Does cold shock treatment extend the shelf life of avocado fruit?

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Abstract

Avocado ripens rapidly after harvest causing the difficulty for fruit export to distant markets. Cold storage at 4 – 6 °C is the most popular method to extend the shelf-life of avocado for 4 weeks. Chen et al. (2017) showed that cold shock treatment (CST) at 0 °C for 30 mins effectively delayed ripening-associated processes, reduced respiration rate, ethylene production and cell-wall enzyme activities. The objective of this thesis was to provide better insight into the effects of CST on delaying avocado ripening by observing the influence of CST on post-storage qualities of avocado.

The experiments replicated the experimental work of Chen et al. (2017). Experiment 1 was to identify the suitable CST in a full matrix of three temperatures (0, 2 and 4 °C) and six durations (15, 30, 45, 60, 90 and 120 minutes). The effects of CST were not observed on firmness retention measured by puncture test, however, there was a consistent trend that colder temperatures and shorter treatments resulted in better firmness outcomes although there are not statistically significant, possibly due to the large fruit variability.

Experiment 2 was to replicate the experiment 1 with increased sample size to 3 times to reduce the influence of fruit to fruit variation on the data analysis. The effects of CST on firmness retention were not seen. The large fruit variability still remained the big issue. Experiment 3 was designed to increase the sample size to 10 times compared to the previous experiment to minimise the large fruit variability using a selected single treatment (0 °C and 60 minutes). The effect of CST treatment on the ripening (including pulp softening, skin discolouration, respiration rate and ethylene production rate) was not statistically significant, however, the considerable reduction of ethylene production rate was observed.

Experiment 4 was designed to provide better insight about the effects of CST on the overall qualities of avocado using 2 different methods for firmness measurements (destructive and non-destructive method). The effects of CST on the ripening was insignificant, however, ethylene production rate significantly reduced with treated fruit. Nevertheless, unlike the experiment results of Chen et al. (2017), in the current study, CST was not found to have any pronounced effects on fruit quality through 4 experiments.

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1-MCP	1-Methylcyclopropene
ACO	1-aminocyclopropane1-carboxylic acid oxidase
ACS	1-aminocyclopropane1-carboxylic acid synthase
AFS	Acoustic Firmness Sensor
CAP	Controlled Atmosphere Packaging
CST	Cold Shock Treatments
DMC	Dry Matter Content
FI	Firmness index
GC	Gas Chromatography
MeJA	Methyl Jasmonate
MeSA	Methyl Salicylate
MAP	Modified Atmosphere Packaging
NIRS	Near-Infrared Spectroscopy
PG	Polygalacturonase
PME	Pectin Methylesterases
RH	Relative Humidity

CHAPTER 1

INTRODUCTION

1. Introduction

Avocado (*Persea americana Mill.)* is a member of the family Lauraceae found in Central America and Southern Mexico. Avocado fruit is divided into three races, including West Indian race (*Persea americana Mill.* var. *Americana,* a tropical race having large variably shaped fruit); Guatemalan race (*P. nubigena* var. *guatemalensis* L. Wms, a subtropical race having mostly round thick-skinned fruit) and Mexican race (*P. americana Mill.* var. *drymifolia Blake,* a semitropical race having smaller and elongated fruit). Due to a large amount of omega fatty acids, avocados were well-known to provide the health benefits to human, for example, reducing cholesterol, cardiovascular diseases and cancer (Ozdemir and Topuz, 2004; Lu et al., 2009; Duarte et al., 2016).

Avocado cultivars for commercial production are ‘Hass’, ‘Bacon’, ‘Fuerte', 'Gwen', 'Ryan', and so on, in which 'Hass' is the most important that makes up more than 85 % of all avocado grown and sold worldwide. Also, ‘Hass’ avocado is the predominant cultivar in the major producing countries, such as Mexico, Chile and the USA (Yahia and Woolf, 2011). The major producing countries of avocado include Mexico, Indonesia, Dominican Republic, Chile, amongst others (Table 1.1).

Table 1.1 Global avocado production in 2018 by country (tonnes) (Anonymous, 2018)

Country	*Area harvested (ha)*	*Production (tonnes)*
Mexico	206,389	2,184,663
Chile	29,166	124,506
Dominican Republic	13,924	644,306
Indonesia	33,393	410,094
USA	21,707	168,528
Australia	13,531	63,486
New Zealand	4,408	25,525

Compared to other avocado varieties, ‘Hass’ avocado fruit is more oval and has irregularly rough and grainy skin’s surface that changes to dark purple colour when ripe. Having a small seed and weighing from 140 to 340 grams on average, a ‘Hass’ avocado fruit contains a good portion of edible flesh (Ayala Silva and Ledesma, 2014). The maturity of the fruit affects the incidence and severity of rots as well as chilling injury. Late-season avocado tends to have a higher number of rot’s incidence, such as stem-end and body rots than the early-season one after storage (Dixon et al., 2003).

Oil content is considered to be the most crucial determinant of avocado’s maturity. Among all three botanical races of avocado, the Mexican race has the highest oil content and are the most cold-tolerant (Yahia and Woolf, 2011). Different cultivars vary in oil content which ranges 7 - 40 % on a fresh weight basis (Ayala Silva and Ledesma, 2014). Oil content and dry matter content (DMC) were well-known for a good correlation (Lee et al., 1983), as a result, DMC was commonly used as a maturity index in Australia, New Zealand, Chile, the United States and Israel (Hofman et al., 2000). The minimum DMC for harvesting requirement is 17 - 25 % which regards to the regulation of each country and depends on the cultivar (Yahia and Woolf, 2011). For example, DMC standard for ‘Hass’ cultivar is 22.8 % in the USA (Gamble et al., 2010), 23.5 % in Colombia (Carvalho et al., 2014) and 24 % in New Zealand (Pak et al., 2003); DMC standard for ‘Fuerte’ cultivar is 21 % in the USA (Lee et al., 1983).

Avocado is known as climacteric fruit which ripe off the tree after picking (Schroeder, 1953), while other fruits belong to the non-climacteric fruit group that the ripening ceases after picking from the plants (Kader and Yahia, 2011). Firmness and skin colour are the major indicators of ‘Hass’ avocado fruit ripening. When 'Hass' avocado fruit ripes, chlorophyll level decreased at the same time with the increase of anthocyanin (cynanidin-3-O-glucoside) level, as a result, skin colour changes from green to purple or black (Schaffer et al., 2013). Although the skin colour is known as ripeness indicator of handlers and consumers, the ripeness of the fruit is sometimes not corresponding to the colour of fruit skin during the ripening due to large fruit to fruit variation (Schaffer et al., 2013). Fruit firmness was also used as an avocado ripeness index in South Africa (Schaffer et al., 2013).

Storage temperature is the critical factor to extend the storage life of avocado. Hopkirk et al. (1994) stated that the optimal temperature for avocado ripening is 15 - 20 °C, which was demonstrated that when the temperature was below 15 °C, the ripening rate decreased, and when the temperature was above 25 °C, rot and uneven ripening might occur. In order to be able to transport avocado to distant markets, avocado is mostly stored in low temperature. Hopkirk et al. (1994) recommended that ‘Hass’ avocado fruit should be stored at 4 - 6 °C for 4 weeks for optimal quality at the distant markets, while fruit maintained at 0 - 2 °C developed symptoms of cold damages and increased rot incidence after storage of 2 weeks. Another study by Woolf et al. (2003) reported that avocado fruit stored under 3 °C developed skin damage, which limited the application of cold storage as a method of avocado preservation. Additionally, the severity of cold damages depends on the cultivar, maturity and ripeness of fruit when stored at 0 - 4 °C (Yahia and Woolf, 2011). The application of low-temperature conditioning at 6 – 8 °C for 3 – 5 days before cold storage at 0°C was proved to prevent skin damage for avocados (Woolf et al. (2003b)).

Cold shock treatment (CST) has been studied to extend the storage life of various crops, for instance, cucumbers (Chen et al., 2015), bananas (Zhang et al., 2010), avocados (Chen et al., 2017) and nectarines (Xiong et al., 2006). Zhang et al. (2010) confirmed that immersion of banana fruit in ice water for an hour effectively inhibited ripening-associated processes, reduced ethylene production rate and respiration rate. The study concluded that CST decreased activities of polygalacturonase, pectin methylesterase and CMCase, which relate to ripening and softening processes. Similarly, Chen et al. (2015) demonstrated that CST reduced weight and firmness loss and increased the activities of antioxidant enzyme and respiration rate of cucumber fruit treated at 3 °C for 40 min. The authors also recommended that the CST condition should be below the threshold to achieve optimal effects.

With the same methodology, Chen et al. (2017) demonstrated that immersion of ‘Hass’ avocado in ice water for 30 minutes effectively delayed the ripening and reduced the rate of respiration and ethylene production. However, due to the high fruit variability, the effects of CST on the storage life extension of avocado need to be validated. The objective of this thesis is to provide better insight to the effectiveness of CST application on firmness retention in avocado by first attempting to replicate the findings of Chen et al. (2017), followed by conducting a storage trial to study the potential effects of CST on post-storage quality. However, the first part of the study (replication of the previous study) served as a critical step, where the level of success in this step would determine the need for a storage trial afterwards.

The thesis focused on the effect of CST on the firmness, colour, respiration rate and ethylene production rate of avocado during the storage time through four experiments reported in chapter 3, chapter 4, chapter 5 and chapter 6 respectively. The effects of treatment duration and temperature on avocado’s firmness were investigated in chapter 3 during the ripening at the 2-day interval. Chapter 4, the sample size was three times higher than that of chapter 3 to reduce the fruit variability and a change in texture analysis to reduce the measurement error. In chapter 5, the study focused on a specific treatment period and temperature to investigate the effect of CTS to reduce the fruit-to-fruit variation when the sample size was ten times higher than the chapter 4 and studied broader about skin colour, respiration rate and ethylene production rate. Chapter 6 replicated the chapter 5 on fruit treatments, with the intensive investigation and measurements the change of fruit firmness, skin colour, ethylene production rate and respiration rate during the storage time of 7 days, combining a non-destructive method for firmness measurement.

CHAPTER 2

LITERATURE REVIEW

2. Literature review

2.1. Introduction

'Hass' avocado is the most common variety of New Zealand's avocado, accounting for 95 % of growing areas. The majority of New Zealand avocado production is exported to distant markets in Australia and Asia (Dixon et al., 2003). As a climacteric fruit, avocado has a relatively short shelf-life, i.e. 5 – 7 days to ripen after harvest (Ozdemir and Topuz, 2004). However, it takes several weeks for avocados to travel in the supply chain, from being harvested from a farm to consumers. As a result, the relatively short shelf life causes trouble for logistic management and fruit export industry. Additionally, rot and chilling injuries are one of the most prevalent problems for avocado industry (Dixon et al., 2003; Zauberman et al., 1973).

To overcome these challenges, the need to extend the shelf-life as well as to reduce the incidence and severity of rot and chilling injuries is urgent and essential for fruit exporting industry. Several methods have been studied to maintain or improve the quality of avocados during the storage and transport, including cold storage at 4 – 6 °C for four weeks (Hopkirk et al., 1994), hot water treatment at 38 °C for 60 minutes (Woolf et al., 1995), low-temperature conditioning at 6 – 8 °C for 3 – 5 days (Woolf et al., 2003), 1-MCP application of 0.45 $\mu L\ L^{-1}$ for 24 hours (Jeong et al., 2002, Woolf et al., 2005, Hershkovitz et al., 2005), controlled and modified atmospheres (Hernández et al., 2017, Eksteen and Truter, 1985).

CST has been found to have positive effects of extending the storage life of many produces, including tomatoes (Zhang et al., 2018), broccoli (Zhang et al., 2009), cucumbers (Chen et al., 2015), bananas (Zhang et al., 2010) and avocados (Chen et al., 2017). The effects of CST include delaying the ripening and the softening of produces, such as the retention of skin colour and fruit firmness retention, and the reduction of respiration rate and ethylene production rate. The methods used to measure quality parameters from these studies were shown in the chapter. The objective of this thesis is to provide better insight into the effectiveness of CST application on delaying the ripening of avocados. The literature review serves as a knowledge basement for developing experimental procedure, fruit quality measurements as well as relating theories to understand the effect of CST.

2.2. Fruit ripening

The ripening of avocado starts with the changes of biochemistry occurred in the fruit, such as the change of fruit firmness relating to cell-wall degradation, skin colour, aroma and the increase of ethylene production rate and respiration rate (Seymour et al., 2012).

2.2.1. Cell-wall degradation during the ripening

Avocado only ripens after being picked off from the tree, and the ripening process of avocado starts 1 – 2 days after harvest (Schroeder, 1953). The fruit ripening is associated with the softening process which involves the modification and degradation of the primary cell wall of the fruit. The cell wall components comprise cellulose, hemicellulose, pectic polysaccharides (or pectin) cross-linked by calcium and structural proteins. The cell-wall degradation is influenced by the significant roles of three hydrolytic enzymes: cellulase, polygalacturonase, pectin methylesterase which are linked to the softening process of avocado during the ripening (Hofman et al., 2013, Defilippi et al., 2018).

Avocado texture significantly changes during the ripening where the mesocarp becomes soft in line with the degradation of cell-wall structure as affected by the increase of cellulase activity (Sakurai and Nevins, 1997). Awad and Young (1979) reported that there was a strong relationship between ethylene production rate, respiration rate and cell-wall degradation caused by hydrolytic enzymes in mesocarp during the ripening. Notably, ethylene production rate significantly increased at the beginning of the climacteric stage resulting in the rise of respiration rate and the activity of cellulase; the activity of polygalacturonase, pectin methylesterase increased three days later in the phase.

Cellulase plays a vital role in avocado ripening. O’donoghue et al. (1994) emphasised on the importance of cellulase (endo-β-1,4-glucanase) at the pre-climacteric phase in which cellulase degraded cellulose at the accessible sites of the microfibril, which initially breaks the cell-wall structure and makes way for the attack of pectin-methylesterase and polygalacturonase at the later stage. polygalacturonase has the least impact on cell-wall degradation among the three enzymes during the ripening. Jeong et al. (2002) reported that polygalacturonase showed a significant effect on fruit softening at the later climacteric stage, which linked to the polyuronide depolymerisation in cell-wall. The result was consistent with the experimental results of Awad and Young (1979) and Huber and O’Donoghue (1993). Wakabayashi et al. (2000) suggested that the de-esterification of polyuronides caused by pectin-methylesterase assisted the degradation of polyuronide by polygalacturonase because the high concentration of methyl-esterification obstructs the activity of polygalacturonase (Yoshioka et al., 1992).

2.2.2. Fruit firmness and measurement methods

Firmness is one of the quality-indicative properties to monitor and assess the shelf-life of fruit besides skin colour, respiration rate and ethylene production rate under a specific storage condition (temperature, relative humidity, atmosphere) (Sierra et al., 2019). In line with the cell-wall degradation, firmness of mesocarp decreases overtime during the ripening. White et al. (1999) described the available destructive methods to measure firmness value of avocado, for example, firmometer (Anderson firmometer), penetrometer (puncture test) using Effegi probes or conical probes and hand squeezing. Amongst the methods evaluated, firmometer showed a good linear correlation to hand squeezing evaluation. Penetrometers have been widely used in many studies, for example, hand-held Effegi penetrometer (Li et al., 2016), firmness penetrometer (Valero et al., 2007), universal testing machine (Sierra et al., 2019), TA.XT Plus Texture Analyser (Gwanpua et al., 2018b, Soteriou et al., 2014).

To monitor the ripeness of fruit during the transport and cold storage, many non-destructive methods for firmness measurement have been introduced, for example, vibration spectrum (Abbaszadeh et al., 2013, Hosoya et al., 2017), acoustic (Duprat et al., 1997, Mao et al., 2016), ultrasonic (Morrison and Abeyratne, 2014, Mizrach et al., 2000), low-mass impact (Howarth et al., 2003) and laser air-puff detector (Hung et al., 1999).

Acoustic measurement is based on the natural resonance frequency analysis, which is produced as the fruit surface is tapped (Estrada-Flores, 2003). Acoustic Firmness Sensor (AFS) developed by AWETA™ (The Netherlands) has been widely used for firmness measurement in many previous studies: avocados (Estrada-Flores, 2003), apples (Fathizadeh et al., 2020, Grimi et al., 2010, Chen and DeBaerdemaeker, 1993), tomatoes (Muramatsu et al., 1996, Schotte et al., 1999, Duprat et al., 1997), plums, nectarines and apricots (Muramatsu et al., 1996), kiwifruits (Muramatsu et al., 1997, Javadi and Nasiri, 2017, Feng et al., 2016), watermelons (Mao et al., 2016) and peaches (Yurtlu, 2012).

Compared to the traditional penetration method (puncture test), the acoustic method can provide better discrimination of ripe fruit (or mature fruit) which have lower firmness compared to unripe fruit (or immature fruit) (Estrada-Flores, 2003, Duprat et al., 1997). By contrast, destructive methods generally can provide better discrimination of unripe (or immature) avocado fruit (with higher firmness values) compared to the acoustic method (White et al., 1999). However, a low correlation coefficient between the acoustic method and penetration method was reported for firmness measurement of avocados (Estrada-Flores, 2003, Galili et al., 1998), apples and peaches (Yurtlu, 2012).

2.2.3. Skin colour evolution during the ripening

The skin colour change of ‘Hass’ avocado is one of the indicators for ripening of avocado fruit from a consumer's perspective. Cox et al. (2004) reported that when ‘Hass’ avocado fruit ripens, the skin colour changes from bright green to dark brown, purple or black associated with the decrease of the value of colour parameters such as L, C^*, h^* indicating lightness, chroma and hue, respectively. The concentration of chlorophyll a and b in the skin significantly declined during ripening while the level of cyanidin 3-O-glucoside increased in line with the change in hue angle (Ashton et al., 2006). The finding was consistent with the findings of Cox et al. (2004) showing that 3 – 6 days after harvest, the cyanidin 3-O-glucoside rose sharply along with the drop of chlorophyll a and b concentration during 4 – 5 days after harvest. A higher storage temperature resulted in a higher amount of cyanidin 3-O-glucoside produced (Cox et al., 2004). It can be explained that high temperature accelerated the activity of enzymes which are responsible for chlorophylls degradation and anthocyanin synthesis (Sierra et al., 2019).

Maftoonazad and Ramaswamy (2008) demonstrated that during the ripening of ‘Hass’ avocado, the skin colour parameters (L^*, a^*, b^*, C^*, h^*) changed significantly corresponding to the change of the colour and brightness of the skin. The decrease of L^*

value indicates the loss in brightness, the increase of a^* value indicates the more redness in colour, the decrease of b^* value indicates the drop of yellowness whereas the reduction of h^* value indicates the change of skin to a more reddish colour. The decrease of C^* value indicates that there is less saturation in colour (Maftoonazad and Ramaswamy, 2008).

Nevertheless, since there is usually high variability in fruit quality including a large variation in skin colour, the assessment of ripeness of fruit solely based on the skin colour may not provide a reliable result. The skin colour varies from tree to tree (Hofman and Jobin-Decor, 1999) and is affected by the production location (Vuthapanich, 2001), sunlight damage (Woolf et al., 1999). Schaffer et al. (2013) showed that avocado fruit may have the ready-to-eat firmness but is not entirely purple or black. Additionally, more mature avocado fruit may have darker skin (Hofman and Jobin-Decor, 1999).

2.2.4. Ethylene production during the ripening

As a climacteric fruit, ethylene production increases significantly in the climacteric phage, leading to a dramatic increase in respiration rate during ripening. Ethylene is a plant hormone which plays an essential role in the interactive signalling and metabolic pathways for the regulation of the ripening of climacteric fruits (Stepanova and Alonso, 2005, Barry and Giovannoni, 2007). The production of a high amount of ethylene is induced by the ethylene biosynthetic genes involving 1-aminocyclopropane1-carboxylic acid synthase (ACS) and 1-aminocyclopropane1-carboxylic acid oxidase (ACO) following an ethylene biosynthesis pathway (Lelièvre et al., 1997).

