in protein folding, transportation and degradation (Wang *et al*., 2002). Moreover, HSPs help in protein refolding (Sabehat *et al*., 1996; Wang *et al*., 2002). There are five families of HSPs have been identified: HSP70, chaperonins, HSP90, HSP100 and small HSP (sHsp). In 'Imperial' tomato fruit, hot water treatment at 42°C for 5 min was found to reduce CI during storage at 5°C for 10 and 20 d and for 7 d at 21°C through increased accumulation of sHsp (Salazar-Salas *et al*., 2017).

Effect of HWT on weight loss

The effect of HWT on weight loss depends on treatment temperature, duration and crop types (Gonza´lez-Aguilar *et al*., 2000b). In 'Bell' pepper, HWT at 53 and 45°C for 4 and 15 min, respectively, increased weight loss after 14 and 28 d of storage at 8°C (Rodov *et al*., 1995; Gonza´lez-Aguilar *et al*., 2000b). However, in the study conducted by Rodov *et al*. (1995), HWT at 53°C for 2 min reduced weight loss in 'Nagami' kumquat fruit stored at 2 and 17°C. Moreover, the use of vapor heat treatments (35 and 38°C both for 12 and 24h) reduced fruit weight loss of cactus pear ('Alfajayucan', 'Amarillo Milpa Alta', 'Christilina', 'Rojo 3589', 'Rojo Pelón' and 'Sangre de Toro') fruit by inducing epicuticle wax layer (López-Castañeda *et al*., 2010).

Effect of HWT on firmness

According to Brummell and Harpster (2001), fruit firmness depends on the activity of hydrolytic enzymes such as lipoxygenase (LOX), polygalacturonase (PG), pectin methyl esterase (PME), cellulase and pectinase (Brummell and Harpster, 2001). These enzymes are responsible for the degradation of cell wall. Previous studies have shown that HWT can either increase or decrease fruit firmness loss depending on treatment and crop types (Abu-Aziz *et al*., 2009; Yuan *et al*., 2013). The use of HWT (53°C for 3 min) maintained firmness of 'Yujinxiang' muskmelon fruit stored at 22± 2°C through reducing the activities of PG and PEM (Yuan *et al*., 2013). In contrast, HWT at 50°C for 5 and 10 min increased firmness loss of 'Hass' avocado fruit due to increased cellulase activity after storage at 5°C for 15 d and followed by 3 d shelf life at 20°C (Abu-Aziz *et al*., 2009).

Effect of HWT on fruit ripening

Ripening process of climacteric fruit is triggered by ethylene production (Bower *et al*., 2003). The biosynthesis of ethylene production is controlled by S-adenosylmethionine synthase, 1-aminocyclopropane-1-carboxylic acid synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) (Wang *et al*., 2002). The enzyme, ACO catalyse the last step of ethylene biosynthesis by converting 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene (Wang *et al*., 2002). The effect of HWT have been found to vary with the treatment and commodity. High temperatures range 34-53°C have been found to inhibit ripening in 'Rutgers' tomato (Biggs *et al*., 1988), 'Magnum 45' muskmelon (Dunlap *et al*., 1990) and 'Mei' mango (Luo, 2006) fruit through reducing ACC oxidase activity caused decrease in ethylene production. Moreover, temperature at 34°C inhibited activity of ACC due to a rapid decline in 1-aminocyclopropane-1-carboxylic acid synthase (ACS) activity in 'Rutgers' tomato fruit (Biggs *et al*., 1988). However, concentration of ACC was higher in 'Hass' avocado fruit heat-treated (6 and 12 h) at lower duration than in longer heat-treatment (24, 36 and 48 h) after removal from storage at 0°C for 21 d and ripened at 20°C (Florissen *et al*., 1996).

Effect of HWT on colour

Colour is determined using objective (*L**, C*and *h**) and subject (eye colour rating) colour parameters. Lightness (*L**) describe the lightness or brightness [*L** = 0 (black) and *L** = 180 (white)], a* specify the greenness or redness (where - a* indicates greenness whereas +a* means redness) and b* indicates yellowness or blueness (where - b* indicates blueness whereas +b* means yellowness). Chroma (C*) value indicates the degree of colour saturation and proportional to the strength of the colour. Whereas, the Hue angle (h^*) is the basic unit of colour (0 = red; 90 = yellow; 180 = bluish-green and 270 = blue). Hue angle (h^*) and Chroma (*C**) values are calculated based on *a** and *b** values (McGuire, 1992).

In previous studies, lower hue angle value of 'Hass' avocado peel was related to peel colour change during ripening (Cox *et al*., 2004; Ashton *et al*., 2006). The study conducted by Cox *et al*. (2004) showed a significantly and strong negative correlation (R^2 = -0.804) between *h** and accumulation of peel colour pigment anthocyanin, mainly cyanidin 3-*O*-glucoside. According Cox *et al*. (2004), cyanidin 3-*O*-glucoside is responsible for colour purple of 'Hass' avocado fruit peel during ripening. The use of HWT at 50°C for 11 min improved colour of 'Keitt' and 'Nam doc mai' mango fruit stored at 7 and 25°C for 10 d through reducing hue angle (*h**) values (Zakariya and Alhassan 2014). Moreover, HWT at 40 or 45°C for 5 or 15 min improved red colour of 'Cerasiform Alef' cherry tomato fruit after removal from cold storage at 5°C for 19 d followed by 6 d shelf life at 20°C through increased *a** value (Yang *et al*., 2009).

Mode of action for HWT on how improve fruit resistance against CI and enhance colour

Chilling injury breakdown the cell membrane which result in the increase in solute, electrolyte content and malondialdehyde (MDA) compound (Martindale and Holbrook, 2002; amauchi *et al*., 2008). The use of hot water treatment of 48°C/10 min enhanced fruit resistance against CI through the reduction of solute leakage and malondiadehyde content in 'Xiahui 5' peach fruit (Huan *et al*., 2017). Hot water treatments also found to increase heat-shock proteins (HSPs) which cooperate with antioxidants towards protection crops against environmental stress (Bassal and El-Hamahmy, 2011). Heat-shock proteins (HSPs); specifically, small ones (sHSPs)

scavenge reactive oxygen species (ROS) and maintain cell membrane quality attributes such as fluidity and permeability (Nakamoto and Vigh, 2007). The application of HWT at 42°C/5 min reduced CI incidence after storage at 5°C/10 for 20 d to 21°C for 7 d and triggered the activities of small HSP (sHSPs) in 'Imperial' tomato fruit (Salazar-Salas *et al*., 2017).

In addition, studies have shown that HWT may trigger the synthesis of plant hormones like methyl jasmonate (MJ), salicylic acid (SA) and abscisic acid (ABA) which are involved in up-regulating encoding protein that promote anthocyanin synthesis (Reyes-Díaz *et al*., 2016). According to Shan *et al*. (2009), MJ up-regulated coronatine-insensitive protein 1 which regulate 'late' anthocyanin biosynthetic enzymes (DFR, LDOX and UFGT) in Arabidopsis plants. In 'Sweet Heart' and 'Sweet Late' cherries, the production of salicylic acid was also found to promote the accumulation of anthocyanin (Gimenez et al., 2014).

2.2.3 Methyl jasmonate as post-harvest treatment

Methyl jasmonate (MJ) is a naturally occurring plant growth regulator and plant stress hormone that responds to both biotic and abiotic stresses through down-regulation of reactive oxygen species (ROS) (Ding *et al*., 2001). The effect of exogenous MJ application has been reported on 'Tommy Atkins' mango (Gonzalez-Aguilar *et al.*, 2000a), 'Beefstake' tomato (Ding *et al*., 2001), 'Elite' zucchini squash (Wang and Buta, 1994), 'Fuji' apple (Öztürk *et al.*, 2013), raspberry 'Heritage' (Wang, 2003), papaya'Sunrise'(Gonzalez-Aguilar *et al.*, 2003) and 'Griff' longkong fruits (Venkatachalam and Meenune, 2015).

Effect of MJ on CI and fruit crops membrane

Methyl jasmonate has been applied on a number of horticultural crops to mitigate CI. Studies have shown that the effect of MJ depends on fruit maturity and concentrations (Öztürk *et al.*, 2013; Venkatachalam and Meenune, 2015). For instance, Ding *et al*. (2001) found that low concentrations (0.01 mM vapour) of MJ reduced CI in 'Beefstake' tomato fruit stored at 5°C for 2-4 weeks. Therefore, Ding *et al*. (2001) suggested that low concentrations (0.01 mM vapour) of MJ induced defense-mechanism response that provides protection against chilling injury. In addition, Venkatachalam and Meenune (2015) found that high concentrations of 30

µMol L^{-1} MJ effectively alleviated chilling injury on 'Griff' longkong fruit during extended storage (stored at 13°C until 75% of CI symptoms appeared on the fruit pericarp) by increasing antioxidant enzyme activities. In 'Beskid' raspberry fruit stored at 4°C for 7 d, the use of MJ (10, 20 and 30 µMol L-1) enhanced antioxidant enzyme activity which resulted in reduced CI and electrolyte leakage (EL) (Ghasemnezhad and Javaherdashti, 2008). Antioxidants protect cell membrane and reduce CI through scavenging free radicals that are formed when plant tissue is under oxidative stress (Bertling *et al*., 2007); and thereby, improving membrane permeability, electrolyte leakage and lipid peroxidation (Martindale and Holbrook, 2002).

According to Eaks (1990), chilling sensitive species have high percentage of saturated fatty acids. Chilling temperatures activate lipid degradative enzymes such as lipoxygenase (LOX) and phospholipase D (*lipophosphodiesterase II*, *lecithinase D*, *choline phosphatase*) (Pinhero *et* al., 1998; Mao *et al*., 2007). In membrane lipids, lipoxygenase causes peroxidation damage, which result in increased membrane fluidity and levels of saturated lipids (Pinhero *et* al., 1998). According to Creelman and Mullet (1997), MJ is derived from enzymatic oxidation of unsaturated fatty acids by lipoxygenase (LOX). Therefore, exogenous application of MJ retains high-unsaturated membrane-lipids that help crops to adapt under oxidative stress (Sayyari *et al.,* 2011). In the study conducted by Venkatachalam and Meenune (2015), the use of MJ (10, 20 and 30 µMol L^{-1}) treatments have been found to reduce chilling injury symptoms by suppressing LOX activity and EL. Moreover, MJ (0.01 and 0.1 mM) protected cell membrane of 'EMb' strawberry exposed to water stress condition through decreased membrane-lipid peroxidation as expressed by malondialdehyde (MDA) content (Wang, 1999).