The increase of respiration follows the rise of ethylene production at the pre-climacteric phase that the increase of ethylene production provokes the elevation of respiration rate (Paul et al., 2012, Adato and Gazit, 1977). Avocado produces a small amount of ethylene and remains unripe when the fruit is still attached to the tree due to the lack of ACS and ACO (Sitrit et al., 1986) or the suppression of ACS activity (Paul et al., 2012). McMurchie et al. (1972) introduced the two-system process of ethylene production of unripe avocado in the pre-climacteric phase: “auto-inhibition” (system 1) and “auto-induction” (system 2) during ripening where a dramatic increase of ethylene production accelerating fruit ripening is observed.

Ethylene production of avocado is relatively low at harvest (about 0.1 µL kg^{-1} h^{-1}), then dramatically increases when the fruit ripens at the climacteric phase reaching up to 100 µL kg^{-1} h^{-1} at 20 °C (Yahia and Woolf, 2011, Adato and Gazit, 1977). The ethylene production depends on the storage temperature. Eaks (1978) demonstrated that ethylene production of avocado fruit declined significantly when the storage temperature increased from 20 °C to 30 °C; ethylene production was unnoticeable at 40 °C as avocado fruit ripened abnormally when the storage temperature rose to 40 °C because high temperature affected the protein synthesis (Eaks, 1978). Respiration and ethylene production significant reduce when avocado fruit is stored or treated at low temperature. Blakey et al. (2015) reported that avocado fruit stored at 1 °C remarkably decreased respiration and ethylene production compared with the traditional cold storage at 5.5 °C for 4 weeks. Chen et al. (2015) showed that cucumber fruit was immersed in cold water at 3 °C for 40 mins suppressed the rise of ethylene production and respiration and reduced the weight and firmness loss.

For some climacteric fruit, when the internal concentration of ethylene reaches a threshold of 1 µL L^{-1}, the receptors are saturated, which induces the ripening response (Paul et al., 2012, Burg and Burg, 1962). The sensitivity of different fruits to ethylene differs. The threshold levels of 0.1 – 0.5 µL L^{-1} of ethylene concentration were reported to initiate the ripening of climacteric fruits such as avocado, banana, pear; a lower threshold level of 0.01 μL L^{-1} for kiwifruit (Wills et al., 2001). Hence avocado fruit should not be stored near other ripe fruits which produce ethylene (Chaplin et al., 1983).

There are several ways to extend the shelf-life of fruits by suppressing the ethylene production process to delay the ripening of fruits. Previous attempts include cold storage, controlled atmospheric storage (Hailu et al., 2013), dynamic controlled atmosphere (Mditshwa et al., 2018, Brizzolara et al., 2017), modified atmosphere packaging (Eksteen and Truter, 1985, Gonzalez et al., 1989, Castellanos et al., 2017), blocking of ethylene receptors (1-MCP treatment) (Watkins, 2006, Hershkovitz et al., 2005, Khan and Singh, 2007, Feng et al., 2000) and the use of ethylene absorbents (O_3, $KMnO_4$, nano-TiO_2, MOFs) (Dutta et al., 1991, Sharma et al., 2012, Jang et al., 2006, Wang et al., 2018).

Several ethylene measurement methods that utilise various types of sensors include gas chromatography (GC), electrochemical sensors (amperometric and electrocatalytic), optical sensor (non-dispersive infrared spectroscopy, laser, Raman spectroscopy), and chemical sensors (photoluminescence, cataluminescence, colourimetric, gravimetric) (Hu et al., 2019, Caprioli and Quercia, 2014, Cristescu et al., 2013, Pereira et al., 2017, Gwanpua et al., 2018, Zaidi et al., 2016).

2.2.5. Respiration rate during the ripening

Avocado is classified as a climacteric fruit due to the significant rise of ethylene production and respiration at the climacteric stage during ripening (Seymour and Tucker, 1993, Eaks, 1978, Nath et al., 2014). Respiration is defined as an oxidation process of sugar, polysaccharides and lipid (which is abundant in avocados) through metabolic pathways to produce adequate energy for living cells. With climacteric fruits, a dramatic increase of respiration at the climacteric stage occurs to supply energy for the later ripening events such as cell-wall degradation, protein and pigments synthesis (Seymour et al., 2012).

The respiration of climacteric fruit decreases after harvest and start to significantly increase at the onset of ripening followed by the rise of ethylene production, in which ethylene acts as a plant hormone triggering and regulating the ripening process such as accelerating the elevation of respiration (Paul et al., 2012, Adato and Gazit, 1977).

The respiratory pattern of avocado fruit was divided into three stages: a pre-climacteric stage where the respiration rate decreases gradually, a climacteric stage where the respiration rate increases substantially, and a post-climacteric stage where the respiration rate declines (Kassim et al., 2013). According to Eaks (1983), avocado stored at 5 °C for 4 weeks, then ripened at 20 °C showed a typical climacteric pattern with the pre-climacteric stage of 4 days, the climacteric stage after 6 days and the post-climacteric stage after 8 days.

The ethylene production and respiration drop sharply when avocado fruit is stored at low temperature. Blakey et al. (2015) reported that avocado fruit stored at 1 °C remarkably decreased the ethylene production and respiration compared with the traditional cold storage at 5.5 °C for 4 weeks. Similarly, Zamorano et al. (1994) reported that avocado stored for 55 days at 7 °C significantly reduced the ethylene production and delayed the ripening of avocado fruit; however, respiration rate increased remarkably after 25 days of storage which concurred with the softening. The storage temperature of under 3 °C could restrain the ripening of avocado fruit. Castellanos et al. (2016) reported that avocado stored at 6 °C had respiration rate of 960 cm^3 kg^{-1} d^{-1} and ethylene production rate of 0.71 cm^3 kg^{-1} d^{-1} which were 4 - 6 times lower than those of avocados stored at 24 °C which had respiration rate of 3030 cm^3 kg^{-1} d^{-1} and ethylene production rate of 3.11 cm^3 kg^{-1} d^{-1}. Similarly, respiration rate of cherry fruit stored at 0 °C for 10 days increased 5 times after being transferred to 20 °C with respiration rate of 10 mg kg^{-1} h^{-1} at 0 °C and 50 mg kg^{-1} h^{-1} at 20 °C (Alique et al., 2005).

2.3. Avocado fruit maturity

Oil content is considered a vital determinant of eating quality of avocado fruit. Due to a strong correlation with a coefficient of R = 0.96 between oil content and dry matter content (DMC) (Lee et al., 1983), DMC may replace oil content and is used as the maturity index for avocado producers in many countries in the world, including New Zealand, Australia, Colombia, Mexico and USA (Woolf et al., 2009, Lee et al., 1983, Chen et al., 2009). The evaluation of DMC was reported to be less time-consuming and it does not require expensive equipment and high technology compared to that of oil content.

The minimum level of DMC was defined as the maturity index that the minimum acceptability of eating quality to consumers (Kader, 1997, Lee et al., 1983; Lee, 1981), as it is impossible to assess the maturity of avocado from its visual appearance (Lee, 1981). The eating quality of avocado is determined by mesocarp flavour and texture. Higher oil content and result in desirable sensory attributes, such as creaminess and smoothness (Magwaza and Tesfay, 2015). Avocado is harvested after the fruit has reached the minimum maturity level. Immature fruit tends to shrivel during storage and not to ripen normally, resulting in a rubbery texture and flavourless taste (Gamble et al., 2010).

There are several advantages of harvesting high maturity index avocados. Avocados with higher DMC are considered a higher quality product in New Zealand

(Yahia and Woolf, 2011). Gamble et al. (2010) reported that there was a positive correlation between maturity index and the liking index and intent-to-buy index of avocado eaters. Additionally, avocados with higher maturity index have been reported to have fewer disorders after long-term cold storage. As a result, the minimum maturity standard is higher in some countries to ensure a better eating quality (Pak et al., 2003), and to bring the benefit for logistic management, handling procedure and marketing.

Late-harvested avocado fruit has a higher incidence of rots and internal disorder (including grey pulp and vascular browning) which were recommended as a major factor for accepting quality instead of oil content and DMC (Hofman et al., 2000). Dixon et al. (2003) found out that late-harvested avocado showed the highest degree of rots and internal disorders, while the mid-season avocados had the best quality with fewer rots and internal disorders. The different storage temperatures were recommended for avocado fruit harvested at different time, for example, 5 °C for early season, 4 °C for mid-season and 7 °C for late-season fruit, to achieve the best quality with fewer rots and chilling injury (Dixon et al., 2003).

Avocado fruit is well known for its high variability in maturity when fruit are sampled for an experiment (Woolf et al., 2003a). The fruit maturity assessment can be performed by many methods, including the traditional destructive methods and novel non-destructive methods. The traditional measurement methods to assess maturity indices are destructive, time-consuming, costly and require a precision in sample preparation (Arpaia et al., 2001). Destructive methods to assess maturity indices were described in many previous studies, for example, DMC (Lee et al., 1983; Pak et al., 2003), oil content (Young and Lee, 1978; Meyer and Terry, 2008), moisture content (Hofman et al., 2013), picking dates (Lee et al., 1983), etc. Due to the high variability of avocado fruit in maturity, the sample size for maturity testing does not always represent a batch leading to the higher risk of having immature or over-mature fruit in a consignment.

There are several non-destructive methods studied for fruit maturity determination (Magwaza and Tesfay, 2015), for example, ultrasonic measurement system (Mizrach and Flitsanov, 1999, Mizrach et al., 2000, Mizrach, 2000), nuclear magnetic resonance imaging (Kim et al., 1999, Marigheto et al., 2005), image processing and analysis (Arzate-Vázquez et al., 2011, Guerrero and Benavides, 2014), hyperspectral imaging (Mehl et al., 2002, Gowen et al., 2007, Pinto et al., 2019), and vis / near-infrared spectroscopy (NIRS) (Blakey et al., 2009, Wedding et al., 2011, Gómez et al., 2006).

Olarewaju et al. (2016) demonstrated the possibility of using NIRS for maturity prediction of avocado fruit. The experimental finding showed that NIRS could predict the moisture content and DMC precisely, with the residual predictive deviation of 2.00 and 2.13 respectively; however, this tool can not use to predict oil content due to the unreliability of the developed model (the residual predictive deviation less than 1.0). The small oil level and high moisture content of early-season fruit could mask the peaks captured by NIRS. The result agreed with the experimental result of Clark et al. (2003). Blakey et al. (2009) developed an equation ($R^2 = 0.92$, SE = 1.8 %) for predicting water content of avocado using NIRS, which had the high potential for online sorting of avocado to reduce a variation in maturity in a consignment.

2.4. Chilling injury

Chilling injury or cold damage happens mostly with tropical and subtropical fruits when they are stored under low temperature, but over their freezing point for a period of time, which weaken the fruit tissues causing an abnormal metabolic process (Wang and

Wallace, 2004). As a subtropical fruit, avocado is very sensitive to chilling injury. External chilling injury appears as skin browning or blackening with irregular dark patches on the skin (Fig 2.1A). Internal chilling injury appears as flesh greying or blackening (diffuse flesh discolouration), vascular browning, vascular leaching, and stringy vascular tissue (Fig 2.1B) (Yahia and Woolf, 2011). The browning of flesh appears when the membranes of flesh cells change in permeability which makes way for polyphenol oxidase to contact and catalyse the substrates (phenols) resulting in the oxidation of phenols to quinones which are eventually polymerised into brown pigments (Hershkovitz et al., 2005).

Figure 2.1 Severe external chilling injury (A) and internal chilling injury (B) (reproduced with permission from Whilte and Woolf (2005))

Cold storage for a long time is a common cause of chilling injury in avocados. Hopkirk et al. (1994) reported that ‘Hass’ avocado should be stored at 4 – 6 °C for up to 4 weeks to avoid or reduce the possibility of chilling injury which may appear in avocados stored at 0 – 4 °C; however, the incidence and severity levels depend on the cultivars, the fruit maturity and the ripeness levels (Zauberman et al., 1973). Skin damage was reported for avocados stored at 2 °C, while avocados stored at 3 – 5 °C for more than two weeks appeared internal chilling injury and ripened abnormally (Yahia and Woolf, 2011).

Exogenous ethylene treatment accelerates the development of chilling injury. Ethylene treatment at the concentration of 10 ppm and storage for 4 – 6 weeks at 1.5 °C and 5 °C increased the incidence and severity level of chilling injury and rots in avocados after transferred to 20 °C for 4 days (Chaplin et al., 1983). Pesis et al. (2002) showed that ethylene treatment at 100 µL L^{-1} for 24 h at 20 °C following by cold storage at 5 °C for 3 weeks increased the chilling injury development (mesocarp discolouration) compared to the control fruit. The application of exogenous ethylene accelerated the chilling injury in various fruits, for example, plums (Candan et al., 2008, Dong et al., 2002), cantaloupe melons (Ben-Amor et al., 1999); however, the application of exogenous ethylene may reduce the CI of pears (Wei et al., 2019).

The assessment of chilling injury is based on the severity level and the incidence of chilling injury occurring on avocado fruit skin and mesocarp. The incidence of chilling injury is the per cent of fruit with signs of diffuse flesh discolouration (internal chilling injury) or discrete patches (external chilling injury) on the skin caused by chill damage (Fig 2.2 & 2.3). The severity level is the index on a scale from 0 to 5 depending on the proportion of chilling damage on the skin or/and on mesocarp (Glowacz et al., 2017, Meir et al., 1997). The severity level was described by Pesis et al. (1994) using a scale (Table 2.1).

Table 2.1 The severity level of chilling injury (Pesis et al., 1994)

Rating score	Description
0	No damage
1	Very light damage
2	Light damage
3	Medium damage
4	Severe damage
5	Very severe damage

However, the severity level assessment table of Pesis et al. (1994) is somewhat subjective depending on the observation of researchers. White and Woolf (2005) introduced the assessment of avocado severity that based on the per cent of chilling damage on mesocarp or skin on a scale from 0 to 3 (Table 2.2). The severity is calculated by per cent of the chilling injury area in the fruit according to the description and visualisation (Fig 2.2).

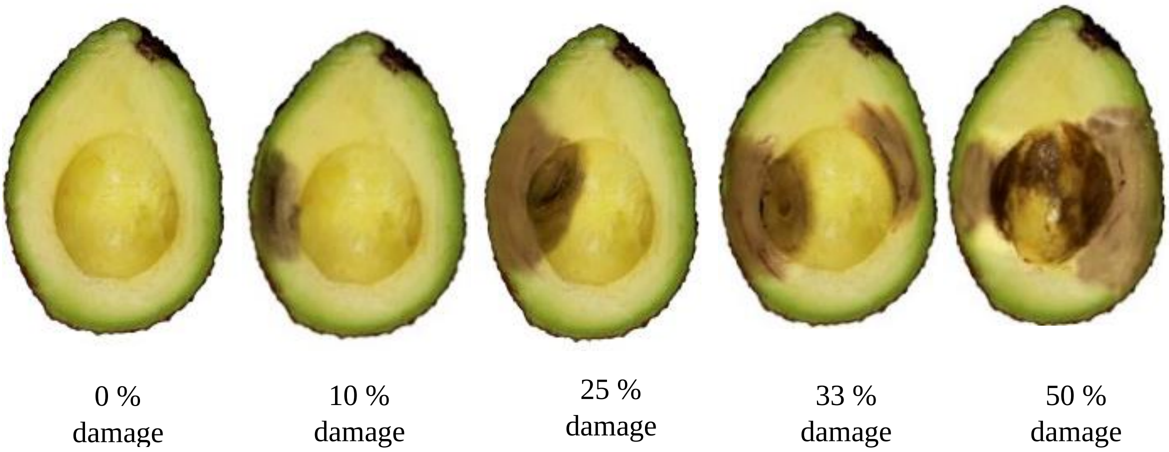
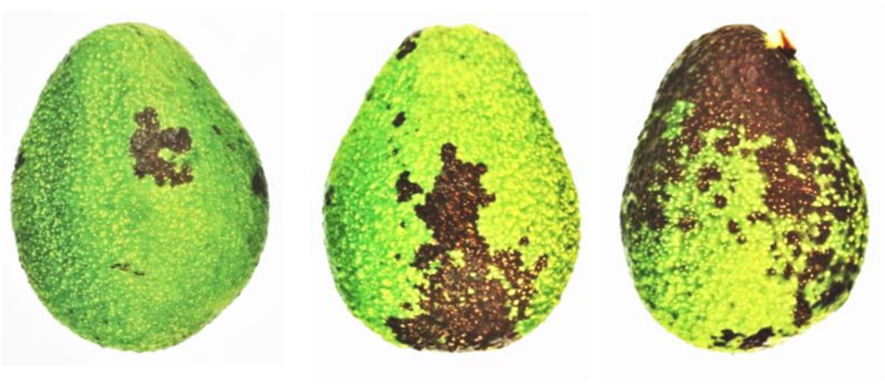


Figure 2.2 The severity level visualized by the per cent of diffuse flesh discolouration (reproduced with permission of White and Woolf (2005))



10 % damage

25 % damage

50 % damage

Figure 2.3 Discrete patches on fruit skin with a rating scale for severity assessment (reprodued with permission of White and Woolf (2005))

Table 2.2 The per cent of damage and the index on a scale from 0 to 3 (reproduced with permission of White and Woolf (2005))

Rating Scale (severity level)	**Per cent of damage**
0	0 %
0.5	5 %
1	10 %
1.5	15 %
2	25 %
2.5	33 %
3	50 %

2.5. Methods improve fruit quality and extend the fruit shelf-life

Several methods have been applied to extend the shelf life of avocado, not only to extend the storage time but also to have some beneficial effects to improve the quality of the fruit, such as reducing the possibility of chilling injury and rots. Methods that have been applied or studied on avocado fruit are summarised in the following sub-sections comprising cold storage (2.5.1), 1-MCP treatment (2.5.2), MeJA and MeSA treatment (2.5.3), high relative humidity storage (2.5.4), controlled and modified atmosphere packaging (2.5.5), temperature treatment (2.5.6) comprising heat treatment (2.5.6.1), low-temperature conditioning (2.5.6.2), and cold-shock treatment (2.5.6.3).

2.5.1. Cold storage

Cold storage was demonstrated to remarkably extend the storage life of avocados and was the most popular method applied for many years. The optimal temperature and relative humidity for avocados vary depending on the cultivars. Rees et al. (2012) showed the recommended storage temperature, relative humidity and storage time for cold storage of various avocado cultivars (Table 2.3). For example, for ‘Hass’ avocado storage, the optimal storage condition should be 3 – 7 °C, relative humidity of 85 – 90 % with the storage time of 2 – 4 weeks. Additionally, the cold storage condition depends on the maturity of the fruit. Rees et al. (2012) recommended that the storage temperature for early-season avocado should be 5 – 7 °C and temperature of 4 – 5.5 °C was recommended for late-season avocados.

Table 2.3 Recommended optimum storage conditions for various avocado cultivars (Rees et al., 2012)

Cultivar	Temperature (º C)	Relative humidity (%)	Storage life (weeks)
Hass	3 – 7	85 – 90%	2 – 4
Fuerte	3 – 7	85 – 90%	2 – 4
Fuchs	13	85 – 90%	2
Pollock	13	85 – 90%	2
Lula	4	90 – 95%	4 – 8
Booth 1	4	90 – 95%	4 - 8

The cold tolerance of avocado fruit to low temperature varies among cultivars, as a result, chilling injury development varies (Zauberman et al., 1973). ‘Nabal’ avocado cultivar was more susceptible to chilling injury than ‘Ettinger’ and ‘Fuerte’ cultivars when these cultivars were stored at 0 – 4 °C for 4 – 6 weeks (Zauberman et al., 1973). A similar chilling response was observed between ‘Fuerte’ and ‘Hass’ avocado cultivar after being stored at 0 and 5 °C for 5 weeks and ripened at 20 °C (Eaks, 1976). The similarity of the variation in chill tolerance among cultivars was seen in other fruits. Phakawatmongkol et al. (2004) showed that ‘Rad’ mango might be stored for 15 days, while ‘Okrong’ mango may be stored for 25 days at 12 °C with the criterion of less than 10 % of skin discolouration.

Mans et al. (1995) proposed a stepped-down cooling regime for the storage of avocado, started with the storage temperature of 7.5 – 8.5 °C and ending at the storage temperature of 4.5 – 6.5 °C. The results suggested that the different temperature regimes did not affect the fruit firmness and the development of chilling injury; however, chilling injury tended to increase for later-season avocado fruit. Avocado stored at 0 – 5 °C for 2 weeks delayed the ripening and displayed a normal climacteric pattern without the development of chilling injury, however, longer storage time from 4 – 6 weeks showed the signs of chilling injury and abnormal ripening with significantly lower ethylene production rate and magnitude of the ethylene peak after transferring fruit to the ripening temperature at 20 °C (Eaks, 1983). It demonstrated that chilling injury depends on the storage time and storage temperature.

2.5.2. 1-methylcyclopropene treatment

1-methylcyclopropene (1-MCP) is known as an inhibitor of ethylene perception by interacting with ethylene receptors. As a result, the physiological responses of fruit induced by ethylene are inhibited, leading to decreasing ethylene production which then delays the climacteric stage, fruit softening and skin discolouration during storage (Watkins, 2006).

The use of 1-MCP to delay the fruit ripening was studied in a variety of fruits, for example, mangos (Razzaq et al., 2016), plums (Khan and Singh, 2007), feijoas (Rupavatharam et al., 2015), bananas (Golding et al., 1998, Jiang et al., 1999), avocados (Jeong et al., 2002, Woolf et al., 2005, Defilippi et al., 2018). 1-MCP was demonstrated to suppress the activities of ethylene biosynthesis enzymes, including ACS and ACO, consequently, the ethylene production was delayed in climacteric fruits resulting in delaying the fruit ripening (Khan and Singh, 2009; Zhu et al., 2015; Razzaq et al., 2016). Additionally, 1-MCP was shown to inhibit the activities of cell-wall enzymes such as polygalacturonase, pectin-methylesterase and 1,4-β-D-glucanase in plum fruit when the fruit was treated at the concentration of 1 – 2 $\mu L\ L^{-1}$ (Khan and Singh, 2007). A similar result reported by Defilippi et al. (2018) where the application of 1-MCP delayed the ripening of treated avocado fruit by 4 days compared to the untreated fruit; 1-MCP restrained the activity of polygalacturonase, pectin-methylesterase which are related to the softening.

1-MCP can inhibit the ripening of avocado fruit induced by ethylene production and ethylene exposure at a low concentration. Feng et al. (2000) reported that the 1-MCP treatment with concentrations of 30 to 70 nL L^{-1} for 24 h at 22 °C could delay the ripening of avocado stored at 22 °C by 10 – 12 days compared to the untreated sample. Similar results have been reported by Hofman et al. (2001) where 1-MCP treatment at a concentration of 25 µL L^{-1} for 14 h at 20 °C inhibited the effects of ethylene treatment at the level of 100 µL L^{-1} which is generally used for fruit ripening acceleration; 1-MCP delayed the ripening of avocado fruit by 4.4 days compared to the control sample. Moreover, Jeong et al. (2002) showed that 1-MCP treatment at a high concentration of 450 µL L^{-1} for 24 h at 20 °C effectively suppressed the activity of cell-wall enzymes and inhibited the activity of polygalacturonase for 12 days.

Furthermore, 1-MCP is demonstrated to decrease the development of chilling injury for avocados after cold storage for 4 – 6 weeks. Hershkovitz et al. (2005) reported that avocados treated with 1-MCP at a concentration of 300 nL L^{-1} for 18 h at 20 °C before being transferred to cold storage at 5 °C for 3.5 weeks, delayed the ripening and softening process after being transferred to 20 °C to ripen. 1-MCP can inhibit the mesocarp browning which associates with the suppression of polyphenol oxidase and peroxidase activities. Woolf et al. (2005) showed that 1-MCP treatment at a concentration of 50 – 1000 nL L^{-1} for 6 – 24 h at 6 °C or 15 °C significantly decreased the development of pulp discolouration for avocado fruit stored at 5.5 °C for 4 – 7 weeks.

Different 1-MCP concentrations are used for different cultivars in the application of 1-MCP. Pesis et al. (2002) showed that ‘Hass’ and ‘Fuerte’ avocado fruit required 1-

MCP treatments at a concentration of 300 nL L^{-1} and 100 nL L^{-1} respectively, for 24 h at 5 °C to reduce the chilling injury and the suppression of polyphenol oxidase activity which were related to the pulp discolouration. On the other hand, 1-MCP treatments caused negative effects for other fruits, for instance, 1-MCP increased the development of cold damage in peaches (Fan et al., 2002, Liguori et al., 2004), plums (Dong et al., 2002) and bananas (Jiang et al., 1999), and resulted in CO_2 damage and pulp browning in apples (Rupasinghe et al., 2000, Murr et al., 2001).

However, 1-MCP treatment may increase the possibility of rot incidence in avocado fruit. Hofman et al. (2001) showed that avocado, custard apple, mango and papaya treated with 1-MCP of 25 µL L^{-1} had prolonged shelf-life compared to the untreated fruit, nevertheless, there was the higher possibility of rot incidence in treated fruits. The negative effects of 1-MCP on the resistance of ripe fruit against fungi and pathogens related to rot development were unclear and inconsistent for different varieties of fruits (Hofman et al., 2001).

2.5.3. Methyl jasmonate and methyl salicylate treatment

Methyl jasmonate (MeJA) and methyl salicylate (MeSA) known as signal molecules in plant stress responses were reported to decrease chilling injury development of fruits. MeJA and MeSa increase the activity of antioxidant enzymes which prevent cell membranes from the dysfunction caused by lipid oxidation (Sayyari et al., 2011) and induce the expression of heat shock protein genes, which protects the fruit from cold damage (Ding et al., 2001).

Glowacz et al. (2017) showed that MeJA and MeSA treatment at the concentration of 100 µmol L^{-1} decreased the incidence and severity of chilling injury by 20% in avocados stored at 2 °C for 21 days. Another experimental work by Meir et al. (1996b) confirmed that 'Hass' avocados treated with MeJA solution with the concentration of 2.5 μmol L^{-1} for 30 s reduced cold damage development for avocado stored at 1 °C for 2 weeks. MeJA and MeSA treatments have been widely applied to reduce chilling injury and the development of rots in a variety of fruits and vegetables, including kiwifruit (Li et al., 2017b), peaches (Meng et al., 2009), capsicums (Fung et al., 2004) and tomatoes (Ding et al., 2001, Ding et al., 2002).

2.5.4. High relative humidity storage

High relative humidity (RH) has been demonstrated to benefit fruit quality. Avocado stored at high RH (above 95 %) can delay the fruit ripening but increase the development of fungi on fruit skin when the RH fluctuates and reaches the saturation level at 100 % (Huysamer and Mare, 2003). Fruits stored and ripened at high RH are shown to have a higher incidence of rots (Dixon et al., 2004). RH of 85 – 95 % was recommended for fresh fruit storage (Handerburg et al., 1986). High RH level can reduce the weight loss in avocado fruit. Avocado fruit stored at an RH of 90 % had decreased a half of weight loss and provided better external qualities compared to fruit stored at 60 % RH (Erickson and Kikuta, 1964). Higher water loss in avocado resulted in the increase of ethylene production rate and respiration rate during ripening, which reduced the storage life by 2 days (Lallu et al., 2004).

2.5.5. Controlled and modified atmosphere packaging

Controlled atmosphere packaging (CAP) and modified atmosphere packaging (MAP) have been widely applied in postharvest of various fruits to delay the ripening, reduce chilling injury, rot and weight loss (Kader et al., 1989). MAP has been applied to extend the storage life of avocado fruit (Meir et al., 1996a, Meir et al., 1997, Gerdes and Parrino-Lowe, 1995, Castellanos et al., 2017, Sellamuthu et al., 2013). The application of MAP using 0.05 mm LDPE bags at 1:1 surface to weight ratio ($cm^2 g^{-1}$), with ethylene and CO_2 absorbers was reported to prolong the shelf-life of avocado fruit stored at 12 °C to 29 days (Illeperuma and Nikapitiya, 2002). A similar result was shown by Meir et al. (1997) where the application of MAP using 30 µm PE bags extended the shelf-life of avocado fruit stored at 5 °C to 9 weeks while delaying ripening and skin discolouration, and reducing chilling injury compared to unwrapped fruit.

2.5.6. Temperature treatment

2.5.6.1. Heat treatment

Heat treatments have been widely used for years as an effective disinfestation method (also called quarantine treatments) to kill pest insects on fruit skin for fruit exports to other countries (Jessup, 1991, Sosa-Morales et al., 2011) along with the cold disinfestation (Hofman et al., 2003). Heat treatments (thermal treatments) followed by low-temperature storage are reported to slow down the ripening of fruit and effectively decrease the chilling injury in many studies. There are three types of thermal treatments: hot water treatments (Woolf and Lay-Yee, 1996, Anwar and Malik, 2007), hot air treatments (Woolf et al., 1995a, Soto-Zamora et al., 2005) and water vapour treatments (Jacobi and Wong, 1992). The application of heat treatment at 41 °C for 30 mins after the cold disinfestation (1 °C, 16 days) reduced cold damage in avocados (Hofman et al., 2002).

Woolf et al. (1995b) reported that hot air treatment at 38 °C for 3, 6, or 10 h and 40 °C for 0.5 h, then stored at 2 °C for 3 weeks decelerated fruit ripening and decreased external chilling injury induced by cold storage, but did not reduce internal chilling injury compared to unheated fruit. The expression of two plant heat-shock protein genes rose with the increase of temperatures and reached the maximum value at 40 °C, which was supposed to prevent the external chilling injury.

Similarly, Woolf et al. (1995a) figured out that hot water treatment at 38 °C for 1 h decreased the external cold damage such as skin discolouration. The application of hot water treatment at 41 °C for 25 – 30 minutes or 42 °C for 25 minutes reduced the vascular discolouration and decay development for avocado fruit stored at 1 °C for 16 days, and also significantly increased 80 % of the skin quality and approx. 20 % of mesocarp quality (Hofman et al., 2002). Similar results were shown by Abu-Aziz et al. (2009) where hot water treatment at 50 °C for 10 minutes significantly reduced the external cold damage for avocado stored at 5 °C for 9 days; however, the level of antioxidants was reduced.

2.5.6.2. Low-temperature conditioning

Low-temperature conditioning was documented as an effective method to reduce the chilling injury symptoms for fruits and vegetables. The effects of low-temperature conditioning depend on the difference between the storage temperature and the conditioning temperature, and the conditioning duration (Wang, 1993). According to Woolf et al. (2003b), low-temperature conditioning at 6 – 8 °C for 3 – 5 days considerably decreased the skin cold damage for 'Hass' avocado stored for 3 weeks at 0 °C. One of the hypotheses for the effects of low-temperature conditioning is the increase of the expression of heat shock protein genes which improve low-temperature resistance of the fruit and prevent the fruit from chilling injury development (Woolf et al., 2003b). Similarly, Hofman et al. (2003) showed that low-temperature conditioning at 6 °C for 3 days before the cold disinfestation (1 °C, 16 days) improved the external and internal quality and reduced chilling injury compared with untreated fruit.

The low-temperature conditioning has been widely applied in various fruits and vegetables, for instance, peaches (Jin et al., 2009, Cai et al., 2010), kiwifruit (Yang et al., 2013), mangos (Pesis et al., 1996, Zhang et al., 2017), avocados (Hofman et al., 2003, Woolf et al., 2003b, Mendieta et al., 2016), pears (Li et al., 2017a, Wang et al., 2017), zucchini squashes (Wang, 1994), and loquat fruit (Cai et al., 2006).

2.5.6.3. Cold-shock treatment

Cold shock treatment (CST) is a new method to extend the shelf life of fruits. CST can be divided into two stages comprising the dynamic stage (the fruit temperature sharply drops) and static stage the fruit temperature decreases slightly) (Chen et al., 2015). The effect of CST is the result of the thermal response of fruit under a low temperature conditions regulated by plant heat shock proteins (Altschuler and Mascarenhas, 1982, Al-Whaibi, 2011). CST was reported to delay the ripening of fruit, reduce weight loss, respiration and ethylene production and decrease cell-wall enzymes activities. Zhang et al. (2010) showed that the immersion of banana fruit in ice-water (0 °C) for 1 h effectively retarded the ripening of banana fruit accompanied by the reduction of respiration and ethylene production rate and cell-wall enzymes activities. Chen et al. (2015) reported that CST at 3 °C for 40 mins reduced the weight loss and firmness loss of cucumber fruit and suppressed the rise of respiration and ethylene production rate.

In the experimental work of Chen et al. (2017), avocado fruit was immersed in chill water at the full matrix of 3 temperatures (0, 2 and 4 °C) and 6 times (15, 30, 45, 60, 90 and 120 minutes) to find the optimal CST to extend the shelf-life of avocados. The results found out that CST at 0 °C and 30 minutes most effectively delayed the ripening of avocado fruit stored at 20 °C for 10 days. The change of skin colour of treated fruit was retarded compared to that of the control, showing by lighter and greener colour, expressed as L-value of approx. 29 and 27, C-value of approx. 8 and 6, h-value of approx. 75 and 40 with treated fruit and control fruit, respectively. Respiration rate and ethylene production rate of treated fruit peaked 2 days later when compared to these of control fruit. Fruit softening was delayed, expressed as the firmness value of approx. 20 and 10 (N) with the treated fruit and the control on day 10.

2.6. Conclusion

Avocado fruit has a relatively short shelf life due to the rapidly ripening and pulp softening. Additionally, the fruit is well-known to have a high fruit variability due to the variation in the preharvest factors (soil, plant nutrition, irrigation, sun exposure), growing and harvesting season and fruit maturity, etc. These problems remain big issues for avocado storage, logistic management and fruit suppliers when exporting avocado to the distant markets and in-store display. The aforementioned postharvest methods have been studied for years with the attempt to prolong the storage time and delay the ripening of fruit.

The CST is seen as a promising method which effectively delays fruit ripening. Moreover, the method is evaluated as simple, low-cost and time-saving that may alternate the abovementioned techniques which are considered sophisticated and high-cost and not suitable for the major avocado production countries in South America, Africa and Asia which are classified as Third World countries. Consequently, the objective of the thesis is to provide better insight to the effectiveness of CST application on the delaying of the ripening of avocado by replicating the experimental work of Chen et al., (2017) to identify the suitable CST and validate the findings.

CHAPTER 3

EXPERIMENT 1

3. Experiment 1

3.1. Introduction

Chen et al., (2017) recently demonstrated the potential to extend the storage life of avocados with CST. In that work, avocado fruit were immersed in ice water for 30 minutes, which delayed fruit ripening process regarding to skin discolouration, pulp softening, respiration rate and ethylene production rate during subsequent storage at 20 °C. The effect of CST on fruit softening was associated with decreased polygalacturonase and endo-β-1,4-glucanase activities (Chen et al., 2017).

The objective of this thesis is to provide better insight into the effects of CST application on firmness retention. As the first step, the aim of this experiment is to replicate the first experiment by Chen et al. (2017). Features of the methodology of the first experiment of Chen et al., (2017) include:

- Sourcing ‘Hass’ avocado fruit from a local fruit market in Haikou, China
- Avocado fruit were dipped in the cold water (0, 2 or 4 °C) for 15, 30, 45, 60, 90 or 120 minutes
- Storage time of 6 days at 20 °C and RH of 85– 90 %
- The sample size of the first experiment was 15 fruit
- Firmness measurement was performed by a puncture test using TA.XT.PLUS fitted with a SMS P/2 needle probe (a stainless steel cylinder of 2 mm in diameter with a conical needle bit)
- Peel color measurement (L, C, h) was conducted using a Minolta Chromometer CR-200

- Five fruits were sealed inside a 6.4 L glass jar for 2 h at 20 °C for measurement of respiration rate and ethylene production rate through change of the gas concentration by the static method

The experiment was expected to identify the suitable CST for avocados, and validate the findings of Chen et al., (2017).

3.2. Material and Methodology

3.2.1. Fruit source

Mature ‘Hass’ avocado fruit (*Persea americana*, Mill., cv. ‘Hass’) were harvested on 30th September 2019 from an orchard in Bay of Plenty, New Zealand. Avocado fruit were picked by hand, taken straight to the packing shed without any chemical application or ethylene treatment, and then stored at ambient temperature (15 – 16 °C). Fruit were then packed into cardboard boxes lined with newspaper, closed with tape, and left overnight in the packing shed at ambient temperature. Fruit were transported in a van at ambient temperature to Massey University (Palmerston North) around 48 h after harvest.

3.2.2. Avocado sample preparation

Upon arrival, all fruit were sorted by weight (200 – 300 g) using an electronic balance (ML-T Balance, Mettler-Toledo, USA). Fruit which were out of the weight range or had physical damage were removed from the populations. Selected fruit were randomised and repacked into plix trays with 15 fruit per tray (Fig 3 – 1). All avocado fruit were stored in a temperature-controlled room at 20 °C and 85-90 % RH overnight before CST application.

Figure 3.1 Fifteen avocado fruit were repacked into a plix tray

3.2.3. Cold shock treatment

The CSTs applied in this experiment were based on the experiment of Chen et al. (2017). Eighteen (18) treatments representing a full matrix of 3 temperatures (0, 2 and 4 °C) and 6 times (15, 30, 45, 60, 90 and 120 minutes) were conducted in random order. The desired CST temperatures were achieved by filling three (3) 60 L barrels with water and placing each of them overnight in three different cool rooms set at 0, 2 and 4 °C respectively. On the day after, the water equilibrated to the temperature of the rooms. For the barrel at 0 °C, ice was added to the barrels at a ratio of 50:50 ice and water to maintain the water temperature at 0 °C during the treatment application.

For each of the 18 treatments, 15 avocados from the same plix tray were immersed in the water barrel for their specific temperature (0, 2 or 4 °C) and specific time (15, 30, 45, 60, 90 and 120 minutes) in combination. After 6 h, when all of the treatments had been completed, the fruit were dried with a cloth, repacked into a plix tray (one tray for a treatment), then stored at room temperature of 20 °C for 3 h before being transferred to the storage room. The fruit were stored in a storage room at 20 °C and 85–90 % RH. After 2, 4 and 6 days of storage, 5 fruit were selected randomly from 15 fruit in the same plix tray for each treatment for firmness measurement. A tray of 15 avocados taken as controls (without going to treatment) was kept in a storage room at 20 °C during the treatment time.

Water temperature used for CST was continually measured (at 30 s intervals) using thermocouples connected to data loggers (1200 series, Grant, UK) (Fig 3 - 2). When the fruit was immersed in the water, the water temperature increased by approx. 0.5 – 1 °C. To maintain the water temperature in the barrels during the treatment period, 250 g of ice was added and thoroughly mixed in the water to decrease the water temperature and prevent local chill temperature around the fruit. To measure the water temperature, 3 thermocouples were dipped into the water at three different locations at the top, middle and bottom of the bath.

Figure 3.2 Avocado fruit were immersed in ice water during CST at 0 °C for 2 h with the thermocouple used for seed surface of the fruit and water temperature observation

To measure seed surface temperature, 15 avocados were punctured by a needle with 2 mm in diameter through the peel from the stem scar until reaching the seed; then a thermocouple was penetrated into the hole to the seed. With the treatments at 0, 2 or 4 °C for 2 h, each set of 5 avocados from spare fruit was taken for fruit core temperature measurement. Seed surface temperature was continually measured (at 30 s intervals) using thermocouples connected to data loggers (1200 series, Grant, UK) (Fig 3.3).

Figure 3.3 Thermocouples connected to data loggers using for seed surface temperature measurement

3.2.4. Fruit Quality Measurement

3.2.4.1. Texture analysis

A puncture test was conducted to determine the firmness of the individual fruit. Firmness was measured at 4 different points around the equator of the fruit, 90° apart. Approximately 1 mm of fruit skin was removed with a mandoline before measurement. The puncture test was performed using a Universal Testing Machine (TA.XT.Plus, Stable Micro Systems Ltd, UK) equipped with an HDP/90 platform, a P/2N needle probe and a

5 kg load cell. The P/2N needle probe is a stainless-steel cylinder of 2 mm in diameter with a conical needle bit (Figure 3.4). The penetration depth of 10 mm with a testing speed of 4 mm s^{-1} and a pre-test speed of 4 mm s^{-1} with the trigger force of 0.01 N were used. The firmness was taken as the maximum break force expressed in Newtons (N). For each fruit, the firmness was considered as the average of four measurements taken at 4 points.