Effect of MJ on weight loss

Fruit crop continues to loss water, therefore, resulting in weight loss after harvest (Mahajan *et al.*, 2008). The efficacy of MJ in reducing weight loss during post-harvest seemed to depend on concentration and crop species (Gonza´lez-Aguilar *et al.*, 2001; Gonza´lez-Aguilar *et al.,* 2003). For instance, exogenous application of MJ (0.2 mmol/L) reduced weight loss of 'Bergarouge' apricot fruit during 8 d shelf-life at 25°C after cold storage at 1°C for 21 d (Ezzat *et al*., 2017). In another study, the use

of MJ vapour (10^{-5} or 10^{-4} M) did not modify water loss of ‘Sunrise’ papaya fruit stored at 10°C for 14-32 d, followed by 4 d shelf life at 20°C when compared with control (Gonza´lez-Aguilar *et al.,* 2003). Moreover, MJ vapour (10^{-5} M) increased weight loss in ‘Kent’ mango fruit after removal from cold storage at 10°C to 20°C for 7 d shelf life (Gonza´lez-Aguilar *et al.*, 2001).

Effect of MJ on firmness

In ‘Kent’ mango fruit, an application of MJ (10^{-5} M) reduced firmness loss after cold storage at 10°C for 14 d followed by 7 days shelf-life at 20°C (Gonza´lez-Aguilar *et al.,* 2001). According to Brummell and Harpster (2001), firmness loss involve an increase in the activity of hydrolytic enzymes such as lipoxygenase (LOX), polygalacturonase (PG), pectin methyl esterase (PME), cellulase and pectinase. Previous studies suggested the efficacy of MJ to maintain firmness is associated with MJ-induced inhibition of lipoxygenase (LOX), polygalacturonase (PG) and pectin methyl esterase (PME) activities (Venkatachalam and Meenune, 2015; Ezzat *et al*., 2017). In ‘Jiubao’ peach fruit, the use of MJ (0.1 mmol/L vapour) reduced PME and increased de-esterification of pectin after storage at 5°C for 21 d to 20°C for 3 d shelf life (Meng *et al*., 2009). However, application of MJ (10^{-4} M vapour) in ‘Tommy Atkins’ mango fruit increased firmness loss during cold storage at 7°C for 21 d and after 5 d shelf life at 20°C (Gonzalez-Aguilar *et al.*, 2000a).

Effect of MJ on ripening

An avocado fruit is highly climacteric. In climacteric fruit, ethylene production triggers the ripening process (Bower *et al*., 2003). Exogenous application of MJ has been found to enhance ethylene production in a climacteric fruit through increased expression and activity of ethylene biosynthesis enzymes (Fan *et al*., 1997; Fan *et al*., 1998). The effect of MJ on ethylene production depend on fruit maturity and treatment concentration (Mukkun and Singh, 2009; Fan *et al*., 1997; Fan *et al*., 1998). In the study conducted by Mukkun and Singh (2009), MJ (50 µM) increased ethylene production in ‘Pajaro’ strawberry fruit stored at 20±1°C for 6 d at fully ripe, half ripe and white stage. In another study, MJ (2×10^{-7} mol L^{-1}) increased ethylene production in ‘Summerred’ apple fruit during storage at 20°C for 15 d (Fan *et al*., 1997). Moreover, in ‘Camarosa’ strawberry fruit, treatment with MJ (50 µM) increased ethylene production and respiration rate at white and pink stage during storage at

25/15°C d/night for 9 d (Perez *et al*., 1997). In contrast, MJ (1 mM MJ for 5 min) increased ethylene production in 'Trad-see-thong' pineapple fruit during cold storage at 10°C for 10 and 15 d when compared with control fruit (Boonyaritthongchai and Supapvanich, 2017).

Effect of MJ on colour change

The skin colour of 'Hass' avocado fruit changes from green to purple then black during ripening (Cox *et al*., 2004). In 'Hass' avocado fruit, the main pigments responsible for skin colour development include; chlorophyll, carotenoids and anthocyanin during ripening (Cox *et al*., 2004). According to Ashton *et al.* (2006), chlorophyll and carotenoids are accountable for the green-yellow colour of mesocarp and extracted oil. However, the purple colouration of 'Hass' skin develops with an increase in the cyanidin 3-*O*- glucoside content as fruit ripen (Cox *et al*., 2004; Ashton *et al*., 2006). Cyanidin 3-*O*- glucoside is an anthocyanin identified to be responsible for purple colour of 'Hass' avocado fruit during ripening (Cox *et al*., 2004). In 'Golden Delicious' apple fruit, application of MJ (8 ppm) vapour has been found to improve skin colour change after 4 h at 25°C through increased accumulation of β-carotene and chlorophyll degradation (Perez *et al*., 1993). In another study, the use of MJ (10^{-5} M) vapour also improved the yellow and red colour development in 'Kent' mango fruit (Gonza´lez-Aguilar *et al.*, 2001).

Mode of action for MJ on how improve fruit resistance against CI and enhance colour

Chilling injury occur when crop is exposed to chilling temperature below critical level. Temperatures below critical level activate lipid degradative enzymes such as lipoxygenase (LOX) and phospholipase D (PLD). Lipoxygenase causes peroxidation damage in membrane lipids, which lead to increased membrane fluidity and levels of saturated lipids (Pinhero *et al*., 1998). In 'Griff' longkong fruit stored at 13°C, application of MJ (10, 20 and 30 µMol L-1) treatments have been found to reduce chilling injury symptoms by suppressing LOX activity and ion leakage in the cell membrane (Venkatachalam and Meenune, 2015). In 'EMb' strawberry under water stress condition, MJ (0.01 and 0.1 mM) treatment protected cell membrane through decreasing membrane-lipid peroxidation as expressed by malondialdehyde (MDA) content (Wang, 1999). Moreover, the application of MJ (16 and 24 µl.l-1) reduced CI

through the enhancement of antioxidant enzyme activity in ‘Beskid’ raspberry fruit after storage at 4°C for 7 d (Ghasemnezhad and Javaherdashti, 2008).

In addition, application of MJ (2.24 g.L-1) decreased hue angle (*h**) of ‘Fuji’ apple fruit stored at 21°C for 7 d which was linked with increased peel anthocyanin content (Rudell *et al*., 2002). Saniewski *et al*. (1998), reported that MJ induces the expression of anthocyanin biosynthesis enzymes in the presence of light. Shan *et al*. (2009), found that MJ application induced anthocyanin accumulation through up-regulation of the ‘late’ anthocyanin biosynthetic enzymes over ‘early’ anthocyanin biosynthetic enzymes in Arabidopsis.

2.3 Research gap

Although concerted research effort has been made to identify preharvest factors and postharvest treatments impacting ‘Hass’ avocado fruit skin colour change during ripening (Mathaba *et al*., 2015; Mathaba *et al*., 2016). Mathaba *et al*. (2015), highlighted that poor skin change of ‘Hass’ avocado fruit during ripening is related to the incidence of external CI and maturity. Hot water and MJ have been used on various fruit crops to mitigate CI (El-Assi, 2004; Ghasemnezhad and Javaherdashti, 2008). However, the effect of hot water and MJ treatments on ‘Hass’ avocado fruit skin colour change during ripening has not yet fully defined for early-season. Therefore, the proposed study would evaluate effect of HWT and MJ on CI and skin colour change of ‘Hass’ avocado fruit harvested during early-season.

CHAPTER 03

EVALUATION OF HOT WATER TREATMENT FOR MITIGATION OF CHILLING INJURY TO IMPROVE 'HASS' AVOCADO FRUIT SKIN COLOUR

3.1 INTRODUCTION

Avocado fruit harvested during early-season are highly susceptible to chilling injury (CI) (Woolf *et al*., 2003). According to Woolf *at al*., (1995), avocado fruit are susceptible to CI when exposed to chilling temperatures below 6°C. Chilling injury symptoms manifest as uneven ripening, mesocarp discolouration, surface darkening and pitting (Cutting *et al*., 1990; Mathaba *at al*., 2015).

In 'Hass' avocado fruit, external CI symptoms may lead to poor skin colour change during ripening (Mathaba *at al*., 2015). 'Hass' avocado fruit skin colour change is used as ripeness or softness indicator by distributors, ripeners and consumers. According to Cox *et al* (2004), the skin of 'Hass' avocado changes colour from green to purple or black during ripening. Therefore, CI and poor skin colour change are the major post-harvest quality defects that result in export losses (Nelson, 2010; Bill *et al.,* 2014*;* Mathaba e*t al*., 2015).

Postharvest hot water treatments (HWT) have been applied to a number of fruit crops and found to enhance CI tolerance (Gonza´lez-aguilar *et al*., 2000b; Khan *et al*., 2007). Hot water treatment have been found to reduce CI through up-regulation of heat shock proteins (HSPs) and antioxidants. However, the physiological response of various fruit species to heat treatments vary with maturity and production site (Amin and Hossain, 2012). Therefore, the present study reports the effect of HWT on CI and skin colour change of 'Hass' avocado fruit during ripening.

3.2 RESEARCH METHODOLOGY AND ANALYTICAL PROCEDURES

3.2.1 Experimental sites

Avocado 'Hass' fruit were harvested from 11 years old trees grown at Nico Swart farm in Kiepersol, (25^{o} 4' 0" S, 31^{o} 2' 0" E), Mpumalanga Province, South Africa. Immediately after harvest, fruit were packed in plastic crates and carefully transported to the Agricultural Research Council – Tropical and Subtropical Crops

(ARC-TSC) (25° 45’ 18’’ S; 30° 96’ 97’’ E) laboratory for storage, ripening, evaluation and analysis.

3.2.2 Experimental procedures and design

Previous reports indicated that HW treatments ranging from 38 to 56°C reduced CI on various horticultural crops. The efficacy of HWT to reduce CI was encourage by temperature and duration (Zakariya and Alhassan, 2014). Generally, fruit are exposed to higher temperatures for shorter period (Boonkorn, 2016). In this study, fruit were randomly harvested (400 fruit) from five selected trees treated alike during early season (April 2018) and transported to the laboratory. At the laboratory, fruit were dipped in hot water at different temperatures of 38°C for 30 min, 42°C for 25 min and 46°C for 20 min, respectively. Untreated fruit were used as control for this experiment. Each treatment had three replicates and 15 experimental units (225 treated fruit). The hot water temperature was monitored by positioning a thermometer in the water bath. Immediately after these treatments, fruit were packaged in export crates and air dried for 60 minutes on ambient temperatures of ±23°C, thereafter, cold stored at 5.5°C for 28 d. After removal from cold storage, fruit were allowed to ripen at ±25°C. Treatments were laid out in a completely randomised design (CRD), replicated 3 times.