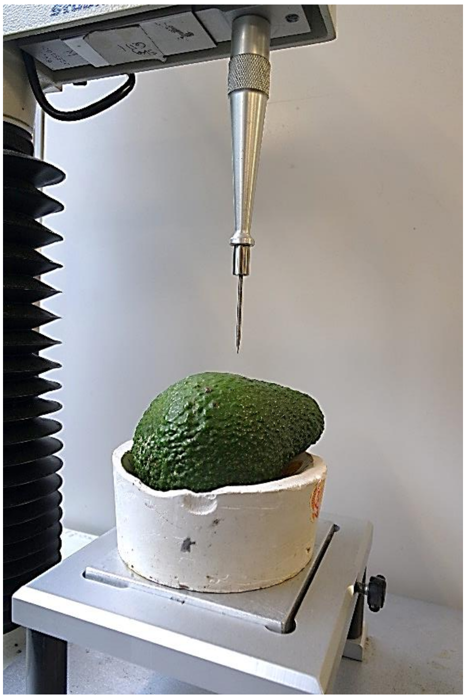


Figure 3.4 Puncture test using TA.XT Plus Texture Analyser with a needle probe

3.2.4.2. Chilling injury evaluation

Given the low temperature used during CSTs, there is potential for CI to develop. CI was assessed based on "*The International Avocado Quality Manual*" (White and Woolf, 2005) as a guide for the avocado industry and retailers. Internal CI is manifested as a greyish-brown flesh, mesocarp discolouration, or grey pulp, particularly at the base of the fruit around the seed (Figure 3.5). The avocado was cut in half to assess the internal

CI. The incidence of internal CI is assessed as the proportion (%) of fruit with signs of mesocarp discolouration and the severity was rated according to the index on a scale from 0 to 3 (Table 3.1) (White and Woolf, 2005)

Table 3.1 The proportion (%) of chilling injury with the index on a scale from 0 to 3 (reproduced with permission of White and Woolf, (2005))

Rating Scale (severity level)	**Rating**
0	0 %
0.5	5 %
1	10 %
1.5	15 %
2	25 %
2.5	33 %
3	50 %

1 = 10% 2 = 25% 3 = 50%

Figure 3.5 Diffuse flesh discoloration caused by chilling injury (reproduced with permission of White and Woolf, (2005))

External chilling injury is manifested as browning or blackening of the skin surface, expressed as irregular dark patches on the skin (Figure 3. 6). Severity assessment based on the per cent of discrete patches on the skin according to Table 3 .1 (White and Woolf, 2005).

1 = 10% 2 = 25% 3 = 50%

Figure 3.6 Discrete patches caused by chilling injury on fruit skin with a rating scale for severity assessment (reproduced with permission of White and Woolf, (2005))

The external chilling injury index (I_{ex}) and internal chilling injury index (I_{in}) were adopted the chilling injury index of Pesis et al. (1994) (Eq. 3.1):

Equation 3.1 Chilling injury index formula

$$I_{in/ex} = \sum_0^3 (s \times r)/n$$

- s: severity level according to Table 3-1
- r: the number of fruit at the severity level (s)
- n: the total number of fruit
- $I_{in/ex}$: internal/external CI index

3.2.4.3. Fruit rot evaluation

The evaluation of incidence and severity of rots was based on "*The International Avocado Quality Manual*" (White and Woolf, 2005) (Table 3.3).

Table 3.2 The proportion (%) of rot with the index on a scale from 0 to 3 (reproduced with permission of White and Woolf, (2005))

Rating Scale (severity level)	Rating
0	0 %
0.5	5 %
1	10 %
1.5	15 %
2	25 %
2.5	33 %
3	50 %

3.2.5. Data analysis

An analysis of variance (ANOVA) was performed by using the general linear model to measure difference among means of fruit firmness. Factors considered in the model were temperature (°C) and time (min). Differences between the means were tested by using Tukey's test. Significant differences were considered at the 95 % level of confidence. Minitab (version 19.0, Minitab Inc., Pennsylvania, USA) was used to conduct all statistical analysis.

3.3. Results

3.3.1. Seed surface temperature during CST

The change of the seed surface temperature during the CSTs at 0, 2 and 4 °C was shown in Fig 3.7. Under the dynamic stage (cooling rate > 0.1 °C/min), the seed surface temperature declined significantly from approx. 22 °C to 5, 8 and 9 °C for the treatment at 0, 2 and 4 °C respectively within the first 30 minutes. The cooling rate during the dynamic stage was approx. 0.33 °C/min for the three treatments. This period was followed by a more gradual decline in the next 30 minutes. Afterwards, the fruit seed surface temperature subsequently remained almost constant at about 1.2, 3.8, 5.0 °C which were higher to some extent from targeted temperature values of 0, 2, 4 °C, respectively. It took approx. 60, 50, and 40 for the CSTs at 0, 2 and 4 °C respectively for fruit core to reach the static stage, and had the same patterns of temperature change achieved by Chen et al. (2017) which reported that it took 60, 30 and 29 minutes. However, the result of Chen did not show a clear separation of the temperature change between the CST at 2 and 4 °C.

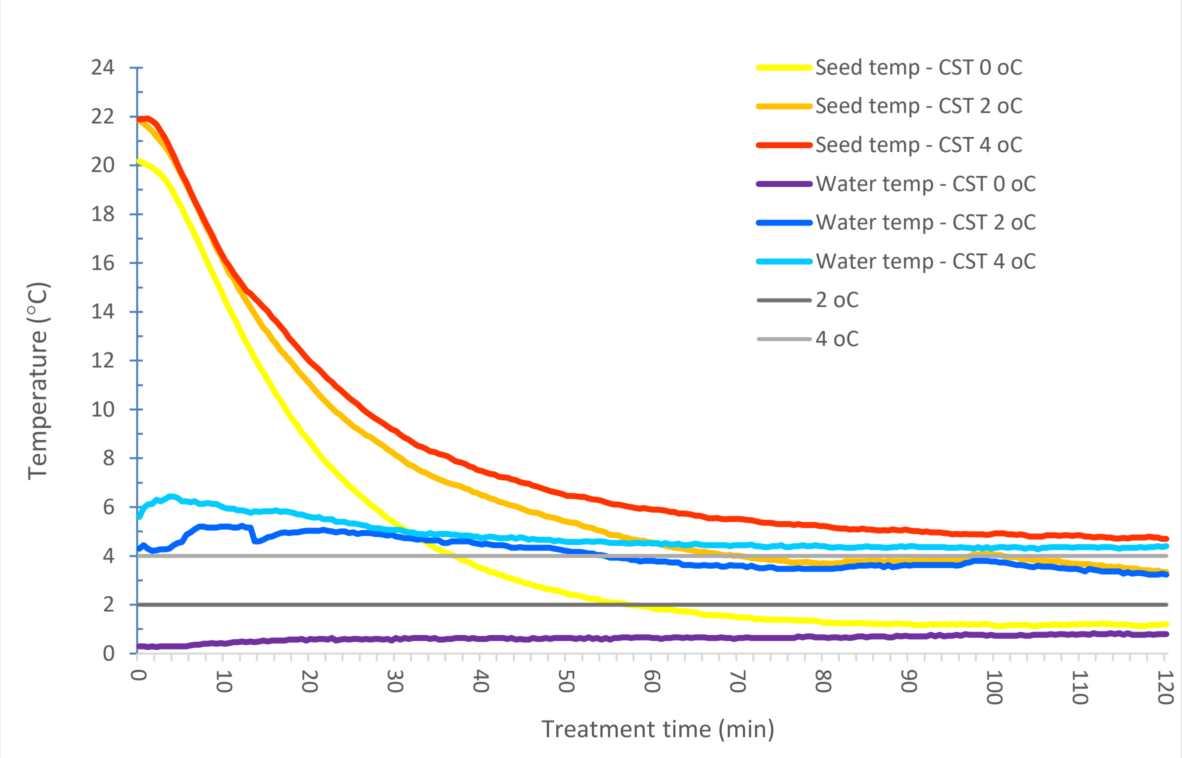


Figure 3.7 The change of the average temperature of fruit seed surface for 5 fruit (± 0.2 °C) and of water at 3 different points (± 0.1 °C) during the CST at 0, 2 and 4 °C in 120 minutes

3.3.2. Fruit firmness

The ANOVA analysis (Table 3.2) indicated that fruit firmness was not influenced by CST temperature nor time of exposure, whereas the longer storage times after treatment resulted in softer fruit. Fruit firmness decreased from the initial firmness value (at harvest) after 6 days of storage. A consistent trend that colder temperatures and shorter treatments resulted in better firmness outcomes although there are not statistically significant (Table 3.2); however, the Fig 1.b of experimental result of Chen et al. (2017) showed the same trend that shorter treatments (CSTs for 15 and 30 minutes) presented a statistically higher in firmness value compared to that of longer treatments (45 to 120 minutes). Additionally, with the treatment for 30 minutes, the colder temperature showed a statistically higher in firmness value, in particular, firmness value of 68, 45, and 35 N for CST at 0, 2 and 4 °C respectively.

It is noticeable that the magnitude of firmness values reported by Chen et al. (2017) are between 35 – 68 N for treatments of 15 and 30 minutes with the lowest values approx. 10 N for other treatments, which are significantly higher than the magnitude of firmness values of the experiment (1 – 10 N) for day 6 (Fig 3 – 8).

Table 3.3 Effects of CST temperature, exposure time and consequent storage time (at 20 °C) on avocado firmness (N). Means with different letter indicate significant differences ($p < 0.05$)

Factor	Value	Mean Firmness (N)
Temperature (°C) n = 90 LSD $_{0.05}$ = 1.23	0 °C	8.03^{a}
	2 °C	7.01^{a}
	4 °C	6.90^{a}
Time (min) n = 45 LSD $_{0.05}$ = 2.12	15 mins	8.42^{a}
	30 mins	7.91^{a}
	45 mins	7.23^{a}
	60 mins	7.16^{a}
	90 mins	6.67^{a}
	120 mins	6.48^{a}
Storage Time (days) n = 90 LSD $_{0.05}$ = 4.65	2 days	11.37^{a}
	4 days	6.72^{b}
	6 days	3.85^{c}

Fig. 3.8 graphically presents the results for each treatment in combination, demonstrating the large distribution of firmness (1 - 8 N) in each of the measured populations while fruit firmness decreased on average from day 2 to day 6. The maturity

variation is likely to contribute to the variability of fruit softening and firmness values, which was previously reported in a number of studies as a result of tree to tree and fruit to fruit variation (Hofman and Jobin-Decor, 1999; Marques et al., 2006; Villa-Rodríguez et al., 2011).

Chen et al. (2017) reported that avocado fruit exposed to CSTs at 0 to 4 °C for 15 to 30 minutes preserved avocado firmness after 6 days of storage, while treatment of 30 minutes and 0 °C resulted in higher firmness values. With the sample size of 15, the firmness values of Chen et al. (2017) showed a low variation (standard errors < 10 %), while that of this experiment showed a high variation (standard errors from 24 – 62 %) with the sample size of 5 (firmness were both measured on day 6). Therefore, increasing the sample size could reduce the fruit to fruit variation.

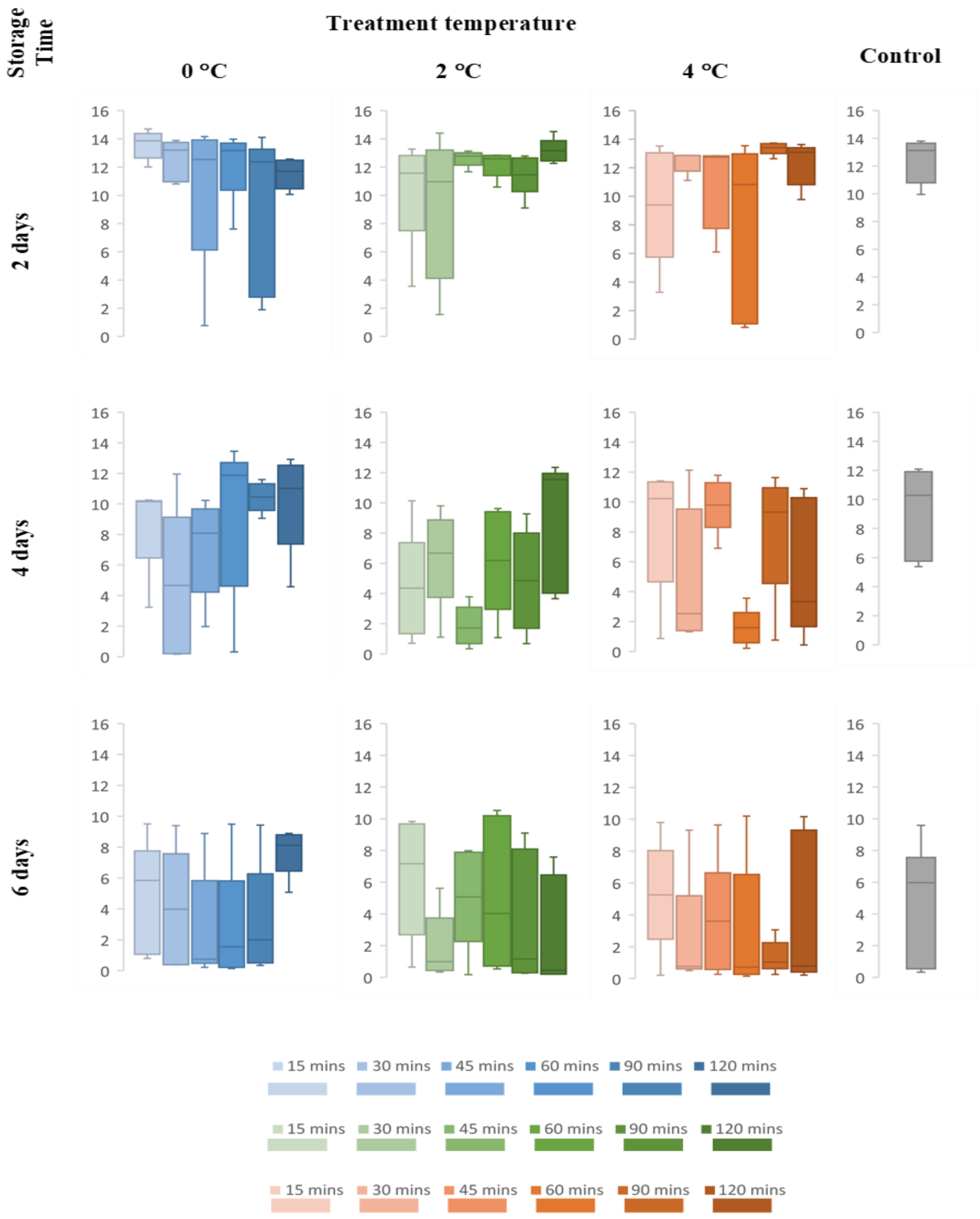
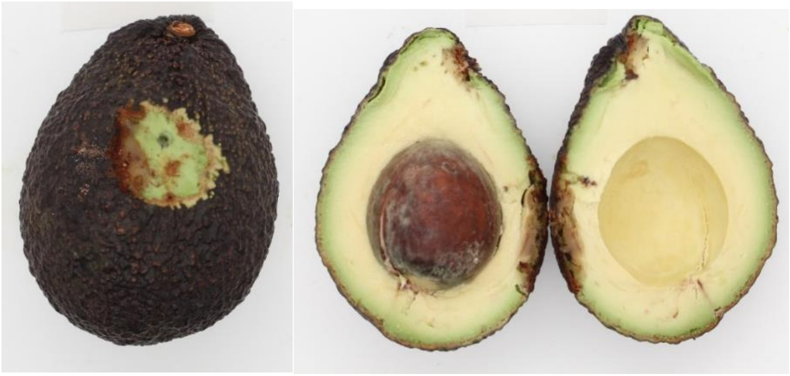


Figure 3.8 Box and whisker plot for fruit firmness (N) after cold shock treatments in a combination of temperature (0, 2, or 4 °C) and time (15, 30, 45, 60, 90 or 120 minutes) and a subsequent 2 days, 4 days and 6 days of storage at 20 °C, relative humidity of 85-90%. Box and whiskers represent a population of 5 individual fruit

3.3.3. Chilling injury and rot

Symptoms of CI were not observed for all treatments after 6 days of storage at 20 °C, which agreed with the finding of Chen et al. (2017). Hence it would seem that CST does not cause CI to avocado fruit, even in the case of the severest treatment at 0 °C for 2 h, when the fruit was later stored at 20 °C.

Fruit rots were observed after 6 days. Stem-end and body rots appeared to be the most severe issue for avocado storage at 20 °C, which were found in some avocado across all the treatments' samples (Fig 3.9). However, rots were not mentioned in the work of of Chen et al. (2017). There was 10 % of total fruit in the experiments that rot appeared both on skin and mesocarp without a significant difference among treatments. Most of rot were classified into the severity level 2 – 2.5 (25 – 33 %) (Table 3.3).



(a) (b)

Figure 3.9 Body rot appeared on skin (a) and in mesocarp (b) with the proportion of rot of 10 %, the index on a rating scale of 1.0 (Table 3.2) (reproduced with permission of White and Woolf, (2005))

3.4. Conclusion

The experiment was conducted in attempt to replicate the experiment of Chen et al. (2017) to provide validation of the effectiveness of CST application on firmness retention in avocado. In contrast to the work performed by Chen et al (2017), the effects of CST in the experiment was not statistically significant with regard to firmness retention after 6 days of storage at 20 °C and RH of 85-90 %. Nevertheless, there was a consistent trend that colder temperatures and shorter treatments resulted in better firmness outcomes although there are not statistically significant. The large variation of firmness values among fruit could be the reason. Hence, in the next chapter, the experiment 2 was conducted to resolve that issue. In particular, some changes to the experimental protocol were:

- increasing the sample size to 15
- changing the puncture probe for firmness measurement to diminish the measurement errors.
- Developing the protocol to evaluate rot incidence and severity.

CHAPTER 4

EXPERIMENT 2

4. Experiment 2

4.1. Introduction

The experimental work of Chen et al., (2017) showed that CST at 30 minutes and 0 °C effectively delayed the pulp softening thanks to the decrease of PG and endo-β-1,4-glucanase activities. From the previous experiment, the effects of treatment temperature and time exposure were not statistically significant on firmness retention since avocado itself has a large variability among fruit when the sample size of 5.

With the sample size of 15, the experiment of Chen et al. (2017) showed smaller variation in firmness values. In an attempt to rectify the differences between the previous experiment (Chapter 3) and the work of Chen et al. (2017), this experiment replicated the work of the previous experiment with some adjustments including increasing of sample size to 15, changing the puncture probe for firmness measurement to diminish the measurement errors and evaluating the incidence and severity of rots.

4.2. Material and Methodology

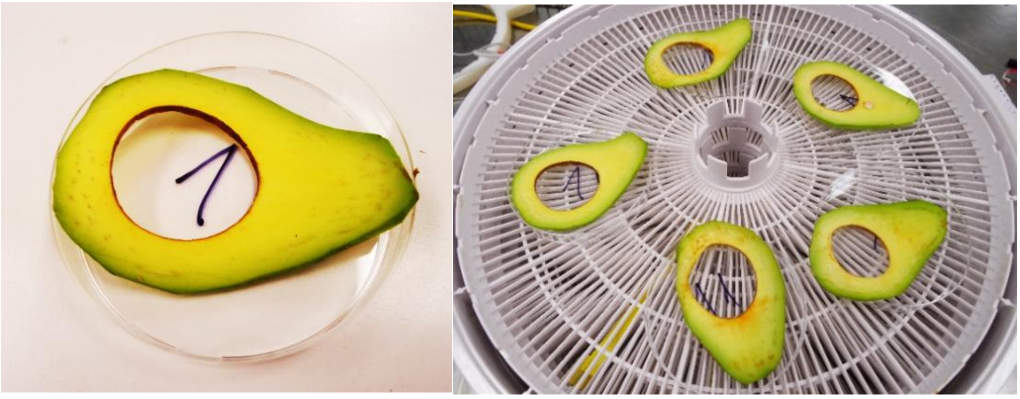
4.2.1. Fruit Source and sample preparation

Mature ‘Hass’ avocado fruit were harvested during the early season on 14th November 2019 from an orchard in Bay of Plenty, New Zealand. Upon arrival, 300 avocado fruit were sorted, repacked on the plix trays and stored in a controlled room as mentioned on the section 3.2.1 and 3.2.2.

4.2.2. At-harvest assessment

Due to the large seasonal variability of avocado (Wedding et al., 2013), the previous experiment showed a large variation in fruit firmness after storage (section 3.4.2). A potential cause of that issue is a wide range of variability in the initial fruit samples. Consequently, an at-harvest assessment of dry matter was therefore conducted to determine the quality and maturity of avocado fruit at the beginning of the experiment, which provided the information of maturity at a specific time of a season.

Fifteen (15) avocados were taken for at-harvest assessment. Adopted the method of Morris and O'Brien (1980) and Bowen et al. (2018), the protocol of DMC measurement was conducted: The fruit were cut lengthwise into halve with 1 mm in thickness, removed the seed, seed coat, and skin (Fig 4.1a). The sample was then cut into pieces and put in the preheated fan dryer at 65°C for 24 h until getting a constant weight (Fig.4.1b).



(a) (b)

Figure 4.1 Dry matter content measurement of avocado fruit using an air fan dryer

DMC was calculated with the following equation:

Equation 4.1 Dry matter content formula

$$DMC\ (\%) = \left(\frac{(m_i\ -\ m_d) - \left(m_f\ -\ m_d\right)}{(m_i\ -\ m_d)}\right) \times 100\%$$

- m_i: initial sample weight
- m_d: dish weight
- m_f: final sample weight

4.2.3. Cold shock treatment

Eighteen (18) treatments representing a full matrix of 3 temperatures (0, 2 and 4 °C) and 6 times (15, 30, 45, 60, 90 and 120 minutes) were treated in random order (Fig 4.3). The establishment of the experiment was similar to the previous experiment (section 3.3.3.2), but with some improvements being:

- Fifteen fruit per treatment in an attempt to get a better measure of variability in the sample and also to enable statistically different separation of treatment means with a small average difference.
- DMC measurement to assess fruit maturity
- The probe used in firmness measurement: P/3 probe replaced P/2 needle probe used in the previous experiment. Compared to P/2 needle probe, P/3 probe provided a larger number in the measurement (Fig 4.2), which reduced the errors in measurement and calculation.

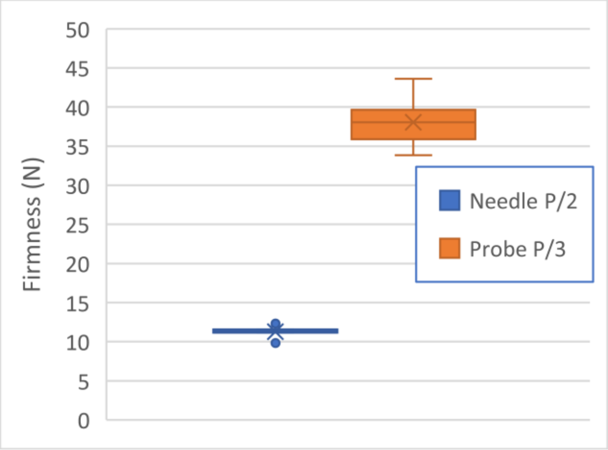


Figure 4.2 Comparison of firmness values performed by P/2 needle and P/3 probe

After the treatment, the fruit were dried with a cloth, repacked on a plix tray, then stored at 20 °C and 85–90 % RH for 6 days. A tray of 15 avocados taken as controls was kept in a storage room at 20 °C during the treatment time.

Figure 4.3 Fifteen fruit were immersed in the cold water at 2 °C with the added ice during the treatment

Fruit core temperature was measured using a set of thermocouple adaptors with squirrel loggers during the treatment (section 3.2.2.2).

4.2.4. Measurement

After 6 days, each box of 15 fruit (one treatment) was taken for evaluation of firmness (section 3.2.4.1) and chilling injury (section 3.2.4.2). Rotted fruit was recorded. After the evaluation, the samples were subsequently eliminated.

4.2.4.1. Texture analysis

A puncture test was again used to determine the firmness value of individual fruit at both the firm (at harvest) and soft (eating ripe) stages (section 3.2.4.1). Notably, a P/3 probe (a cylinder of 3 mm in diameter) was used in this experiment to replace the P/2 needle probe used in experiment 1 and the study of Chen et al. (2017).

Fruit firmness was measured at three different points along the equator of the fruit, 120° apart (more details in section 3.2.4.1). The firmness was taken as the maximum break force (Fsk) and expressed in Newtons (N). For each fruit, the firmness was considered as the average of three measurements taken at three points.

4.2.5.2. Chilling injury evaluation

Chilling injury was assessed based on *"The International Avocado Quality Manual*" (White and Woolf, 2005) as a guide (section 3.2.4.2).

4.2.5.3. Data analysis

An analysis of variance (ANOVA) was performed by using the general linear model to measure difference among means. Factors considered in the model were temperature (°C) and time (min). Differences between the means were checked by using

Tukey’s test. Significant differences were considered at the 95% level confidence. Minitab (version 19.0, Minitab Inc., Pennsylvania, USA) was used to conduct all statistical analysis.

4.3. Results

4.3.1. The change of temperature of the fruit seed’s surface during CSTs

The temperature change at the fruit seed’s surface is shown during the CSTs at 4 °C (Fig.4.4), at 2 °C (Fig.4.5), and 0 °C (Fig.4.6). Under the dynamic stage, the seed’s surface temperature declined significantly from approx. 22 °C to 5, 8 and 9 °C for fruit treated at 0, 2 and 4 °C respectively within the first 30 minutes and followed by a gradual decline in the next 30 minutes. Thereafter, under the static stage, the temperature subsequently remained almost constant at about 0.5, 3.0, 4.5 °C for fruit treated at 0, 2, 4 °C respectively. It is seen that the fruit core temperatures were higher to some extent than the treatment temperatures (water temperatures) of 0, 2, 4 °C.

The cooling rate during the dynamic stage was approx. 0.4 °C/min for treatment at 0 °C and approx 0.33 °C/min for the treatment at 2 and 4 °C. It took about 45 minutes for treatment of 0 °C and 50 minutes for the treatment of 2 and 4 °C for fruit seed surface to reach the static stage. CST with ice water had better heat transfer efficacy with higher cooling rate. Compared to the previous experiment, CSTs of this experiment worked properly having the same patterns with the experiment of Chen et al. (2017) which reported that it took 60, 30 and 29 minutes for the treatment at 0, 2 and 4 °C to reach the static stage. However, the result of Chen did not show a clear separation of the temperature change between the CST at 2 and 4 °C.

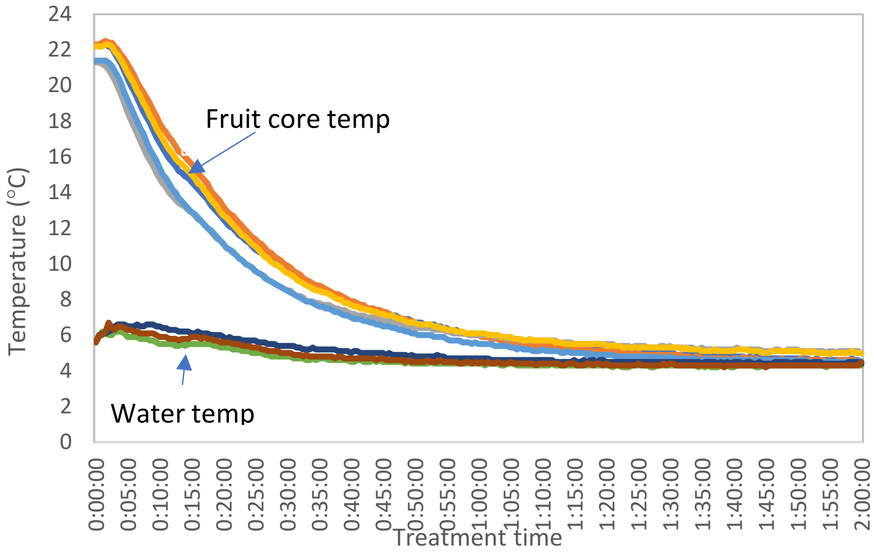


Figure 4.4 The temperature change of fruit seed surface and water during the CST at 4 °C

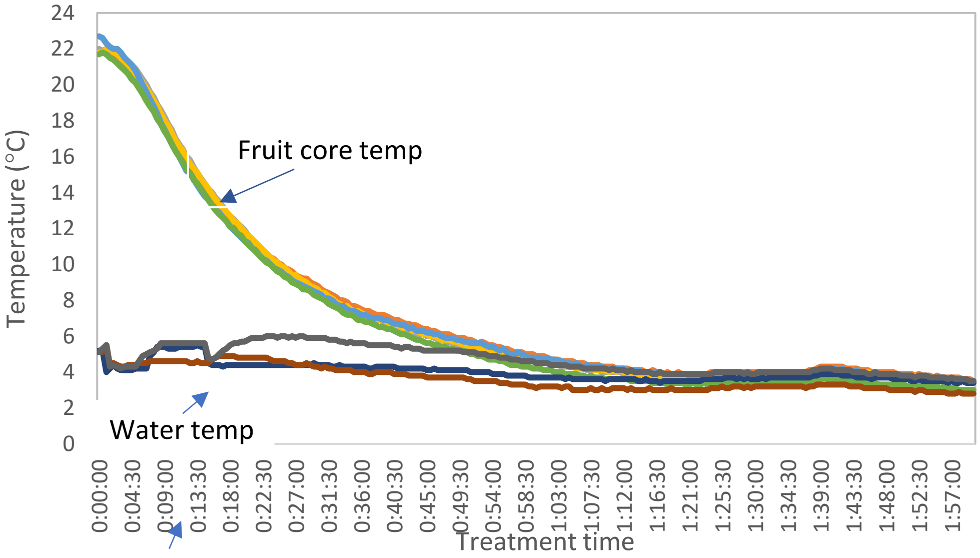


Figure 4.5 The temperature change of fruit seed surface and water during the CST at 2 °C

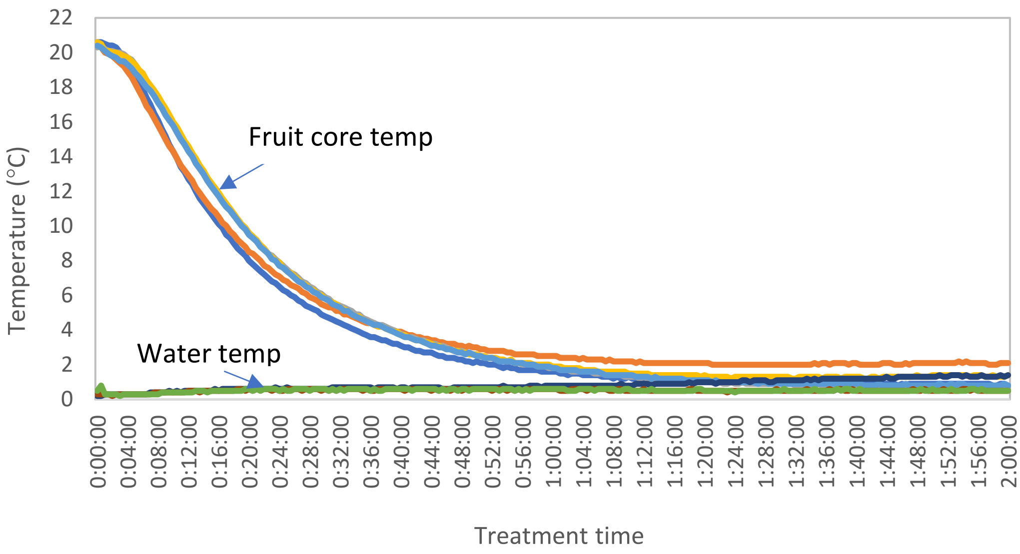


Figure 4.6 The temperature change of fruit seed surface and water during the CST at 0 °C

4.3.2. Firmness

In this experiment, the results of firmness measurement were shown in Fig. 4.7 and Fig. 4.8. It has been observed that fruit firmness decreased from the initial value (at harvest) of 38.1 N to 2.8 N after 6 days. The ripening process initiates by the activity of cellulase which partially modifies cell wall structure, followed by pectin-methylesterases and finally polygalacturonase (Jeong et al., 2002).

Among the 18 treatments studied, no statistical differences in firmness were observed ($p > 0.05$). The statistical analysis also showed substantial standard error among fruit within each treatment sample. The statistical analysis result of this experiment has the same result of the previous experiment which reported the large firmness value variation of fruit within each treatment sample (section 3.4.2). In an attempt to minimize the effect of fruit to fruit variability on disguising treatment effects a larger sample size may be required in the study.

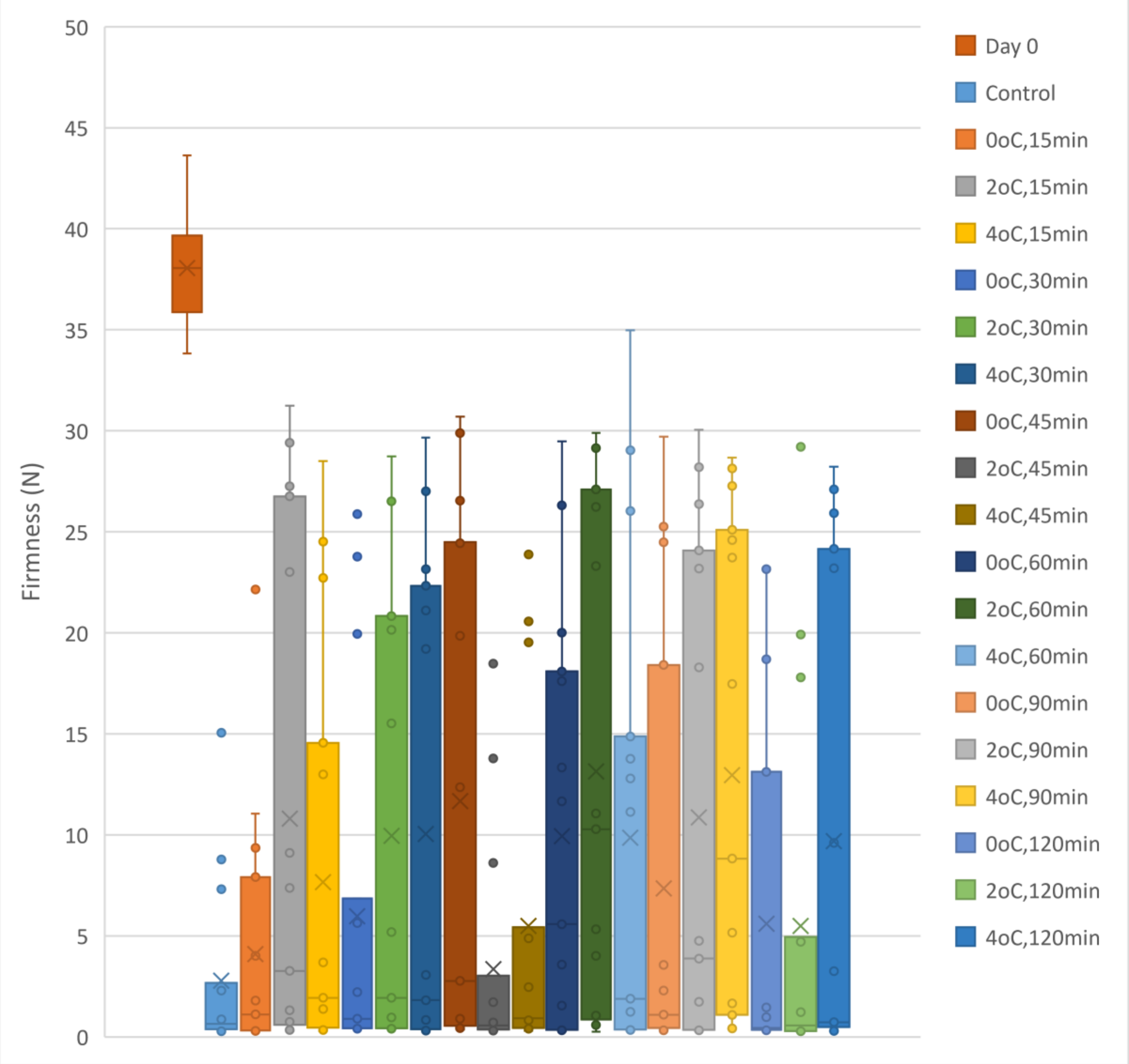


Figure 4.7 Fruit firmness (N) with different treatment temperature (0 to 4°C) in a range of treatment time (15 to 120 mins) after storage of 6 days

The average of DMC was 33.1 % ranging from 30.6 % to 40.2 %. The at-harvest assessment reported that the maturity of fruit was different from fruit to fruit, which was possibly one of the contributors of the variability of avocados. High at-harvest variability of avocado has previously been reported in a number of studies: tree to tree variability and fruit maturity (Hofman and Jobin-Decor, 1999; Marques et al., 2006). Avocado fruit is picked off the trees when fruit is physiologically mature but unripe. Some physiologically mature avocado fruit possibly left on trees for later harvest gains a higher

DMC over time and higher at later harvest (Ozdemir and Topuz, 2004, Lu et al., 2009, Gaydou et al., 1987).

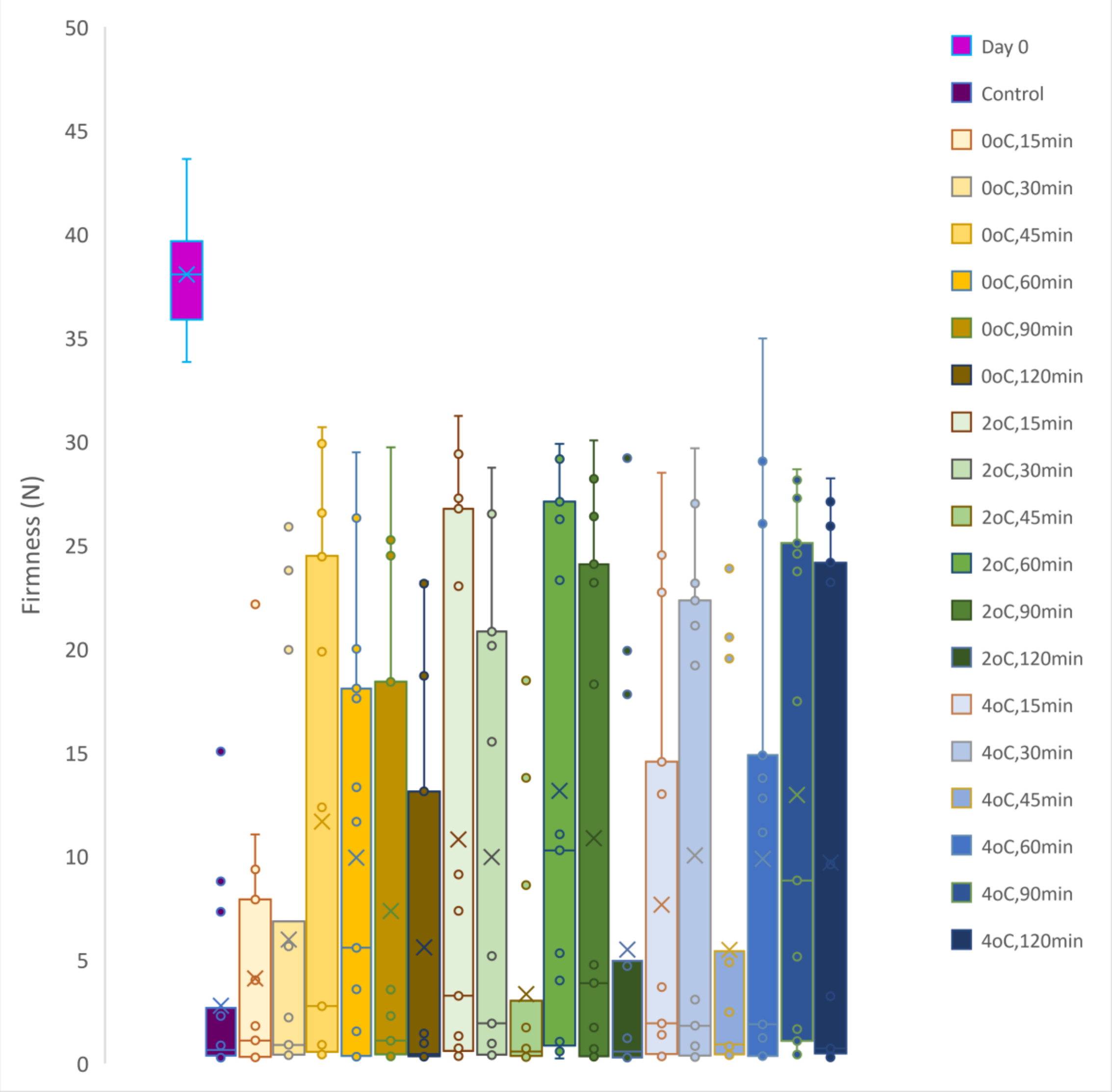


Figure 4.8 Fruit firmness (N) with different treatment times (15 to 120 mins) in a range of treatment temperature (0 to 4°C) after storage of 6 days

Fruit size can contribute to the variability of fruit sample which may increase the random error for this experiment. Hofman and Jobin-Decor (1999) reported that smaller fruit had a higher DMC and ripened more quickly. Avocados from the same orchard which had higher DMC had shorter time to ripen (Osuna-García et al., 2010). However, Hernández et al. (2016) argued that DMC does not correlate on a fruit-to-fruit basis with time to reach ready-to-eat firmness due to the high heterogeneous postharvest ripening.