3.2.3 Data collection

Objective colour

Colour parameters were assessed using a Chromameter (CR-400, Konica Minolta, Osaka, Japan). Values were obtained on the basis of CIELAB colour system (*L**, *a**and *b**) where L describe the lightness or brightness [*L** = 0 (black) and *L** = 180 (white)], *a** specify the greenness or redness (where - *a** indicates greenness whereas +a* means redness) and *b** indicates yellowness or blueness (where - *b** indicates blueness whereas +*b** means yellowness). Chroma (*C**) value indicates the degree of saturation of colour and is proportional to the strength of the colour whereas Hue angle (h^*) is the basic unit of colour (0 = red; 90 = yellow; 180 = bluish-green and 270 = blue). The measurements were taken from 3 replications of each treatment and the average of *L**, *a** and *b** values were used. Hue angle (h^*) and Chroma (*C**) values were calculated based on *a** and *b** according to the following

formulas: Hue angle (h^*) = tan-1($\frac{b*}{a*}$) and Chroma (C*) =$\sqrt{a^2 + b^2}$) as described by (McGuire, 1992). The values *L**, *C**, *a** and *b** are measured in NBS units, hue angle h◦ in degrees from 0 to 360°. Colour was measured on daily basis until the fruit were declared fully ripe.

Subjective colour

Skin colour of 'Hass' avocado fruit was assessed visually and recorded on a scale from 1 to 6, whereby 1 = emerald green; 2 = forest green; 3 = approximately 25% coloured; 4 = approximately 75% coloured; 5 = purple and 6 = black according to White *et al*. (2007).

Fruit firmness

Fruit firmness was measured using a non-destructive Sinclair firmometer (Sinclair IQTM International, Norwich, United Kingdom) on 0, 2 and 4 ripening days until were fully ripe. Firmness of each fruit was measured at three points along the equatorial region of the fruit and was expressed in SU. Fruit were considered commercial ripe upon reaching 25 SU or less.

Weight loss

Fruit weight was measured after fruit removal from cold storage using a weighing scale (SBA 61, Scaltec, Helingenstandt, Germany). The percentage of weight loss was calculated using the following equation:

WL (%) = $\frac{Wi-Wf}{Wi}$ ×100

Where: WL (%) = weight loss in percentage, W_f= final fruit weight, W_i= initial fruit weight

Ripening percentage

Ripening percentage was calculated based on the number of fruit that reached firmness value less or equal to 25 SU. Ripening percentage was calculated as follow:

Ripening percentage (%) = $\frac{\sum Rf}{Tf} \times 100$

Where R_f= ripe fruit, T_f= total number of fruit evaluate

External chilling injury index

External chilling injury was assessed on skin lesions using a scale of 0 to 3, and was expressed in percentage whereby 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). Chilling injury was expressed as a chilling injury (CI) index, and calculated using the following formula:

$$\text{CI index (from 0 to 3)} = \frac{\sum((CI\ level) \times (Number\ of\ fruit\ at\ the\ CI\ level))}{Total\ number\ of\ fruit\ in\ the\ treatment}$$

Electrolytic leakage

Electrolytic leakage (EC) was determined immediately after fruit were removed from cold storage. Four samples were removed from each avocado fruit using cock borer (10 mm diameter), thereafter; rinsed with distilled water at least four times to avoid recording an inaccurate EC values. These samples were placed in 20 ml of distilled water. Electrolyte leakage (EC_1) was measured using electrical conductivity meter (model: Hi991301N, El- Hamma Instruments, Israel) after shaking these samples for 3 h. Afterward, samples were boiled for 1 h, and after the samples were cooled before measuring final electrolyte leakage (EC_2). Electrolytic leakage was calculated using the following formula:

$$\text{EC index} = \frac{\sum EC2 - EC1}{n}$$

Where EC_2= electrolytic leakage of boiled samples, EC_1= electrolytic leakage of samples shaken for 3 h, and n= number of the samples

3.2.4 Data analysis

The data was subjected to analysis of variance (ANOVA) using STATISTICX 10.0. Means were separated using LSD, at the 5% level of significance. Tables and figures were developed using values of mean separation.

3.3 RESULTS AND DISCUSSIONS

Treatment showed non-significant effect ($P > 0.05$) for chroma (*C**) during ripening (Appendix 1). According to Cox *et al*. (2004), *C** values decrease as 'Hass' avocado fruit skin change colour from green to purple then black during ripening. Hot water treatments at 42°C/25 min and 46°C/30 min decreased *C** with the progress of ripening in 'Hass' avocado fruit skin (Table 3.1). In this study, the reduction in *C** values could be attributed by increased in weight and firmness loss, which later affected colour (Hong *et al*., 2007).

A significant difference ($P < 0.05$) of lightness (*L**) was also observed during ripening (Appendix 2). Hot water treated fruit showed *L** values that were lower than the control fruit (Table 3.1). The decrease in *L** was correlated with the accumulation of cyanidin 3-*O*-glucoside on skin of 'Hass' avocado fruit during ripening (Cox *et al*., 2004). The accumulation of cyanidin 3-*O*-glucoside on 'Hass' avocado fruit skin was associated with the development of purple colour during ripening (Cox *et al*., 2004; Ashton *et al*., 2006). This suggest that the lowest *L** values that were observed for HWT at 42°C/25 min and 46°C/30 min could enhanced 'Hass' avocado fruit skin colour change (Cox *et al*., 2004; Ashton *et al*., 2006).

The treatment had no significant effect ($P > 0.05$) on hue angle (*h**) (Appendix 3). The control fruit had high *h** values when compared with treated fruit (Table 3.1). Hot water treatment at 42°C/25 min was most effective in reducing *h** during ripening when compared with other applied treatments and control (Table 3.1). A study by Cox *et al*. (2004) revealed that 'Hass' avocado peel colour change could be evident by a decrease in hue values. They also found a significantly strong negative correlation ($R^2 = -0.804$) between *h** and accumulation of peel colour pigment anthocyanin, mainly cyanidin 3-*O*-glucoside (Cox *et al*., 2004). Previous research work has shown that HWT may trigger the synthesis of plant hormones like methyl jasmonate (MJ), salicylic acid (SA) and abscisic acid (ABA) which are involved in up-regulating encoding protein that promote anthocyanin synthesis (Reyes-Díaz *et al*., 2016). Methyl jasmonate was found to up-regulate coronatine-insensitive protein 1 which regulate 'late' anthocyanin biosynthetic enzymes (DFR, LDOX and UFGT) in *Arabidopsis* plants (Shan *et al.,* 2009). In 'Sweet Heart' and 'Sweet Late' cherries,

the production of salicylic acid was also found to promote the accumulation of anthocyanin (Gimenez *et al*., 2014).

Treatment had a significant effect on subjective (eye) colour of 'Hass' avocado fruit peel during ripening (Appendix 4). Hot water treatment at 42°C/25 min and 46°C/30 min reached average subjective (eye) colour rating of 4.47 and 4.36, respectively; confirming that fruit peel changed colour from emerald green to purple, as previously reported by Mathaba *et al*. (2015). The change in skin colour of 'Hass' avocado fruit during ripening results from an increase in the levels of the anthocyanin pigment (cyanidin 3-*O*-glucoside) (Cox *et al*., 2004). This suggests that in the present study, HWT triggered the production of anthocyanin pigment (cyanidin 3-*O*-glucoside) which in turn caused the fruit to change peel colour to purple (eye colour rating 4.47 and 4.36) when compared with the control.

Table 3.1 Effect of hot water treatment on objective (*C*, h*, L**) and subject (eye) colour on 'Hass' avocado peel fruit during ripening

Treatment (HWT)	Chroma (*C**)	Hue angle (*h**)	Lightness (*L**)	Eye colour (1-6)
38°C /30 minutes	10.42a	76.60a	28.67a	3.72b
42°C/25 minutes	12.73a	68.80a	27.33b	4.47a
46°C /20 minutes	13.01a	78.29a	26.75b	4.36a
Control	12.10a	80.15a	29.51a	3.50b
P-value at 0.05	0.45^{ns}	0.26^{ns}	0.00*	0.01*
LSD=	1.6531	5.5820	0.3798	0.2596

Values with same letters were not significantly different at 0.05 probability level * Significantly different at P = 0.05, ns Not significant at P ≤ 0.05

Hot water treatment exerted a significant effect (P < 0.05) on weight loss of 'Hass' avocado fruit during ripening (Appendix 5). An increase in weight loss was observed on 'Hass' avocado fruit treated with HWT due to an increasing HWT temperatures (Figure 3.1). Our results showed that all studied HWT had a contributing effect on fruit weight loss (Figure 3.1). All treated (HWT) fruit recorded the highest weight loss when compared with untreated (control) fruit. The effect of HWT on weight loss is

presumed to be dependent on treatment temperature and duration (Gonza´lez-Aguilar *et al*., 2000b). In the present study, HWT at higher temperatures (42°C/25 min and 46°C/20 min) resulted in high fruit weight loss during ripening. These results confirmed the findings of Amin and Hossain (2012), who reported that HWT at 47 °C for 3 and 21 min increased water evaporation rate on 'BARI Kola 1' and 'Sabri Kola' banana fruit, which led to high weight loss during ripening. In contrast, López-Castañeda *et al*. (2010) found that vapor heat treatments (35 and 38°C both for 12 and 24 h) reduced fruit weight loss of cactus pear ('Alfajayucan', 'Amarillo Milpa Alta', 'Christilina', 'Rojo 3589', 'Rojo Pelón' and 'Sangre de Toro') fruit by inducing epicuticle wax layer. Epicuticle wax layer protect plants against water loss and limits the loss of substances from the internal tissue (Dominguez *et al*., 2011). In this study, the increase in weight loss for fruit HWT treated at higher temperature (42°C/25 min and 46°C/20 min) might be due to water loss through the large fissures (cracks) which developed on epicuticle wax layers when fruit were subjected to higher temperatures (López-Castañeda *et al*., 2010).