4.3.3. Chilling injury and rot

Symptoms of chilling injury including internal and external chilling injury of avocado fruits were not observed in treated fruits for all treatments after storage of 6 days at 20 °C. This result agrees with the results of the previous experiment and Chen et al. (2017). Hence it would seem that CST does not cause chilling injury to avocado.

Fruit rots were observed after 6 days (Fig 4.9). The incidence of rots across 18 treatments is shown in Table 4.1. Stem-end and body rots appeared to be the most severe issue for avocado fruit stored at 20 °C and found across all the treatments' samples. Reducing the incidence of rot can be achieved by storing at low temperature (< 5 °C) (Mazhar et al., 2018). When avocados were rotten, the flesh became over-soft and mushy, which could contribute to the variation of the firmness values. Therefore, rotten fruit must be removed out of the samples.

Table 4.1 The rot level of fruit samples after 6 days assessed based on the International Avocado Quality Manual" (White and Woolf, 2005)

Treatment	Number of rotten fruit	The average severity level
0°C, 15 min	3	2.33
0°C, 60 min	1	2.0
2°C, 90 min	2	2.25
2°C, 120 min	1	2.0
4°C, 15 min	1	2.5
4°C, 30 min	1	2.5
4°C, 45 min	1	3.0
4°C, 60 min	2	2.3
4°C, 120 min	1	2.0

4.4. Conclusion

This experiment aimed to replicate the previous experiment with some improvements to reduce the variability of fruit. Similar to the previous experiment, the statistical analysis of all the treatments from this experiment still showed that the results were less reliable. Furthermore, the effects on firmness retention of different treatment time and treatment temperature were not clear for this experiment due to the large fruit variability. The next experiment would attempt to solve this issue by increasing the sample size.

CHAPTER 5

EXPERIMENT 3

5. Experiment 3

5.1. Introduction

In previous experimental work, CSTs at the temperatures (0, 2, 4 °C) and treatment time (15, 30, 45, 60, 90 and 120 mins) did not show clear effects on firmness retention after storage. The results of the previous experiment presented that fruit variability remained substantial and potentially too significant for any effects of temperature and time of a CST treatment to be observed.

Firmness measurement from the last two experiments showed a large magnitude of standard deviation. In an attempt to reduce that, this experiment was designed to focus on increasing the sample size from 15 to 150. With the large fruit number, investigating a wide range of conditions is no longer possible. A single treatment with a temperature of 0 °C and duration of 60 minutes was chosen as the treatment showed the highest mean value of firmness after storage time of 6 days although there is no statistical difference among the means of those treatments (Fig 4.7).

In addition to firmness measurement, experiment 3 aimed to observe the effects of CST on skin colour, respiration rate, ethylene production rate and chilling injury. Chen et al., (2017) reported that CST effectively delayed fruit ripening such as skin discolouration, pulp softening, respiration rate, and ethylene production. Hence, the additional measurements on quality parameters would help to validate the effects of CST on the overall quality maintenance of avocado. In summary, this experiment replicated the previous work in Chapter 4 to identify the suitable CST for avocados with an attempt to reduce the large inherent variability by increasing the sampling size, and validating the findings of Chen et al., (2017) by measuring more relating parameters.

5.2. Material and Methodology

5.2.1. Fruit source and sample preparation

Mature 'Hass' avocado fruit were harvested during the early season on 12th December 2019 from an orchard in Bay of Plenty, New Zealand. Upon arrival, 315 avocado fruit were sorted, repacked on the plix trays and stored in a controlled room as mentioned on the section 3.2.1 and 3.2.2.

On the treatment day, each set of 5 fruit from the same plix tray was packed into a labelled mesh bag (Figure 5.1) prior to CST.

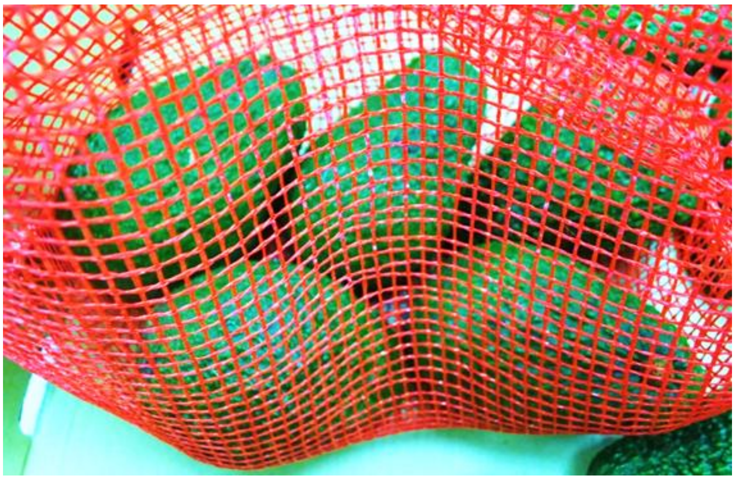


Figure 5.1 Fruit were contained in a mesh bag during CST and repacked on a plix tray thereafter

5.2.2. At-harvest assessment

DMC and initial firmness measured by a texture analyser were the quality parameters for at-harvest assessment (section 4.2.3).

5.2.3. Cold shock treatment

The CST was based on the previous experiment (section 4.3.2.1), however, in this experiment, only one CST (at 0 °C for 60 minutes) was used. Each set of 5 avocados contained in mesh bags were immersed into ice water for 60 minutes. There was a total of 150 avocado fruit used for the treatment and 150 fruit used as control sample. Fruit were packed into plix trays, kept in a storage room at 20 °C and 85-90 % RH for 6 days after CST.

5.2.4. Fruit Quality Measurement

5.2.4.1. Texture analysis

A puncture test was conducted by using a TA.XT.Plus texture analyser with a P/3 probe (see section 4.2.4.1).

5.2.4.2. Skin color measurement

Skin color measurement was performed using a spectrophotometer (CM-2600D, Minolta, Japan). The method was adopted from the experiment of Chen et al. (2017). The settings of the spectrophotometer used for the experiment are shown in Table 5.1.

Table 5.1 Setting parameters for the spectrophotometer

Setting parameter	Value
Spectral component	SCI
UV setting	100% (Full)
UC cut	0 - non
Observer angle	10°
Light source	D65
Measurement area - MAV	8 mm
Measurement area - SAV	3 mm

The spectrophotometer was calibrated with a standard white plate available with the equipment. Three measurements were taken at three different points along the equator and were averaged to represent the average color parameter per fruit. The skin color parameters measured for the experiment were presented in Table 5.2.

Table 5.2 Skin colour parameters measured after storage time of 6 days by a spectrophotometer

Skin color parameters	**Represent**	**Range**
L^*	Lightness	Black to White
C^*	Color saturation	Dull to Vivid
h^*	Hue angle	Color wheel

5.2.4.3. Respiration rate measurement

Respiration rate was measured after storage of 6 days. Fifteen (15) avocados were randomly picked from treated samples and 15 avocados from the control samples. The measurement was conducted in a temperature-controlled room at 20 °C. A set of 3 avocados was kept in a cylindrical container of 3850 mL with an airtight lid fitted with two rubber septa at both ends (Fig. 5.2 A) for 20 minutes. After that time, a 6 mL syringe was used to 'pump' 4 - 5 times to mix the air in the container so that the representative gas was homogeneously sampled. Two 1 mL gas samples were taken from each end of the container for the measurements. The measurement result for each replicate was the average of these two gas samples.

(A) (B) (C)

Figure 5.2 Gas sample was taken after 20 minutes from a cylindrical container contained 3 avocados (A) and injected into an O_2/CO_2 analyser (B). The integrator (C) is where the signals were sent, and the CO_2 level was printed

An O_2/CO_2 analyser developed by Massey University using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK). Oxy-free nitrogen was the carrier gas with the flow rate of 30 - 35 mL min^{-1}. The output signals were analysed using an integrator (Hewlett–Packard, model 3396A) (Fig. 5.2 C). Commercially CO_2 standard of 0.5 % (BOC, Palmerston North, New Zealand) was used for analyser’s calibration. The containers were checked for leaks before the measurements.

Respiration rate was calculated, regarding time between samples, fruit weight and the volume of the containers. The final result of respiration rate was the average of five replicates each consisting of three fruit. Respiration rate calculated with the following the equation (Eq 5.1) based on the ideal gas law, expressed as nano-moles CO_2 evolved per kg fruit per second (nmol kg^{-1} s^{-1}):

Equation 5.1 Respiration rate formula

$$R(CO_2) = \left(V_j - \frac{m}{\rho}\right)\left(\frac{P(C_1 - C_o)(1 \times 10^{-5})}{8.314(T + 273)m(t_1 - t_o)}\right)$$

R (CO2)	Respiration rate (nmol kg^{-1} s^{-1})
V_J	Container volume (mL)
m	Fruit weight (g)
ρ	Fruit density (g/mL)
P	Atmospheric Pressure (Pa)
T	Temperature (°C)
C_o	Concentration of CO_2 at t_o (%)
C_1	Concentration of CO_2 at t_1 (%)
t_o	Time zero (first sampling time, initial time)
t_1	Time one (second sampling time)
t_1 -t_o	Accumulation Time (s)

5.2.4.4. Ethylene production measurement

A laser-based photo-acoustic ethylene detector (ETD-300, SensorSense B.V., Nijmegen, The Netherlands) was used to measure the ethylene concentration (Fig. 5.3). The Sample method was applied which measured the ethylene concentration by comparing the concentration of the sample and the reference sample (gas standard with a known concentration) (Gwanpua et al, 2018). The standards were injected to the ETD-300 several times until the measured concentrations were relatively equal to the actual concentration of 92 ppb to achieve the standard curve.

The gas sample volume of 1 ml was taken from the cylindrical container of 3850 mL which contained 3 avocados, which were the same for respiration rate measurements (section 5.2.5.3). The accumulation time for ethylene measurement was hence 20 minutes.

Figure 5.3 ETD-300 was used to measure the ethylene concentration

A volume of 1 ml of gas sample was injected into a small cuvette connected to ETD-300 via a valve controller. The period of 4 minutes and the flow rate of 4 L h^{-1} were set for the measurement. The gas flushed the accumulated ethylene through the detector. The gas was passed through a CO_2 scrubber (soda-lime) to absorb CO_2 and through Drierite desiccant to absorb moisture. When the cuvette was fully flushed, the ethylene concentration in the cuvette reached an equilibrium in the detector and showed a curve similar to a chromatographic peak. The production of ethylene in the cuvette was represented by the area under the plot. The results of the measurement obtained from Valve Controller software expressed as *ppbv*.

Ethylene production rate is calculated with the following the equation (Eq. 5.2) based on the ideal gas law, expressed as pico-moles C_2H_4 evolved per kg fruit per second (pmol kg^{-1} s^{-1}):

Equation 5.2 Ethylene production rate formula

$$R(C_2H_4) = \left(V_j - \frac{m}{\rho}\right)\left(\frac{P(E_1 - E_o)(1 \times 10^{-11})}{8.314(T + 273)m(t_1 - t_o)}\right)$$

R (C_2H_4)	Ethylene production rate (pmol kg^{-1} s^{-1})
V_J	Container volume (mL)
m	Fruit weight (g)
ρ	Fruit density (g/mL)
P	Atmospheric Pressure (Pa)
T	Temperature (°C)
E_o	Concentration of C_2H_4 at t_o (ppb)
E_1	Concentration of C_2H_4 at t_1 (ppb)
t_o	Time zero (first sampling time, initial time)
t_1	Time one (second sampling time)
t_1 -t_o	Accumulation Time (s)

5.2.4.5. Chilling injury and Rot

Chilling injury and rot were assessed according to “*The International Avocado Quality Manual*" (White and Woolf, 2005) (section 3.2.4.2).

5.2.5. Data analysis

A two-sample t-test was performed to determine the significant difference between treatment and control samples using Minitab (version 19.0, Minitab Inc., Pennsylvania, USA) with data set transformed before the test. A chi-square test was performed to determine the significant difference between the treatment and control samples for each level of rot severity.

5.3. Results and Discussion

The average DMC of the mesocarp of fruit sample for the experiment was 38.4 % with the highest number of 46.2 % and the lowest number of 30.7 %.

5.3.1. Seed surface temperature during CST

The change of the seed surface temperature during the CST at 0 °C was shown in Fig 5.4. The experiment achieved the same patterns of temperature change achieved by the previous experiment. The fruit seed surface temperatures of a set of 5 fruit declined significantly from approx. 20 °C down to between 1.1 and 3.8 °C after 60 minutes of CST, with an average value of 2.7 ± 0.5 °C. The CST achieved the similar temperature pattern from the work of Chen et al. (2017) reporting the decline of temperature from approx 20°C to the average temperature of 2.5 °C after 60 minutes.

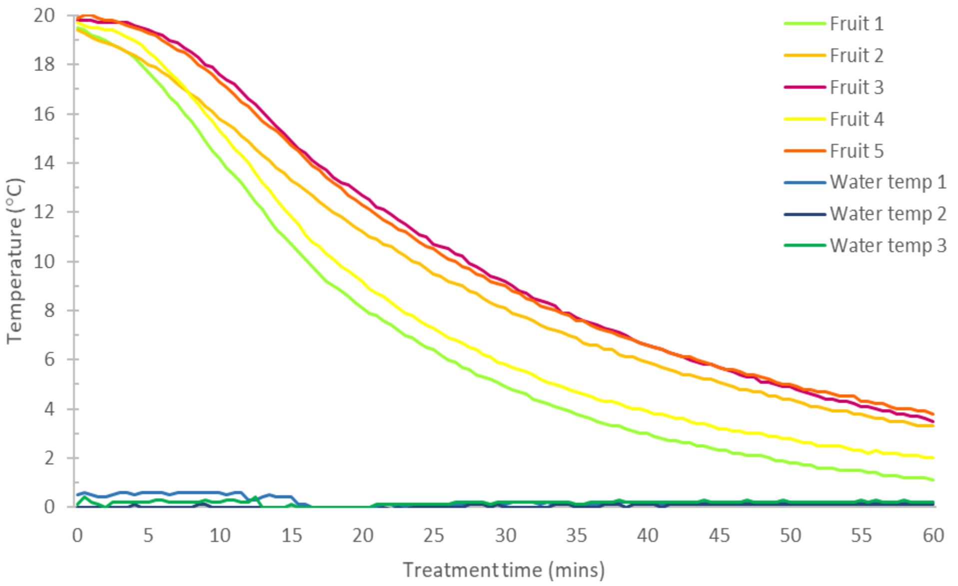


Figure 5.4 The change of average temperature (°C) of fruit seed surface for 5 fruit (± 0.2 °C) and of water at 3 different points (± 0.1 °C) during cold shock treatment at 0 °C in 60 minutes

5.3.2. Firmness

After 6 days of storage at 20 °C, firmness values decreased from the initial firmness of 38.05 ± 0.69 (N) to 3.99 ± 0.66 (N) and 4.44 ± 0.70 (N) for treatment and control samples, respectively (Fig. 5.5).

Fig. 5.6 shows that the firmness data sets of the two samples have large variation, in which 85.2 % of treated avocados and 81.0 % of control samples had the firmness values from 0.23 – 6.73 (N) which is evaluated as “soft ripe”. More than 10 % of fruit had a firmness value from 19.7 – 32.7 (N) for both sample sets which represented the percent of unripe fruit in the both samples. Less than 7 % of avocados were pretty ripe with firmness values from 6.7 – 13.2 (N) which is evaluated as “ripe to firm ripe”.

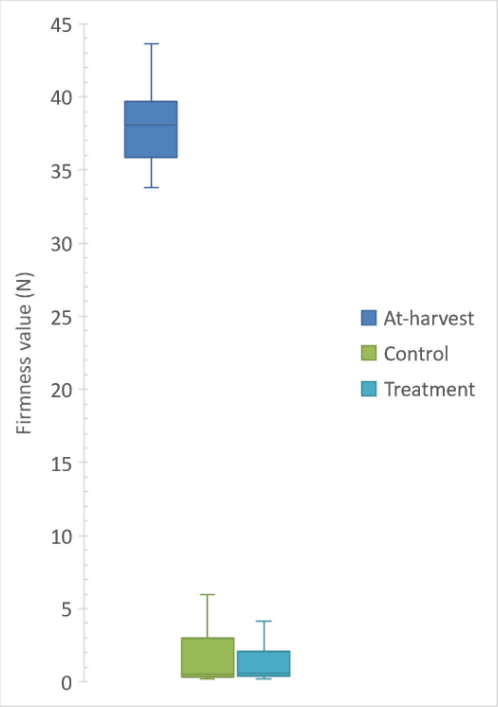


Figure 5.5 The box and whisker plots for fruit firmness (N) of at-harvest, treatment and control samples after storage of 6 days at 20 °C and RH of 85-90 %

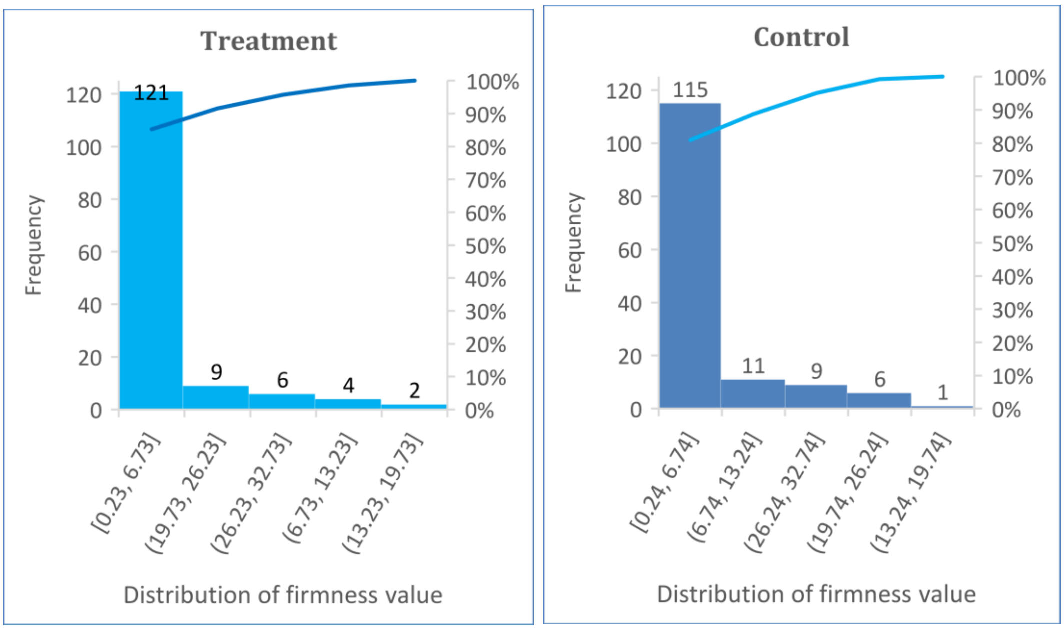


Figure 5.6 The distribution of firmness values of treatment and control samples after a storage time of 6 days at 20 °C and 85-90% RH presented in pareto charts

Due to the non-normal distribution of firmness data set, the data transformation was performed using the Box-Cox method. The histogram graphs for the transformed values of firmness were presented in Fig 5.7.

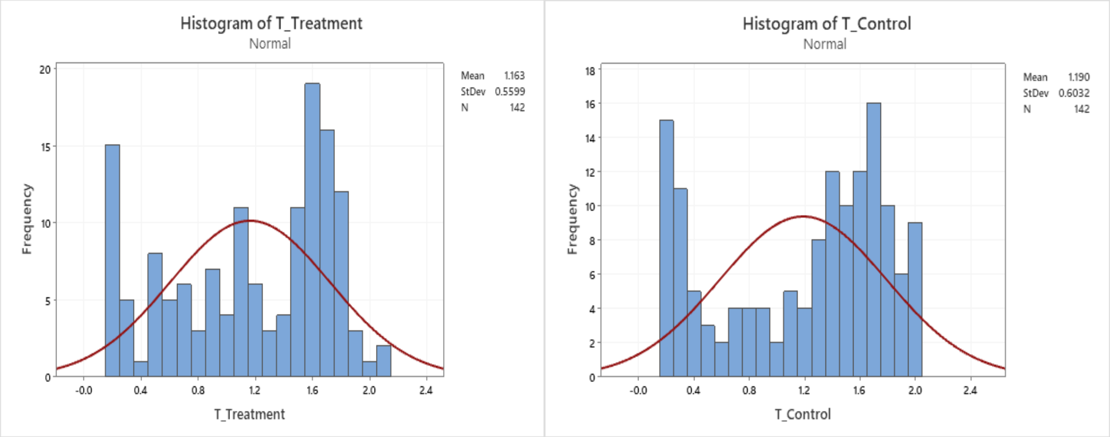


Figure 5.7 The distribution of transformed firmness data of treatment and control samples using the Box-Cox method in histogram charts

Using the transformed data, the t-test analysis showed that CST did not have significant effects on firmness retention compared to control (*p-value > 0.05*). The statistical analysis results confirmed the results of the last experiment that CST showed insignificant effects on fruit firmness and fruit variation was still the most influential factor to data sets with the coefficient of variation of the two sets are 198 % and 188 % for treatment sample set and control sample set respectively. However, with the sample size of 15, the results of Chen et al. (2017) showed a relatively small standard derivation in firmness data set (~ 10%).

5.3.3. Skin colour

When ‘Hass’ avocados ripen, skin color changes from green, to purple, dark brown or black with the changes of pigments in the skin tissue observed as a decrease in values of lightness (L^*), chroma (C^*) and hue (h^*) (Fig. 5.8). Figure 5.9 reported the values of skin color parameters (L^*, C^*, h^*) of the treatment sample and control sample with L^* value of 24.48 ± 0.26 (treatment) and 25.61 ± 0.26 (control); C^* value of 14.18 ± 0.35 (treatment) and 12.91 ± 0.35 (control); h^* value of 92.82 ± 0.92 (treatment) and 94.14 ± 1.06 (control).

The statistical analysis showed that there was no significant difference between the two sample sets which agreed with the result of fruit firmness suggesting no effect of the treatment. However, the study of Chen et al. (2017) reported that treated avocados remained the green skin color longer than the control ones and hence the measured color parameters (L^*, C^*, h^*) of the treated avocados decreased compared to the control ones after 6 days.

Unlike firmness values, skin color parameters showed small variability (Fig. 5.9) with coefficient variance approx. 12.0 % (L^* value); 32.0 % (C^* value); 12.5 % (h^* value).

Figure 5.8 'Hass' avocados changed skin color during their ripening from green color of unripe fruit (left) to dark brown or black color of ripe fruit (right) after a storage time of 6 days at 20 °C and 85-90% RH. Rots appeared on a few avocados with the white to yellow marks on the skin

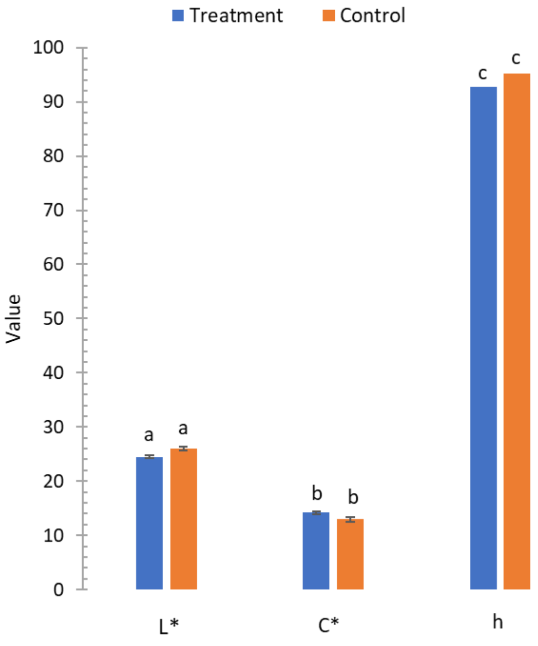


Figure 5.9 The box and whisker plots for color parameters of avocado skin after a storage time of 6 days at 20 °C and 85-90% RH

5.3.4. Ethylene production rate

Avocado fruit produce significant ethylene level during ripening from 1-aminocycclopropane-1-carboxylic acid, as induced by 2 enzymes (ACS and ACO), which initiates the physiological change in firmness and color (Nath et al., 2014; Yahia and Woolf, 2011). Fig 5.10 shows that the ethylene production rate of treatment sample of 12.94 ± 2.04 pmol kg^{-1} s^{-1} was significantly lower than that of control sample of 25.51 ± 3.06 pmol kg^{-1} s^{-1} (*p-value* = 0.014). This showed that CST suppressed the activities of ACS and ACO. The result agreed with the finding of Chen et.al (2017), resulting in delaying the ethylene peak from day 6th to day 8th, but not significantly reduce the magnitude of the ethylene production rate peaks which were approx. 41 and 38 μL kg^{-1} h^{-1} for control and treatment samples respectively.

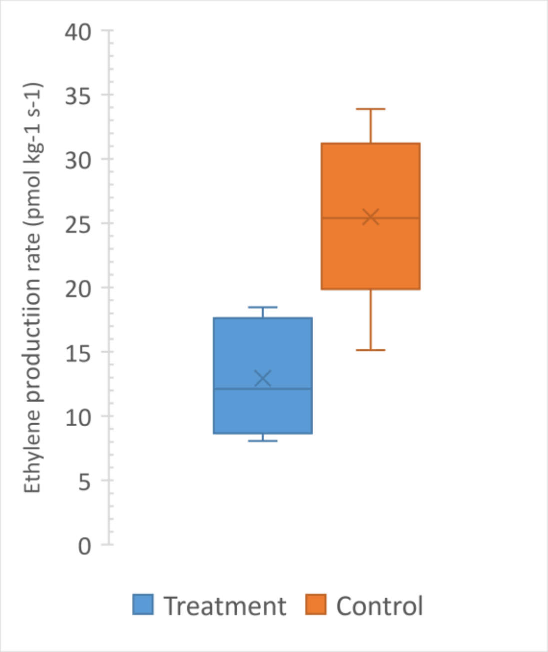


Figure 5.10 Ethylene production rate of treatment and control sample after a storage time of 6 days at 20 °C and 85-90 % RH. 15 avocados were randomly chosen for the measurement from the population of 150 fruit of each data set

5.3.5. Respiration rate

Fig 5.11 shows that respiration rate of treatment sample was 797.2 ± 54.5 nmol kg^{-1} s^{-1} and that of control sample was 841.2 ± 41.0 nmol kg^{-1} s^{-1}. The lower ethylene production rate could result in a delay of or be a cause of a lower respiration rate (Yang et al, 1994). However, the respiration rate of treatment and control samples observed in the experiment were not statistically different from each other (*p-value* > 0.05). This result conflicted with that of Chen et al. (2017) showing that CST delayed the peak of respiration rate from day 6th to day 8th of storage, but CST did not significantly reduce the magnitude of the respiration rate peaks which were approx. 81 and 79 mg kg^{-1} h^{-1} for control and treatment samples respectively.

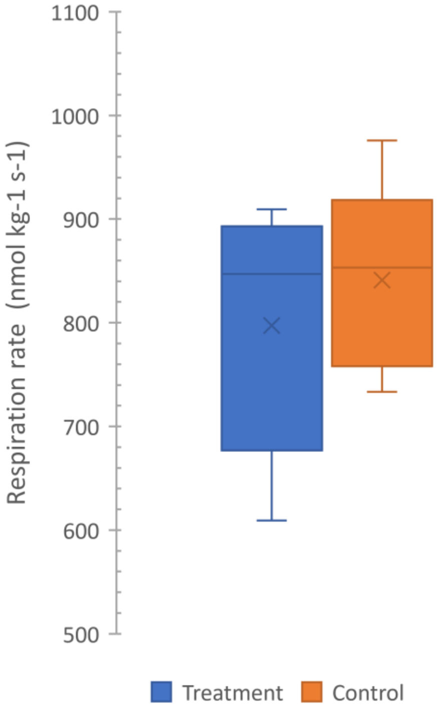


Figure 5.11 Respiration rate of treatment samples and control sample after a storage time of 6 days at 20 °C and 85-90 % RH. 15 avocados were randomly chosen for the measurement from the population of 150 fruit of each data set

5.3.6. Chilling injury and rot

Symptoms of chilling injury were not observed in treated fruit after the storage at 20 °C an 85-90% RH. The result agreed with the study of Chen et al (2017). On the other hand, fruit rot including stem-end and body rots appeared after 6 days. The number of rotten fruit incidences in 3 different severity levels of treatment samples and control samples were shown in Table 5.3, in which the ratios of rotten fruit were 27.3 % and 18.6 % for treatment and control samples respectively.

The statistical analysis using Chi-Square Test reported that there was not statistically different between the number of rotten fruit in each level of severity of both sample sets (*p-value* > 0.05). The percent of rotten fruit in the experiment was much lower than that of a study of Hopkirk et al. (1994) which reported the rot incidence of 67 % for avocados stored and ripened at 20 °C. Fungi (*Botryosphaeria spp.* and *Colletotrichum spp*.) were the major organism causing rot in avocados (Hopkirk et al., 1994).

Table 5.3 The incidence and the severity of rots appearing on the skin and in the flesh of avocados after 6 days at 20 °C and 85-90% RH based on the “The International Avocado Quality Manual" (White and Woolf, 2005)

	Severe Rot (50%)	Medium Rot (25%)	Less Rot (10%)	The total number of rotten fruit	Average point of rot severity
Severity level	3	2	1		
Treatment	17	11	13	41	2.10
Control	11	7	10	28	2.04

5.4. Conclusion

This experiment aimed to replicate the previous experiment with some improvements to reduce the variability of fruit. The statistical analysis of measurement values of firmness, skin color parameters and respiration rate showed that the difference between the means of the CST and the control samples were not different (p-value > 0.05). An effect of CST on ethylene production rate was observed, in which CST treated fruit were had a lower ethylene production rate compared to the control samples (p-value = 0.014). Therefore, while ethylene physiology may have changed, the CST did not show other effects in comparison to the control. The ripening process of avocado during the storage time of 6 days at 20 °C was not markedly affected by a CST prior to this time period.

While this experiment suggest that a CST has little effect on avocado postharvest physiology at 20 °C, this remains in contrast to the findings of Chen et al. (2017). One explanation for this is that the fruit were treated at a different maturity or simply that the time point in which a comparison between the treatment and the control was made is a point either prior to or after differences in the product quality are evident. Hence, in an attempt to validate the findings of Chen et al., (2017), for the next experiment, in addition to the current measurement methods, firmness, respiration rate, ethylene production rate and skin color parameters measurement were performed on a 2-day interval.

Additionally, to understand the change of fruit firmness during the storage time, Aweta Acoustic Firmness Sensor was used for non-destructive measurements that the same avocados could be measured without damaging the fruit. The correlation of both destructive method (puncture test) and non-destructive method was shown. The next experiment was performed to further clarify the effects of CST (if any) on avocados in subsequent 20 °C storage.

CHAPTER 6

EXPERIMENT 4

6. Experiment 4

6.1. Introduction

In the previous experimental work, a significant decrease (*p-value* = 0.014) in the ethylene production rate of the CST treatment sample in comparison to the control sample was observed after 6 days at 20 °C (12.94 and 25.51 pmol kg^{-1} s^{-1} for treatment and control samples respectively). However, the observed reduction in ethylene production did not convert to effects on delaying other fruit ripening properties such as respiration rate, peel colour and firmness (Chapter 5).

The study of Chen et al., (2017) reported that the ethylene production peaked on day 6 for the control sample and day 8 for treatment sample without a significant difference of magnitude of the two values (41 and 38 µL kg^{-1} h^{-1}). However, from the previous experiment, 81 and 85 % of fruit in both samples were "soft ripe" stage at day 6, meaning that the possibility to evaluate fruit qualities to day 8 was impractical because mesocarp becomes too soft for puncture test to distinguish the difference between control and treated fruit. Hence, to understand the change of quality parameters over storage time as well as to clarify the effects of CST on avocado's physiology, this experiment replicated the previous experimental work in Chapter 5 with some improvements:

- An air-flow system was set up to prevent a high level of ethylene accumulated in the storage room during the storage time which could affect the fruit.
- Fruit firmness measurement was performed by both non-destructive method (Acoustic Firmness Sensor - AFS) and destructive method (puncture test) during the storage time on day 2, day 4, day 6 and day 7. The use of the non-destructive method allows for some avocados to be repeatedly measured and kept during the storage to observe the change of fruit firmness for 7 days. A

correlation of the puncture test and AFS method was used to allow comparison of the non-destructive measures to previous results.

- Skin colour parameters were measured using spectrophotometer during the storage time on the same day of firmness measurement.
- Respiration rate and ethylene production rate were measured on single fruit respiration in an enclosed glass jar during the storage time on day 1, day 3, day 5 and day 7.
- To reduce fruit variability, avocados were sorted and graded at the factory based on weight and size.

In summary, the experiment was expected to fully understand the effects of CST on the overall qualities of avocado over the storage time of 7 days to identify the suitable CST for avocados and clarify the experimental results of the work of Chen et al., (2017).

6.2. Material and Methodology

6.2.1. Fruit source

Mature ‘Hass’ avocado fruit were harvested during the mid-season on 29th January 2020 from an orchard in Bay of Plenty, New Zealand. Upon arrival, 315 avocado fruit were sorted, repacked on the plix trays and stored in a controlled room as mentioned on the section 3.2.1 and 3.2.2. On the treatment day, each set of 5 fruit from the same plix tray was packed into a labelled mesh bag (Figure 5.1) prior to CST.

6.2.2. At-harvest assessment

DMC and firmness continued to be the parameters for at-harvest assessment. A total of 20 fruit were used for DMC performed by the avocado supplier and other 15 fruit were performed in the lab. The average dry matter of the fruit sample was 31.93 % (ranging from 27.54 % to 41.07 %). Fruit weight ranged from 173 to 282 g.

6.2.3. Cold shock treatment

The CST applied in this experiment replicated the CST used in the previous experiment (section 5.2.4) in which CST was applied at 0 °C for 60 minutes. There was a total of 300 avocados used for the experiment, in which 150 fruit used as treatment sample and 150 fruit used as the control sample. After the treatment, fruit were kept in an airflow system at 20 °C and 85-90 % RH by setting up barrels.

6.2.4. Fruit storage settings

To avoid the cross-contamination of ethylene gas which potentially produced differently by treated and control fruit, a new setup for fruit storage was established. Six (6) sets of 5 avocados (each set contained in a mesh bag) were stored in a barrel, in other words, each barrel contained 30 fruit (i.e. 6 bags). There were in total 10 barrels, 5 each for treated and control fruit respectively.

Before entering the barrels, each gas flow was bubbled into the glycerol solution contained in a glass jar through a tube. A 40 % (w/w) glycerol in water solution was used to generate the required RH of 85 - 90% inside the barrels (Forney and Brandl, 1992). The airflow rates flowing through the barrels were controlled by a manifold system that ensured the pressure in the barrels was high enough to exhaust the ethylene produced by fruit (Fig 6.1). By establishing this flow-through system potential cross-contamination of ethylene produced by one treatment impact the fruit quality change of the other treatment was eliminated.

6.2.5. Fruit Quality Measurement

6.2.5.1. Texture analysis

The puncture test was conducted by using a TA.XT.Plus texture analyser equipped with a P/3 probe (section 4.2.4.1).

Parallelly, Acoustic Firmness Sensor (AFS) (AWETA™ Impact & Acoustic Firmness System, Nootdorp, The Netherlands) was used to measure the firmness at three different points along the equator (120° apart) and averaged to present one measurement per fruit. The method for acoustic firmness measurements used was that described in White and Woolf, (2005). The settings for the AFS for avocados (Table 6.1) was adapted from the AFS settings of Woolf et al. (2013).

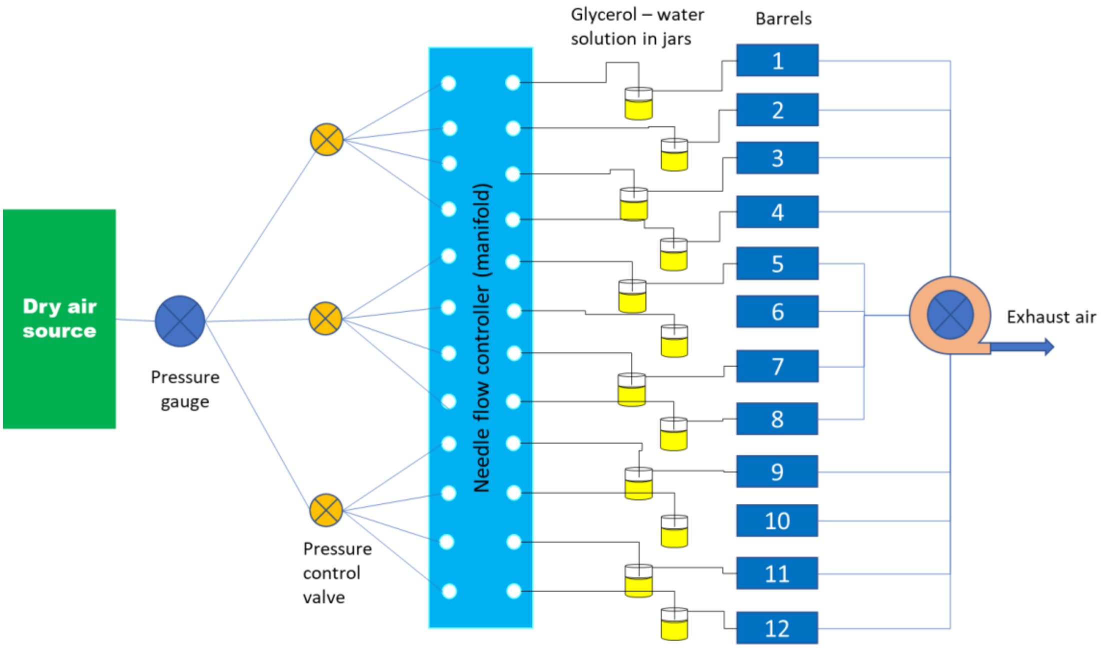


Figure 6.1 The schematic diagram of the storage setting, in which fruit samples were stored in barrels ventilated with humidified air to generate the RH of 85 – 90 % in the barrels. Dry air through the manifold bubbled in glycerol-water solutions to be humidified

Table 6.1 Acoustic Firmness Sensor setting parameters for the avocado fruit

Setting parameter	**Value**
Microphone Gain	50
Tick power	16
Peak selection	Highest peak
Alternative firmness	Impact
Min. FF	0
Max. FF	60

Firmness measurement performed by both non-destructive and destructive methods were conducted on day 2, 4, 6, 7 and at harvest (day 0). To observe the change in firmness in the same population during the storage time, 30 fruit from the treatment and control samples were measured repeatedly using the AFS method only on day 0, 2, 4, 6 and 7. The unit of the puncture test is Newton (N), while that of AFS test is firmness index FI (10^4 $kg^{2/3}$ s^{-2}). Categorization of avocado ripeness according to firmness measured using AFS was previously reported by Woltering et al., (2019) (Table 6.2).