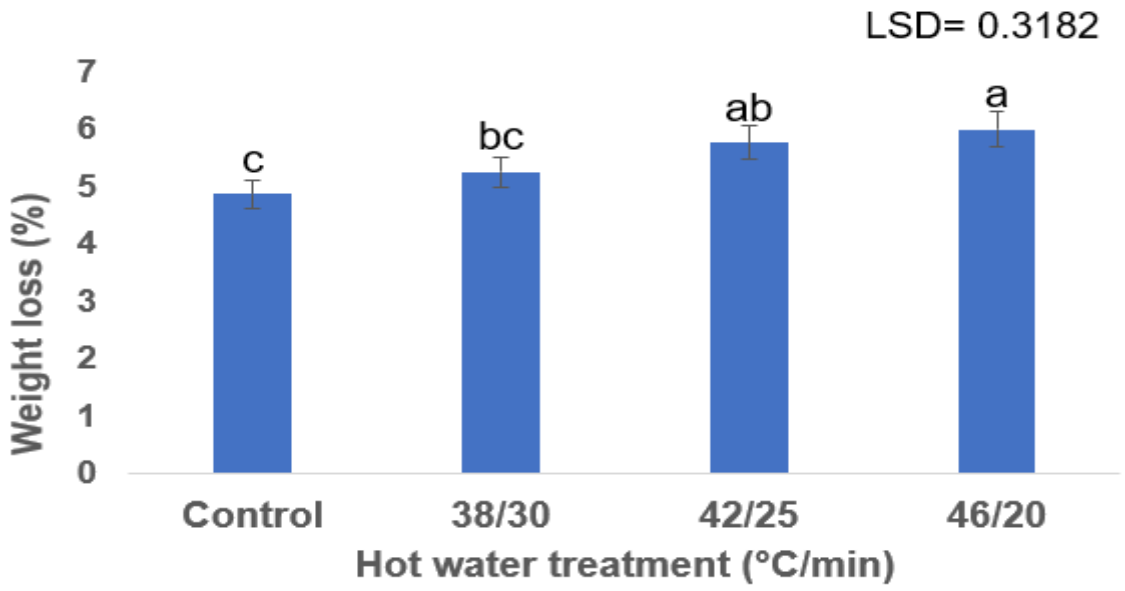

Figure 3.1 Effect of hot water treatment on weight loss of 'Hass' avocado fruit peel during ripening; LSD= 0.3182

The treatment had significant effect ($P < 0.05$) on firmness of 'Hass' avocado fruit peel during ripening (Appendix 6). Hot water treatment of 38°C/20 min, 42°C /25 min and 46°C/20 min resulted in higher firmness when compared with control (Figure

3.2). Fruit under these treatments (38°C/20 min, 42°C/25 min and 46°C/20 min) were softer, reaching average values of 15.69, 17.17 and 14.61 SU, respectively. Studies have shown that HWT can either increase or decrease fruit firmness depending on treatment and product (Abu-Aziz *et al*., 2009; Yuan *et al*., 2013). In the present study, HWT significantly reduced firmness, but 42°C/25 min appeared to have maintained fruit firmness during ripening when compared with 46°C/20 min and 38°C/20 min. It is presumed that HWT increased pectin enzyme activities leading to cell wall disassembly, subsequently; loss in fruit firmness and ultimately softening (Abu-Aziz *et al*., 2009). In addition, the present results suggested that HWT for 'Hass' avocado would hasten firmness loss, which eventually affects fruit quality.

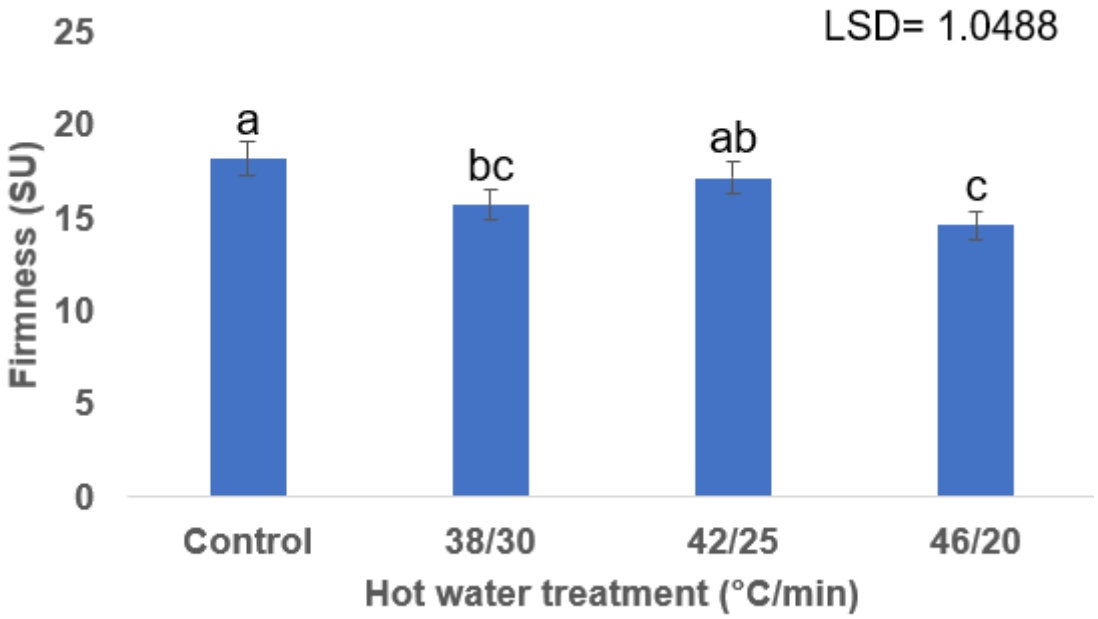

Figure 3.2 Effect of hot water treatment on firmness loss of 'Hass' avocado fruit peel during ripening; SU, Sinclair Units

Hot water treatment had a significant effective ($P < 0.05$) external chilling injury index (Appendix 7). The control fruit recorded the highest incidence of ECI when compared with HWT treated fruit (Figure 3.3). Amongst HWT, 42°C/25 min resulted in a 0% incidence of ECI, while HWT of 46°C /20 min resulted in higher ECI symptoms (Figure 3.3). These results were in agreement with the finding of Özdemir *et al*. (2009), whereby, HWT at 50°C/10 or 20 min and 55°C/10 min reduced the incidence of CI on 'Fuyu' persimmon fruit when compared with control. It has been found that chilling injury breakdown the cell membrane, therefore; resulting in the rise in solute

and electrolyte content (Martindale and Holbrook, 2002). Chilling injury also found to increase malondialdehyde (MDA) compound, which result from peroxidation of membrane fatty acids and used as indicator of membrane integrity (Yamauchi *et al*., 2008). In contrast, hot water treatment of 48°C/10 min induced chilling injury tolerance by maintaining cell membrane integrity while reducing solute leakage and malondiadehyde content in 'Xiahui 5' peach fruit (Huan *et al*., 2017). In this study, HWT significantly reduced the incidence of external chilling injury on 'Hass' avocado fruit peel. It is presumed that HWT upheld cell membrane integrity and malondiadehyde content in 'Hass' avocado peel.

In another study by Bassal and El-Hamahmy (2011), it was reported that HWT reduces CI by up-regulation of heat-shock proteins (HSPs). According to Rossel *et al*., (2002), HSPs were found to cooperate with antioxidants towards protection of *Arabidopsis* plants under high light stress. In another study, HSPs; specifically, small ones (sHSPs) maintain cell membrane quality attributes such as fluidity and permeability also scavenge reactive oxygen species (ROS) in Cyanobacteria strain under oxidative stress (Nakamoto and Vigh, 2007). In addition, Salazar-Salas *et al*. (2017) found that HWT of 42°C/5 min repressed the incidence of CI after storage at 5°C/10 for 20 d to 21°C for 7 d and triggered the activities of small HSP (sHSPs) in 'Imperial' tomato fruit. In the present study, we therefore postulate that a similar effect was exerted in 'Hass' avocado fruit since HWT treated fruit showed reduced in CI symptoms following cold storage.

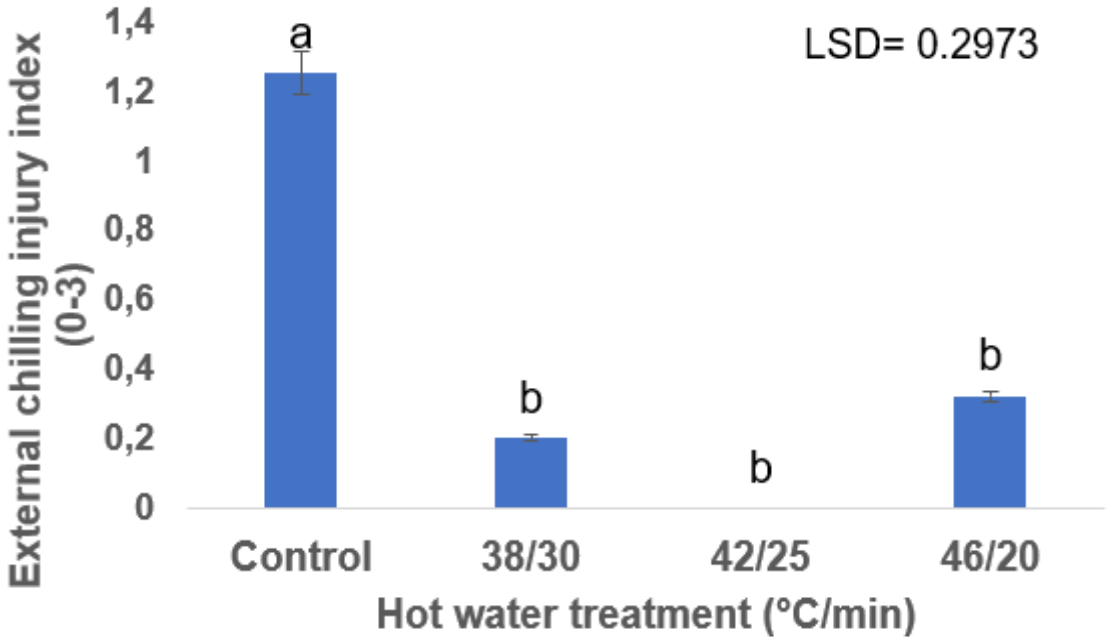

Figure 3.3 Effect of hot water treatment on external chilling injury index of 'Hass' avocado fruit peel

Treatment showed non-significant effect ($P > 0.05$) on electrolyte leakage (EL) (Appendix 8). Hot water treatments reduced EL when compared with control fruit after withdrawal from cold storage (Figure 3.4). The efficacy of HWT on EL depends on treatment temperature and rise in storage temperature (Nyanjage *et al*., 1999). In the study conducted by Huan *et al*. (2017), HWT at 48°C/10 min reduced solute leakage in 'Xiahui 5' peach fruit during storage at 4°C. In contrast, Nyanjage *et al*. (1999) found that 'Kent' mango fruit exposed to HWT at 46.5°C for 120 min had higher EL after 10 d of storage at 13°C. In this study, it is presumed that HWT reduced skin damage leading to lower EL.

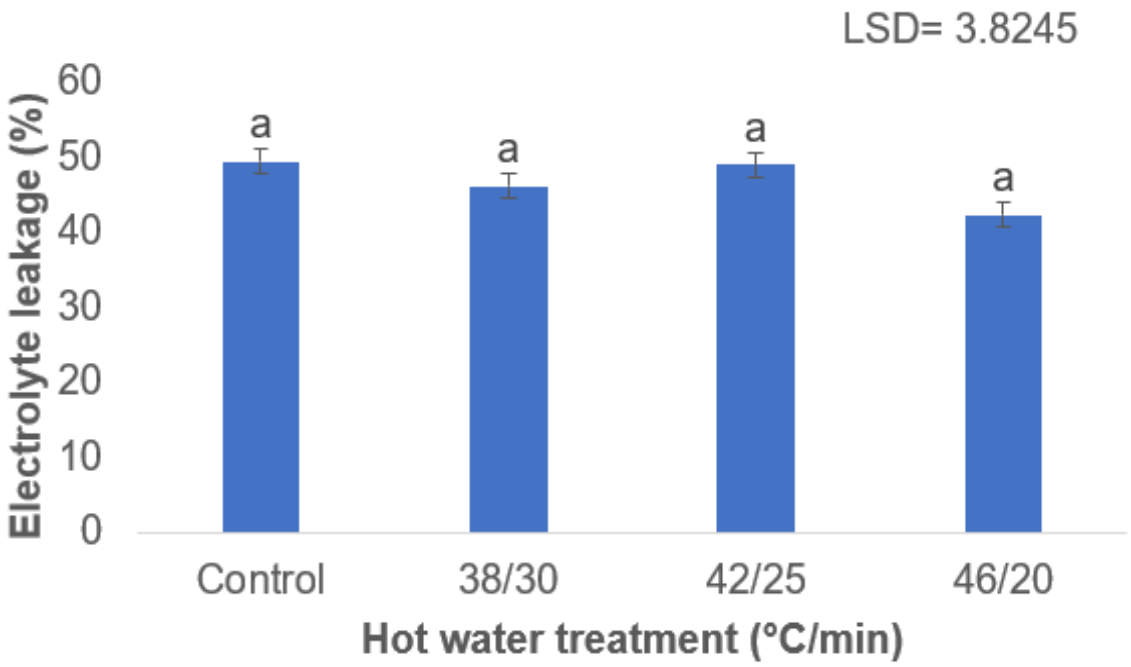

Figure 3.4 Effect of hot water treatment on electrolyte leakage recorded of 'Hass' avocado fruit peel

Hot water treatment had no significant effect (P > 0.05) on 'Hass' avocado fruit during ripening. Fruit exposed to 42°C/25 and 46°C/20 min were all ripe (100%) at the final day of evaluation whereas 38°C/30 min and control fruit recorded 96.67% (Figure 3.5). Furthermore, HWT treated fruit showed a gradual increase in ripening percentage after d 2 of evaluation (Data not shown). This trend of fruit ripening observed was in accordance with the results of Mayani *et al*. (2017), who found that 50°C/20 min HWT delayed ripening on 'Kesar' mango fruit until 30 days when compared with control which reached 100% ripening percentage earlier within 25 days. Some studies have established that exposing 'Rutgers' tomato (Biggs *et al*., 1988), 'Magnum 45' muskmelon (Dunlap *et al*., 1990) and 'Mei' mango (Luo, 2006) fruit to high HWT of 34-53°C may inhibit ripening by reducing 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity resulting to decrease in ethylene production. This could be explained in the present study, whereby; HWT treated fruit showed a delay in ripening from d 0 to d 2 (Figure 3.5). Previous study conducted by Biggs *et al*. (1988), found that the activity of ACC was inhibited by the temperature at 34°C due to a rapid decline in 1-aminocyclopropane-1-carboxylic acid synthase (ACS) activity in 'Rutgers' tomato fruit. In this study, it is plausible that a delay ripening in

HW treated fruit via inhibiting ACC action could in turn have affected ethylene production and ripening (Biggs *et al*., 1988).

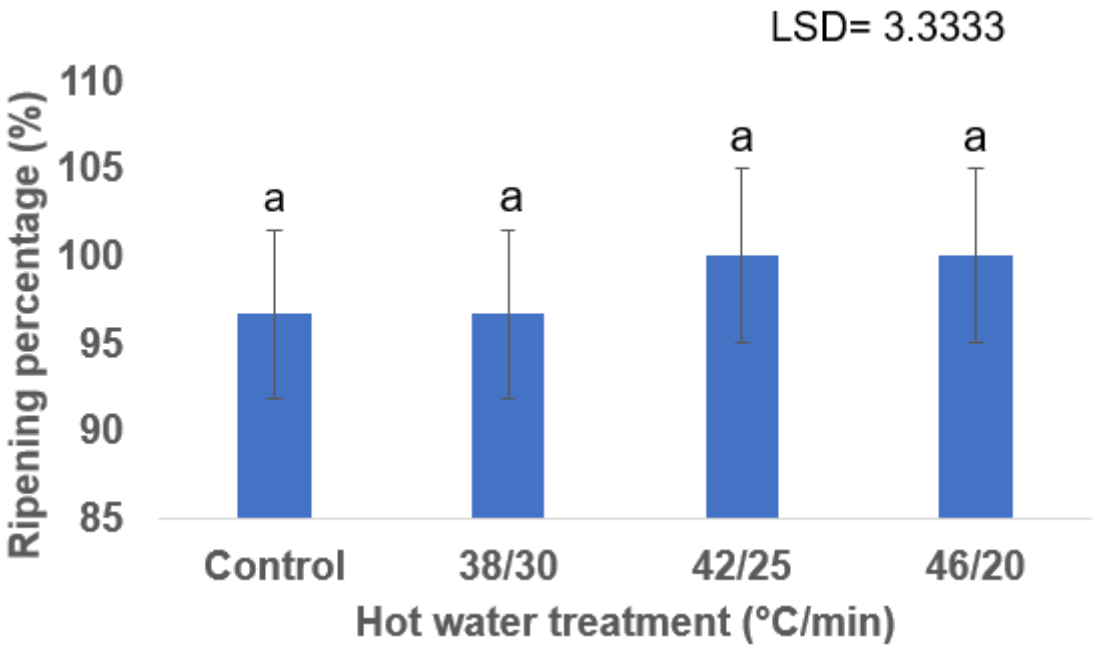

Figure 3.5 Effect of hot water treatment on ripening percentage of 'Hass' avocado fruit during ripening; LSD= 3.3333

3.4 Conclusion

Hot water treatment effectively reduced chilling injury and improved skin colour development of 'Hass' avocado fruit but negatively impacted quality parameters such as weight loss, firmness and physical appearance. Hot water treatment at 42°C/25 min or 46°C/20 min were the most effective to improve 'Hass' avocado fruit skin colour.

CHAPTER 04

EVALUATION OF METHYL JASMONATE TREATMENT FOR MITIGATION OF CHILLING INJURY TO IMPROVE 'HASS' AVOCADO FRUIT SKIN COLOUR

4.1 INTRODUCTION

The use of methyl jasmonate (MJ) as postharvest treatment have been applied to various fruit crops and was found to enhance chilling injury (CI) tolerance and colour development (Perez *et al*., 1993; Meng *et al*., 2009; Aghdam and Bodbodak, 2013). However, there is lack of empirical evidence on the use of MJ treatment to enhance CI tolerance and improve skin colour change of 'Hass' avocado fruit during ripening. Several studies showed that the storage life of 'Hass' avocado fruit is limited by chilling injury (CI) under low temperature (Woolf *et al*., 2003; Meng *et al*., 2009; Aghdam and Bodbodak, 2013).

According to Woolf *et al*., (1995), long storage of avocado fruit at temperature near or below 6°C result in development of CI. In avocado fruit, CI symptoms appear as uneven ripening, mesocarp discolouration, surface darkening and pitting (Cutting *et al*., 1990). Furthermore, it has been reported that external CI symptoms lead to poor skin colour development of 'Hass' avocado fruit (Mathaba *et al*., 2015). The skin colour of 'Hass' avocado fruit change from green to purple then black during ripening (Cox *et al*., 2004). Therefore, objective of the study was to determine effect of MJ as post-harvest treatment on chilling injury and skin colour change of 'Hass' avocado fruit during ripening.

4.2 RESEARCH METHODOLOGY AND ANALYTICAL PROCEDURES

4.2.1 Experimental sites

Same as explained in Chapter 03 (Section 3.2.1).

4.2.2 Experimental procedures and design

Studies have shown that the effect of MJ depends on types of crop, maturity and concentrations (Öztürk *et al.*, 2013; Venkatachalam and Meenune, 2015). High concentrations of 30 µMol L^{-1} MJ were found effective to alleviate CI in longkong fruit during extended storage (Venkatachalam and Meenune, 2015). Therefore, there are

few reports about exogeneous application of MJ liquid concentrations on various crops. In this study, fruit were randomly harvested (400 fruit) from five selected trees treated alike during early season (April 2018) and transported to the laboratory. At the laboratory, fruit were dipped into methyl jasmonate (MJ) solution at a concentration of 10 and 100 µmol/L for 2 min. Untreated fruit were used as control for this experiment. Each treatment had three replicates and 15 experimental units (135 treated fruit). After treatment, fruit were packaged in export crates and air dried for 60 min on ambient temperature of ±23°C and cold stored at 5.5°C for 28 days. After removal, from cold storage fruit were allowed to ripen at ±25°C. Treatments were laid out in a completely randomised design (CRD).

4.2.3 Data collection

All the data collected for objective colour, subjective colour, weight loss, fruit firmness, ripening percentage, external chilling injury and electrolyte leakage were done as explained in Chapter 03 (Section 3.2.2).

3.2.4 Data analysis

It was analysed using STATISTICX 10.0 as explained in Chapter 03 (Section 3.2.4)

4.3 RESULTS AND DISCUSSIONS

A significant effect ($P < 0.05$) was observed on fruit skin *L**, *h** and eye colour (Appendix 11, 12 and 13). However, there was no significant effect ($P > 0.05$) on fruit *C** (Appendix 10). With respect to treatment effects, MJ treated fruit showed low values for *L**, *C** and *h**, except for eye colour when compared with control fruit. In 'Kent' mango fruit, the use of 10^{-5} M MJ improved red and yellow colour development by increasing *L** and *h** after cold storage at 5°C followed by 7 d shelf life at 20°C (Gonza´lez-Aguilar *et al.*, 2001). In this study, the skin colour of 'Hass' avocado fruit treated with MJ (10 and 100 µmol/L) were dark purple as indicated by lower *h** (67.32 and 70.23) values and higher eye colour rating (5.39 and 5.19) values, whereas; control fruit were approximately 75% coloured with higher *h** (84.12) and lower eye colour rating (4.25) values (Table 4.1). Similarly, MJ (2.24 $g.L^{-1}$) decreased *h** of 'Fuji' apple fruit stored at 21°C for 7 d and this response was associated with increased peel anthocyanin content (Rudell *et al*., 2002).

According to Saniewski *et al*., (1998), MJ induces the expression of anthocyanin biosynthesis enzymes in the presence of light. 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). 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). The application of MJ induced anthocyanin accumulation through up-regulation of the 'late' anthocyanin biosynthetic enzymes over 'early' anthocyanin biosynthetic enzymes in *Arabidopsis* (Shan *et al*., 2009). While, coronatine-insensitive protein 1 plays a vital role in regulating 'late' anthocyanin biosynthetic enzymes in MJ-induced anthocyanin accumulation (Shan *et al*., 2009). Therefore, dark purple colour observed on MJ treated fruit could be associated with increased an accumulation of cyanidin-3-*O*-glucoside (Cox *et al*., 2004: Ashton *et al*., 2006).

Table 4.1 Effect of methyl jasmonate on objective (*C*, h*, L**) and subject (eye) colour on 'Hass' avocado peel fruit during ripening

Treatment (MJ)	Chroma (*C**)	Hue angle (*h**)	Lightness (*L**)	Eye colour (1-6)
10 µmol/L	9.18a	67.32b	27.52a	5.39a
100 µmol/L	9.05a	70.23b	26.46b	5.19a
Control	10.14a	84.12a	27.52a	4.25b
P-value at 0.05	ns 0.62	0.04*	0.003*	0.005*
LSD=	1.1797	5.1106	0.2748	0.2245

Values with same letters were not significantly different at 0.05 probability level * Significantly different at P = 0.05, ns Not significant at P ≤ 0.05

Methyl jasmonate treatment had a significant effect ($P < 0.05$) on fruit weight loss (Appendix 14). The avocado fruit 'Hass' treated with 100 µmol/L MJ showed higher weight loss percentage when compared with control fruit (Table 4.2). Interestingly, the low concentration of MJ (10 µmol/L) reduced weight loss percentage when compared with control fruit. According to Mahajan *et al.* (2008), fresh produce continues to lose water, thereby, resulting in weight loss during post-harvest. The ability of MJ to reduce weight loss seemed to depend on concentration and crop species (Gonza´lez-Aguilar *et al.*, 2001; Gonza´lez-Aguilar *et al.,* 2003). In 'Bergarouge' apricot fruit, post-harvest application of MJ (0.2 mmol/L) reduced weight loss during 8 d shelf-life after 21 d cold storage (Ezzat *et al*., 2017). In contrast, post-harvest application of MJ did not change water loss of 'Sunrise' papaya fruit during storage for 14-32 d at 10°C and 4 d shelf life at 20°C (Gonza´lez-Aguilar *et al.,* 2003).

The ripening of fruit involves the breakdown of pectins, celluloses and hemicelluloses in cell wall, which result in softening later to fruit weight loss (Marin-Rodriguez *et al.* 2002). In 'Fuyang' loquat fruit, the application of MJ (10 µM) maintained higher pectins (CDTA-pectins) and lower levels of celluloses and hemicelluloses which could lead to lower weight loss during storage at 1°C for 35 d (Cao *et al*., 2010). In this study, the increase in weight loss on MJ (100 µmol/L) treated fruit was associated with increased ripening rate and firmness loss (Conchan *et al*., 2013).

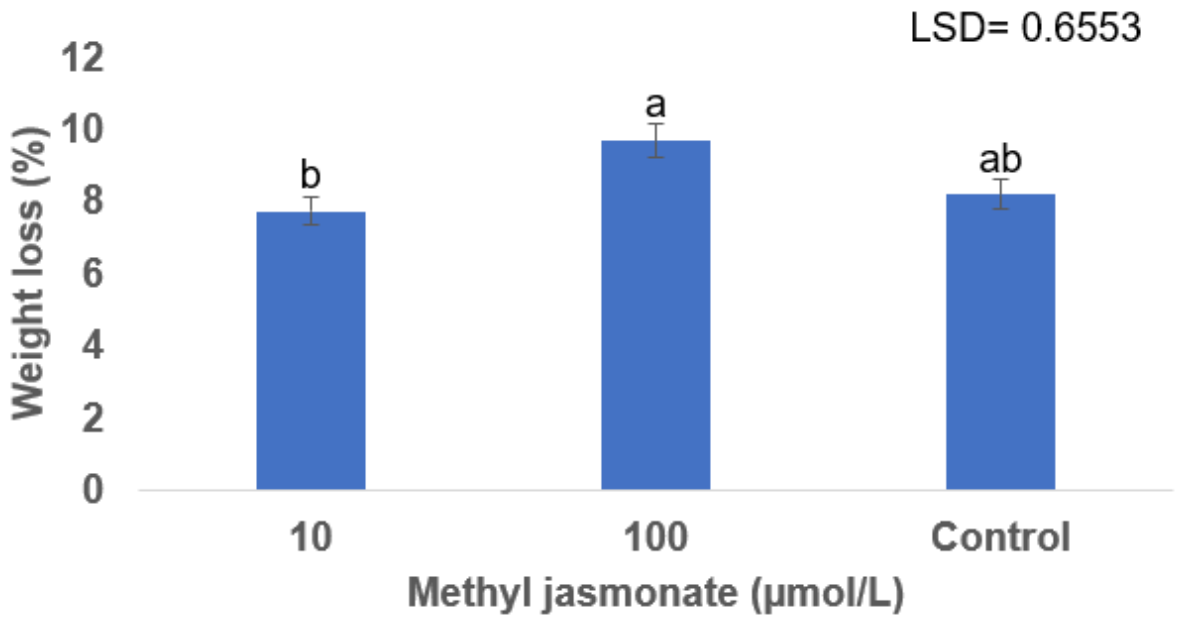

Figure 4.1 Effect of methyl jasmonate (10 and 100 µmol/L) treatment on weight loss of 'Hass' avocado fruit peel during ripening

The treatment (MJ) had no significant effect ($P > 0.05$) on fruit firmness during ripening (Appendix 15). Numerically, the control fruit had high firmness values when compared with MJ treated fruit (Figure 4.2). The effect of MJ on fruit firmness is presumed to be dependent on concentrations (Gonza´lez-Aguilar *et al.,* 2001). In this study, the lowest dose of MJ (10 µmol/L) maintained high fruit firmness than 100 µmol/L MJ and control during ripening.

These results confirmed the findings of Gonza´lez-Aguilar *et al.* (2001), who reported a reduced of firmness loss in 'Kent' mango fruit treated with MJ (10^{-5} M), thereafter; stored for 14 d at 10°C followed by 7 d shelf life at 20°C. The loss in fruit firmness results from the breakdown of pectin, cellulose and hemicellulose in cell wall during ripening (Marin-Rodriguez *et al.* 2002). The exogenous application of MJ (10µM) maintained higher pectins (CDTA-pectins) and lower levels of celluloses and hemicelluloses in 'Fuyang' loquat fruit during storage at 1°C for 35 d (Cao *et al*., 2010). In this study, the reduction in firmness loss on MJ (10 and 100 µmol/L) treated fruit might be due to the suppression of cell wall degradation (Meng *et al*., 2009).

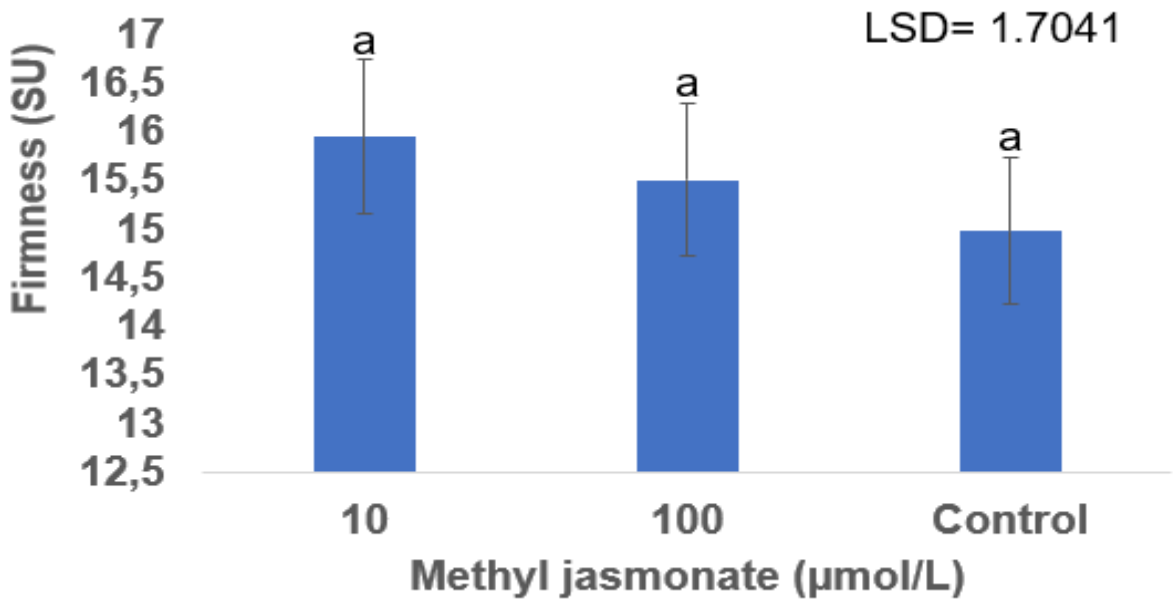

Figure 4.2 Effect of methyl jasmonate (10 and 100 µmol/L) treatment on firmness of 'Hass' avocado fruit peel during ripening; SU, Sinclair Units

Methyl jasmonate treatment had no significant effect ($P > 0.05$) on external chilling injury index on 'Hass' avocado fruit peel after withdrawal from 28 d cold storage (5.5°C) (Appendix 16). Chilling injury symptoms were approximately 1.06 on a scale of 0 to 3 for control fruit (Figure 4.3). Methyl jasmonate treatments alleviated chilling injury, irrespective of concentration (10 or 100 µmol/L).

In the membrane lipids, the ratio of unsaturated to saturated fatty acids varies with chilling-resistant and chilling-sensitive species (Eaks, 1990). Higher percentage of saturated fatty acids were found on chilling-sensitive than on chilling-resistant species (Eaks, 1990). Retaining high-unsaturated membrane-lipids helps to acclimatize crops to low temperatures (Sayyari *et al.,* 2011). Chilling injury begin when crop is exposed to chilling temperature below critical level, where lipid degradative enzymes such as lipoxygenase (LOX) and phospholipase D (PLD) are activated (Pinhero *et* al., 1998; Mao *et al*., 2007). Lipoxygenase causes peroxidation damage in membrane lipids, which result in increased membrane fluidity and levels of saturated lipids (Pinhero *et* al., 1998). According to Creelman and Mullet, (1997), endogenous methyl jasmonate is derived from enzymatic oxidation of unsaturated fatty acids by lipoxygenase (LOX). Therefore, application of MJ (10, 20 and 30 µMol L^{-1}) treatments have been found to reduce chilling injury symptoms by suppressing

LOX activity and ion leakage in the cell membrane of ‘Griff’ longkong fruit stored at 13°C (Venkatachalam and Meenune, 2015). In ‘EMb’ strawberry under water stress condition, MJ (0.01 and 0.1 mM) treatment protected cell membrane through decreasing membrane-lipid peroxidation as expressed by malondialdehyde (MDA) content (Wang, 1999).

In addition, chilling temperatures increase reactive oxygen species (ROS) which causes damage in plant cell membrane during cold storage (Lee *et al*., 2007). High production of ROS cause peroxidation of lipids, enzyme inhibition, nucleic acids damage and activation of programmed cell death (PCD) pathway, which later lead to cell death (Martindale and Holbrook, 2002). In plants, numerous antioxidants protect against ROS (Lee *et al*., 2007). According to Bertling *et al*. (2007), antioxidants protect cellular structure by scavenging free radicals that are formed when plant tissue exposed to oxidative stress. In ‘Beskid’ raspberry fruit, exogenous application of MJ (16 and 24 $\mu l.l^{-1}$) protected cell membrane, and thereby; reducing chilling injury after storage at 4°C for 7 d, through the enhancement of antioxidant enzyme activity (Ghasemnezhad and Javaherdashti, 2008). Moreover, MJ (10, 20 and 30 $\mu Mol\ L^{-1}$) treatments reduced chilling injury through enhanced antioxidant enzyme activity on ‘Griff’ longkong fruit stored at 13°C (Venkatachalam and Meenune, 2015). In this study, the alleviation of CI by MJ treatment could be associated with the suppression of membrane enzymes degradation that reduced ion leakage (Meng *et al*., 2009).

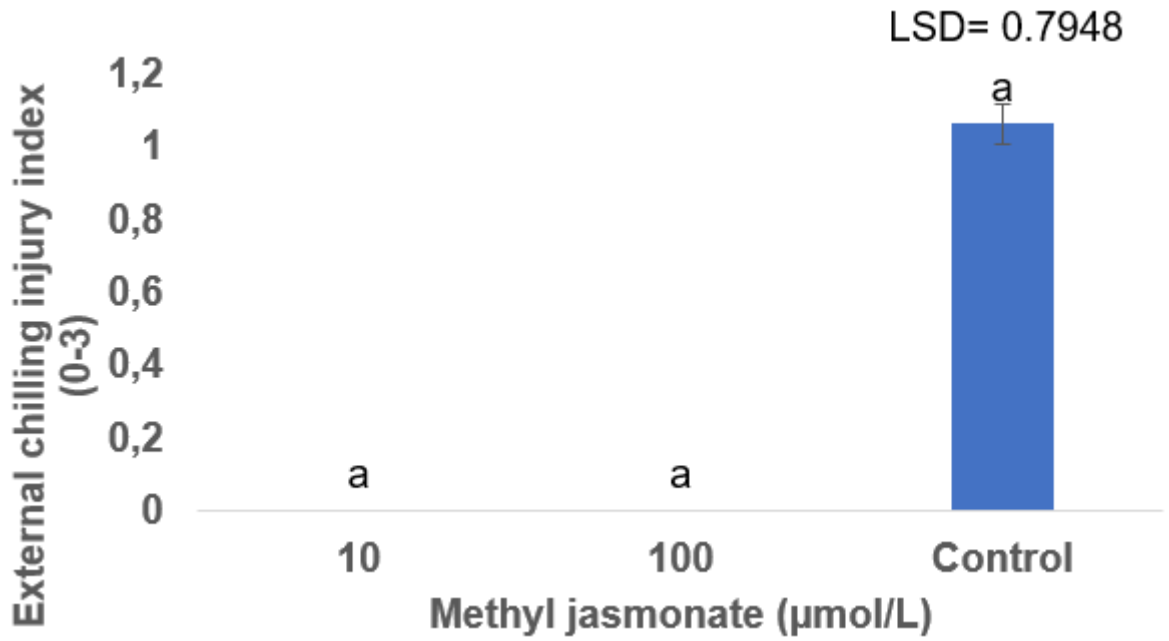

Figure 4.3 Effect of methyl jasmonate (10 and 100 µmol/L) treatment on external chilling injury index of 'Hass' avocado fruit peel

Treatment (MJ) had no significant effect ($P > 0.05$) on electrolyte leakage on 'Hass' avocado fruit peel after 28 d storage at 5.5°C (Appendix 17). Fruit treated with MJ had lower electrolyte leakage when compared with control (Figure 4.4). These results were in agreenment with of Junmatong *et al.* (2012), whereby; MJ (0.1 and 1 mM) treatments reduced EL in 'Nam Dok Mai No.4' mango fruit stored at 5±1°C for 42 d. Moreover, MJ (10, 20 and 30 µMol L^{-1}) treatments reduced electrolyte leakage through enhanced antioxidant enzyme activity on 'Griff' longkong fruit stored at 13°C (Venkatachalam and Meenune, 2015). In this study, the reduction in EL could be due to maintained in membrane integrity and enhanced antioxidant activity (Meng *et al*., 2009).

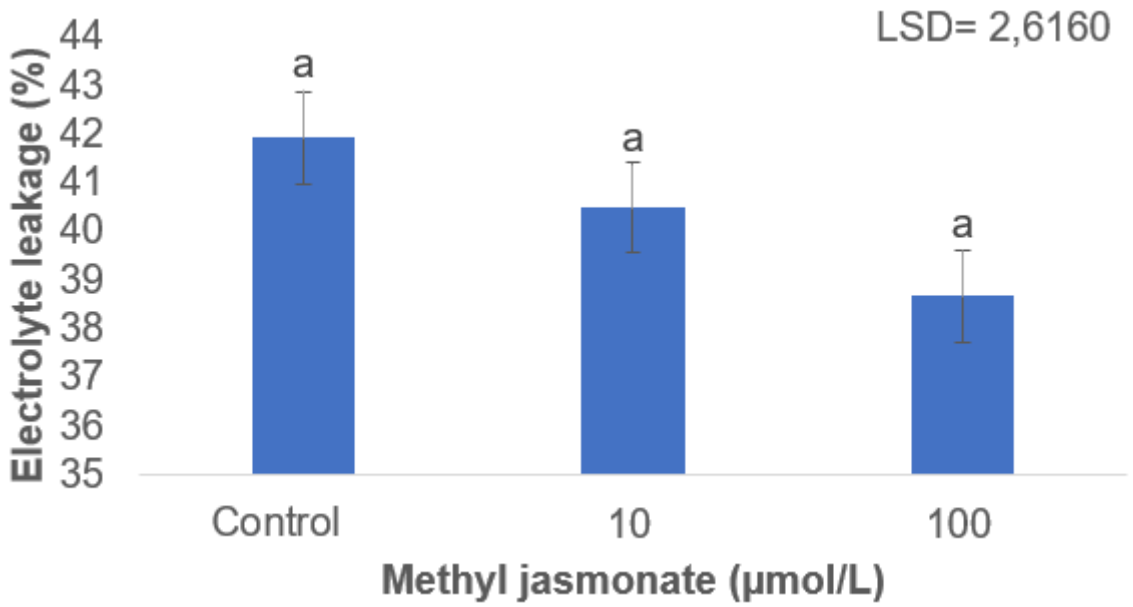

Figure 4.4 Effect of methyl jasmonate (10 and 100 µmol/L) treatment on electrolyte leakage of 'Hass' avocado fruit peel

Treatment showed non-significant effect ($P > 0.05$) on ripening percentage of 'Hass' avocado fruit after withdrawal from 28 days cold storage (5.5°C) (Appendix 18). In general, fruit started to ripen from day 2 after withdrawal from cold storage (Data not shown). At the final day of evaluation, a lower ripening percentage was observed in 'Hass' avocado fruit treated with low concentration (10 µmol/L) of MJ when compared with MJ (100 µmol/L) treated and control fruit (Table 4.3). The ripening process of climacteric fruit is triggered by ethylene production (Bower *et al*., 2003). The application of MJ stimulates ethylene production in a climacteric and non-climacteric fruit through increased expression and activity of ethylene biosynthesis enzymes (Fan *et al*., 1998; Bower *et al*., 2003). The efficacy of MJ treatment on ethylene production, 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activities and ACC synthase differed with fruit developmental stage and MJ concentration (Mukkun and Singh, 2009; Fan *et al*., 1997; Fan *et al*., 1998). In 'Pajaro' strawberry fruit, the highest ethylene production was observed with treatment of MJ (50 µM) and stored at 20±1°C for 6 d at fully ripe, half ripe and white stage (Mukkun and Singh, 2009). In 'Summerred' apple fruit, the use of MJ (2×10^{-7} mol L^{-1}) increased ethylene production during storage at 20°C for 15 d (Fan *et al*., 1997).

Moreover, in ‘Camarosa’ strawberry fruit, treatment with MJ (50 µM) increased ethylene production and respiration rate at white and pink stage during storage at 25/15°C day/night for 9 d (Perez *et al*., 1997). According to Perez *et al*. (1997), respiratory rate activity was associated with ripening rate. In this study, ripening percentage could be associated with increased ethylene production (Mukkun and Singh, 2009; Fan *et al*., 1997; Fan *et al*., 1998) and inhibition of polygalacturonase (PG) activity (Venkatachalam and Meenune, 2015).

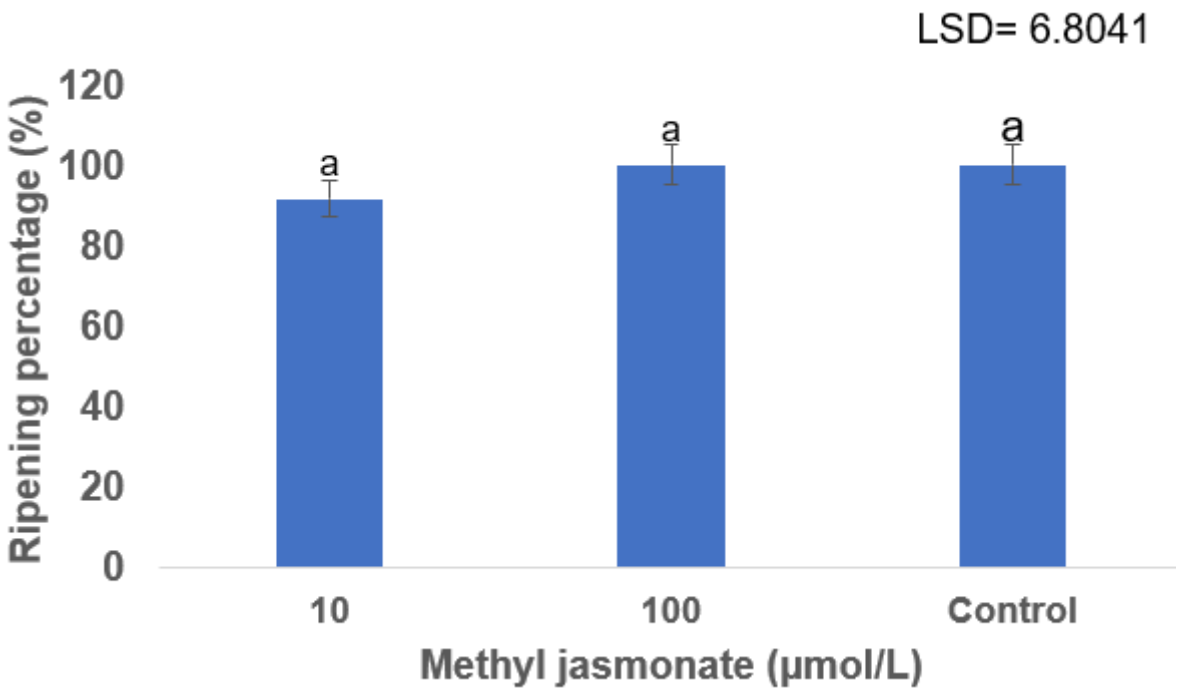

Figure 4.5 Effect of methyl jasmonate (10 and 100 µmol/L) treatment on ripening percentage of ‘Hass’ avocado fruit during ripening

4.4 Conclusion

Methyl jasmonate effectively alleviated chilling injury and enhanced skin colour of ‘Hass’ avocado fruit harvested at early-season. Methyl jasmonate treatment also improved fruit firmness, electrolyte leakage but for weight loss varied with concentrations. Low concentration (10 µmol/L) reduced weight loss whereas high concentration (100 µmol/L) increased weight loss.

CHAPTER 05

SUMMARY, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

Exposing fruit to HWT reduced external chilling injury (ECI) index and improved skin colour but negatively impacted fruit quality such as weight loss, firmness and physical appearance. Hot water treatment at 42°C/25 min or 46°C/20 min were the most effective to improve ‘Hass’ avocado fruit skin colour.

The results indicated that ‘Hass’ avocado fruit can be harvested at early-season and treated with MJ to alleviate CI and improve skin colour. Methyl jasmonate at low concentration was most effective in maintaining fruit quality when compared with high concentration. Treating fruit with MJ improved physical appearance with the exception of high concentration (100 µmol/L) on weight loss.

5.2 Recommendations

i) hot water treatment reduced CI and improved skin colour development but aggravated fruit firmness and weight loss, therefore; HWT (42°C/25 min or 46°C/20 min) should be combined with postharvest that maintain firmness and reduce weight loss to get fruit of a good quality.

ii) high concentration (100 µmol/L) of MJ showed to alleviated CI and improve skin colour of ‘Hass’ avocado fruit but impaired weight loss when compared with low concentration (10 µmol/L) in terms of quality parameters, therefore, experiments with different MJ-concentrations must be conducted to get optimum concentration.

iii) further experiments are required to determine the mechanism of colour change on MJ-treated fruit during ripening.

iv) HWT (42°C/25 min and 46°C/20 min) and (100 µmol/L) increased firmness and weight loss, therefore, experiment of MJ and HWT should be carried out to determine the effect combined treatments on these quality parameters.

5.3 Conclusions

Hot water treatment effectively reduced chilling injury and enhanced skin colour development of ‘Hass’ avocado fruit during ripening. Hot water treatment at 42°C for

25 min or 46°C for 20 min were the most effective treatments to improve ‘Hass’ avocado fruit skin colour. Generally, HWT reduced chilling injury and improved skin colour but impaired other quality parameters like weight loss and firmness loss. Therefore, treatments that reduced CI also enhanced skin change of ‘Hass’ avocado fruit during ripening.

Treating ‘Hass’ avocado fruit with MJ alleviated chilling injury and improved skin colour development during ripening. The effectiveness of MJ treatment varied with treatment concentration for skin colour change. Low concentration (10 µmol/L) was the most effective on improving ‘Hass’ avocado skin colour development during ripening when compared with high concentration (100 µmol/L). In terms of CI index, MJ effectively alleviated ECI, irrespective of concentration. Methyl jasmonate treatment also improved general appearance of fruit. The benefits of MJ lie in its ability to extend storage time recommended for export marketing while maintaining fruit quality and can be incorporated into the pack-house sorting line.

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APPENDICES

Appendix 1 Analysis of variance (ANOVA) for HWT on chroma (*C**)

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	12.1556	4.05188	0.99	0.4457
Error	8	32.7935	4.09918		
Total	11	44.9491			

Appendix 2 Analysis of variance (ANOVA) for HWT on lightness (*L**)

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	14.2379	4.74596	21.93	0.0003
Error	8	1.7312	0.21640		
Total	11	15.9691			

Appendix 3 Analysis of variance (ANOVA) for HWT on hue angle (*h**)

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	223.981	74.6602	1.60	0.2648
Error	8	373.908	46.7385		
Total	11	597.889			

Appendix 4 Analysis of variance (ANOVA) for HWT on subjective (eye) colour

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	2.03816	0.67939	6.72	0.0141
Error	8	0.80893	0.10112		
Total	11	2.84709			

Appendix 5 Analysis of variance (ANOVA) for HWT on weight loss

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	2.30060	0.76687	5.05	0.0298
Error	8	1.21507	0.15188		
Total	11	3.51567			

Appendix 6 Analysis of variance (ANOVA) for HWT on firmness

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	22.2361	7.41203	4.49	0.0397
Error	8	13.2008	1.65010		
Total	11	35.4369			

Appendix 7 Analysis of variance (ANOVA) for HWT on external chilling injury index

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
Treatment	3	2.76749	0.92250	6.96	0.0128
Error	8	1.06033	0.13254		
Total	11	3.82783			

Appendix 8 Analysis of variance (ANOVA) for HWT on electrolyte leakage

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	3	94.889	31.6297	1.44	0.3012
Error	8	175.520	21.9400		
Total	11	270.409			

Appendix 9 Analysis of variance (ANOVA) for HWT on ripening percentage

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	3	33.333	11.1111	0.67	0.5957
Error	8	133.333	16.6667		
Total	11	166.667			

Appendix 10 Analysis of variance (ANOVA) for chroma (*C**) of 'Hass' avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	2.1570	1.07849	0.52	0.6209
Error	6	12.5261	2.08769		
Total	8	14.6831			

Appendix 11 Analysis of variance (ANOVA) for lightness (*L**) of 'Hass' avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	3.85637	1.92818	17.02	0.0034
Error	6	0.67970	0.11328		
Total	8	4.53607			

Appendix 12 Analysis of variance (ANOVA) for hue angle (*h**) of 'Hass' avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	483.549	241.775	6.17	0.0350
Error	6	235.067	39.178		
Total	8	718.617			

Appendix 13 Analysis of variance (ANOVA) for subjective (eye) colour of ‘Hass’ avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	2.22685	1.11343	14.72	0.0048
Error	6	0.45370	0.07562		
Total	8	2.68056			

Appendix 14 Analysis of variance (ANOVA) for weight loss of ‘Hass’ avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	6.3450	3.17248	4.93	0.0542
Error	6	3.8647	0.64412		
Total	8	10.2097			

Appendix 15 Analysis of variance (ANOVA) for firmness of ‘Hass’ avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	1.4213	0.7106	0.16	0.8531
Error	6	26.1343	4.35571		
Total	8	27.5556			

Appendix 16 Analysis of variance (ANOVA) for chilling injury index of ‘Hass’ avocado treated with MJ

Source	**DF**	**SS**	**MS**	**F-value**	**P≤ 0.05**
treatment	2	2.22840	1.1142	1.18	0.3708
Error	6	5.68519	0.94753		
Total	8	7.91358			

Appendix 17 Analysis of variance (ANOVA) for electrolyte leakage of 'Hass' avocado treated with MJ

Source	DF	SS	MS	F-value	P≤ 0.05
Treatment	2	16.0867	8.0433	0.78	0.4985
Error	6	61.5933	10.2656		
Total	8	77.6800			

Appendix 18 Analysis of variance (ANOVA) for ripening percentage of 'Hass' avocado treated with MJ

Source	DF	SS	MS	F-value	P≤ 0.05
treatment	2	138.889	69.4444	1.00	0.4219
Error	6	416.667	69.4444		
Total	8	555.556			

Appendix 19: Paper presented at national conference as part of this research project

a) **Oral presentation** - M Munzhedzi, N Mathaba, TP Mafeo and J Mlimi, "Ripening physcochemical properties of new 'Fuerte-type' avocado selections, *5th University of Limpopo Faculty of Science and Agriculture Research Day*, 3-4 October 2015, Polokwane, Limpopo, South Africa**.**

b) **Poster presentation** - M Munzhedzi, TP Mafeo, MR Masevhe, N Mathaba, J Mlimi and MJ Ntandane, "Evaluation of post-harvest storage temperature (5.5°c) and shelf-life of newly developed 'Fuerte-type' avocado selections 'ITSC selection', 'Calshad', 'BL1058' and 'Wurtz'", *Combined congress*, 19-22 January 2015, George, Western Cape, South Africa.