Table 6.2 Classification of RTE ripeness according to avocado firmness using AFS

Class RTE	**Description**	**Aweta value (FI)**
1	Not OK – Too hard	> 22
2	OK - but still hard	18.3 - 22
3	OK - RTE	11.3 – 18.3
4	OK - but getting too soft	9 – 11.3
5	Not OK – Too soft	< 9

6.2.5.2. Skin colour measurement

Skin colour measurement was performed using a spectrophotometer (CM-2600D, Minolta, Japan). Three measurements were taken at three different points along the equator and were averaged to represent the average colour parameter per fruit. Lightness (L^*), chroma value (C^*), and hue angle (h^*) were reported. The skin colour measurements were performed at harvest, on day 2, day 4, day 6 and day 7 (section 5.2.5.2).

6.2.5.3. Respiration rate measurement

Ten (10) avocados each from treated and control samples were randomly picked for measuring the respiration rate and ethylene production rate. The measurement was conducted in a temperature-controlled room at 20 °C. Each avocado was kept in a glass jar of 1050 ml with an airtight lid fitted with a rubber septum on the lid for 15 minutes (Fig. 6.2). At the beginning ($t_o = 0$), 1-ml samples were taken from the empty jars for initial CO_2 concentration. After 15 minutes (t = 15 mins), a 6-ml syringe was used to 'pump' 4 - 5 times to mix the air in the jar, so that the representative gas was homogenously sampled. Two 1-ml gas samples were taken from the jar ready for CO_2 and ethylene measurements.

Figure 6.2 Gas samples were taken for respiration rate and ethylene production rate from a set of 10 glass jars which contained one fruit after 15 minutes

An O_2/CO_2 analyser developed by Massey University using an O_2 electrode (Citicell C/S type, City Technology Ltd., London, UK) in series with a miniature infrared CO_2 transducer (Analytical Development Company, Hoddesdon, UK) was used. The output signals were analysed using an integrator (Hewlett–Packard, model 3396A). The respiration rate is calculated using Eq. 5.2, expressed as nmol kg^{-1} s^{-1} (section 5.2.5.3). The measurements were performed at harvest, day 1, day 3, day 5 and day 7.

6.2.5.4. Ethylene production measurement

A laser-based photo-acoustic ethylene detector (ETD-300, SensorSense B.V., Nijmegen, The Netherlands) was used to measure the ethylene concentration. The Sample method was applied (section 5.2.5.4) which the gas samples were taken at the same time for respiration rate measurement (section 6.2.6.3). The accumulation time for ethylene measurement was hence 15 minutes. The ethylene production rate is calculated using Eq. 5.3, expressed as pmol kg^{-1} s^{-1}.

6.2.5.5. Chilling injury and rot

Chilling injury and rot (section 3.2.4.2) were assessed according to "*The International Avocado Quality Manual*" (White and Woolf, 2005).

6.2.6. Data analysis

A two-sample t-test was performed to determine any significant difference between treatment and control samples using Minitab (version 19.0, Minitab Inc., Pennsylvania, USA) with data set transformed before the test. A chi-square test was performed to determine the significant difference between the treatment and control samples for each level of rot severity.

6.3. Results

6.3.1. Seed surface temperature during CST

The change of the seed surface temperature during the CST in 0 °C water is shown in Fig. 6.3. The experiment achieved the same patterns of temperature change obtained in the previous experiment (Fig. 5.4). The seed surface temperature of the 5 avocados declined from 20 °C to between 1.2 and 3.5 °C after 60 minutes of CST, with an average value of 2.8 ± 0.5 °C. The CST achieved a similar temperature pattern compared to the work of Chen et al. (2017) reporting the decline of temperature from approx 20 °C to an average temperature of 2.5 °C after 60 minutes.

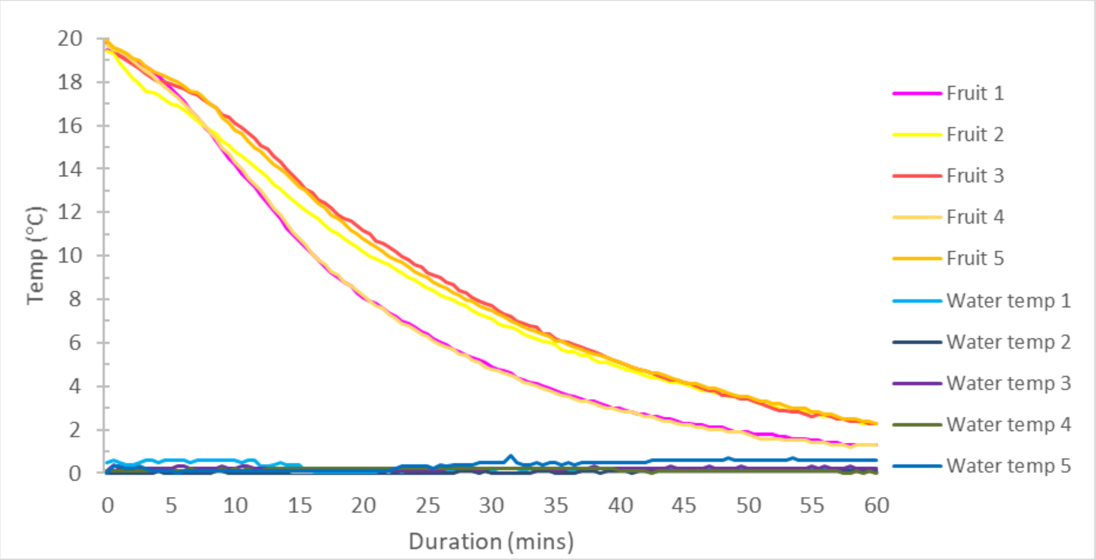


Figure 6.3 Temperature of fruit seed surface for 5 fruit and of water at 3 different points during the CST at 0 °C in 60 minutes

6.3.2. Firmness

6.3.2.1. Firmness values measured by the penetrometer and AFS

The firmness of fruit at harvest was 35.41 ± 0.63 N for puncture test and 54.80 ± 1.27 FI for AFS test. Fig. 6.4 shows that the changing patterns of firmness values measured by both methods for treatments and control samples are relatively similar. With measurements performed by puncture test (Fig. 6.4), during ripening at 20 °C, the firmness values decreased gradually from 35.5 N to approx. 28 N on the first 2 days, followed by a sharp drop to approximately 2 N after 4 days. Firmness values continued to decrease slightly and reached approx. 0.4 N for both the treatment and control sample after 7 days.

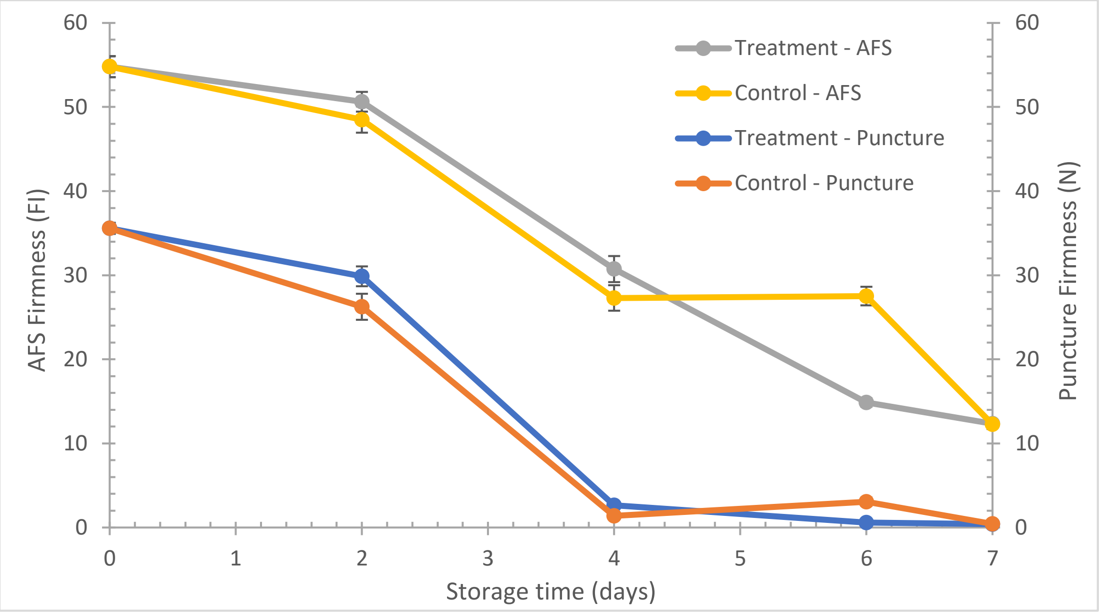


Figure 6.4 Fruit firmness of treatment and control samples were measured by puncture test (N) and AFS (FI = 10^4 $kg^{2/3}$ s^{-2}) of treatment and control samples during storage for 7 days at 20 °C and 85-90% RH

With firmness measurements performed by AFS (Fig. 6.4), the firmness values decreased gradually from approx. 55 FI to approx. 50 FI for both the treatment and control sample respectively after 2 days. The FI profoundly dropped to approx. 28 FI after 4 days for both the treatment and control samples. On day 6, firmness values showed a significant difference (*p-value* < 0.00) with the treated fruit being softer (14.87 ± 0.50 FI) than the control samples (27.52 ± 1.10 FI). On day 7, treatment and control samples reached a similar firmness value (approx. 12.3 FI). Given that it was the only occasion in the experiment in which firmness differences between the CST and the control was detected, it remains unclear whether the differences in the firmness values on day 6 represent an actual CST effect or whether it was an outlier due to fruit to fruit variation. Irrespective of this, the significant result suggests that the control fruit maintain firmness better than the CST fruit, opposite to the result suggested by Chen et al., (2017).

Firmness values reach “ready-to-eat” stage when they range from 4.4 – 6.7 N for the penetration test or 11.3 – 18.3 FI for AFS (Table 6.2). From Fig 6.4, puncture test showed that fruit reached ready-to-eat stage on day 4 while AFS data suggested that fruit reached that on day 6. The difference between the measured values of AFS and puncture test may be attributable to one of several reasons. With the puncture test, 1 mm of fruit skin was removed with a mandoline before the measurement, hence, the measurement value was the pulp firmness. The rough skin of avocado did not affect the firmness values. With AFS, the measurement was performed on the rough skin of the avocado, the measurement surface on the skin was an irregular shape sometimes.

The Pearson’s correlation coefficient (R = 0.393) was found between the AFS and penetration method which showed a moderate positive relationship (*p-value* = 0.038) for firmness values of treatment samples on day 6 (Fig 6.5). However, firmness values measured by both methods on day 7 for control and treatment samples did not show a strong correlation with R = –0.088 and 0.119 for control and treatment sample respectively. Galili et al. (1998) reported the correlation coefficient between the two methods for avocado was 0.659 to 0.806. Pearson’s linear correlation between AFS and penetration method for avocado firmness measurement was reported (R = 0.695) (Shmulevich et al., 2003). Nevertheless, with other fruit, for example apples, there was a good correlation between AFS and puncture test (R = 0.96); the firmness index (FI) was consistent and correlated with physiological properties during the storage life of apple (Fathizadeh et al., 2020).

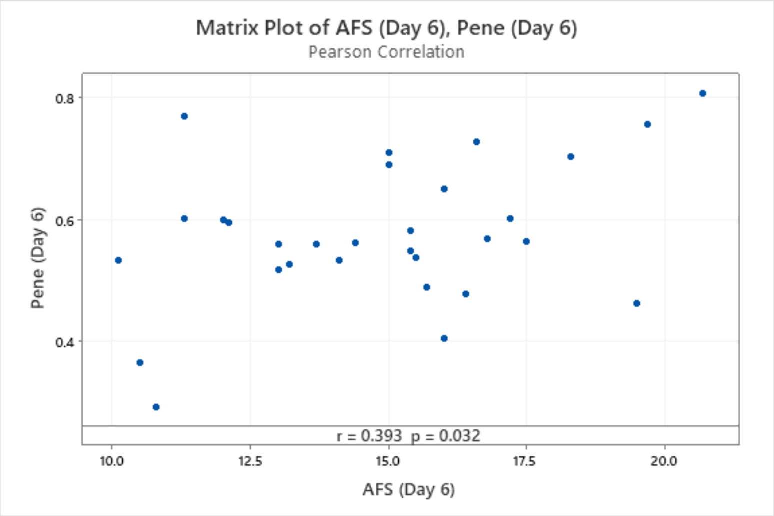


Figure 6.5 The matrix plot of AFS and penetration method for Pearson's correlation

6.3.2.2. Firmness values of treatment and control samples measured by AFS

To observe the change in firmness in the same population during the storage time of 7 days, 30 fruit from the treatment and control samples were kept and measured repeatedly using the AFS method only. Fig. 6.6 showed that the firmness decreased gradually from 54.80 ± 1.25 (FI) to 50.63 ± 1.17 (FI) and 48.51 ± 1.56 (FI) in the first 2 days; then profoundly dropped to 30.73 ± 1.55 (FI) and 27.30 ± 1.51 (FI) on day 4, then sharply dropped to 15.13 ± 0.43 (FI) and 15.34 ± 0.54 (FI) on day 6, and slightly decreased to 13.58 ± 0.50 (FI) and 13.40 ± 0.54 (FI) on day 7 for treatment and control sample respectively.

Firmness values of both sample sets were changing with the same trend without a significant difference. It was concluded that CST had no effect on firmness retention for avocados which is opposite with the conclusion of Chen et al. (2017) that CST delayed the ripening process including pulp softening.

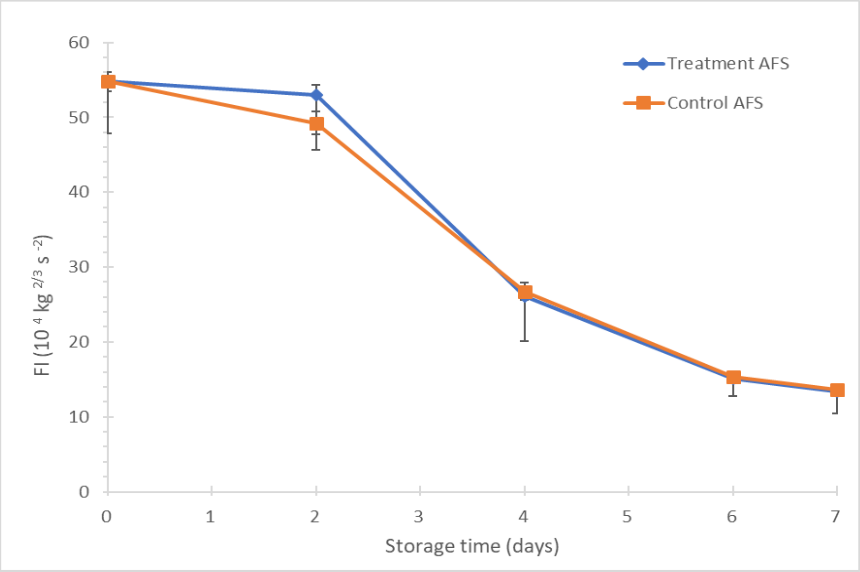


Figure 6.6 The change of fruit firmness performed by AFS of treatment and control samples during the storage time of 7 days at 20 °C and 85-90% RH. Fruit firmness was expressed by FI (10^4 kg $^{2/3}$ s^{-2})

6.3.3. Skin colour

During the ripening of 'Hass' avocados, skin colour changed from green to black described by a decrease of lightness (L^*), chroma (C^*) and hue (h^*). The study of Chen et al. (2017) showed downward trend of peel colour parameters during the storage time of 10 days. Fig 6.7 showed that L^*, C^* and h^* decreased during the storage time with the L^*-values of 21.49 ± 0.33 and 22.20 ± 0.28; C^*-values of 2.79 ± 0.16 and 3.48 ± 0.2; h^*-values of 41.18 ± 1.27 and 44.74 ± 1.09 for treatment sample and control sample respectively on day 7.

The statistical analysis found that there was no significant difference between the two sample sets in terms of skin colour parameters (*p-value* > 0.05), which agreed with the result of fruit firmness suggesting no effect of CST. This was opposite to the results of the work of Chen et al., (2017) as the study reported that CST retained the green and light colour for the treatment sample compared to the control sample.

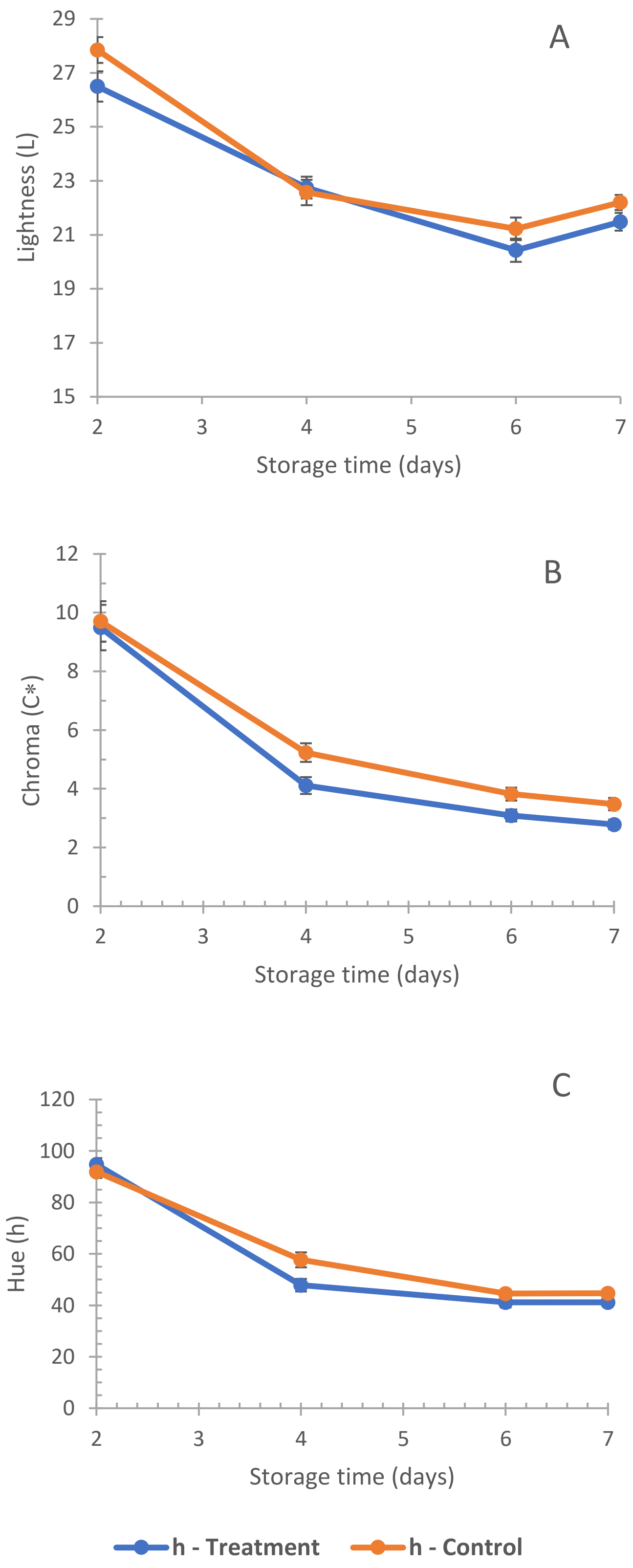


Figure 6.7 The change of skin colour parameters (L*, C*, h*) during the storage time of 7 days at 20 °C and 85-90% RH for treatment and control samples (n = 15)

6.3.4. Respiration rate

Figure 6.8 showed that the respiration rate oscillated from day 1 to day 5 before rapidly increasing to the highest values of 1705.17 ± 90.41 nmol kg^{-1} s^{-1} and 1652.01 ± 59.09 nmol kg^{-1} s^{-1} for treatment and control samples respectively. However, the respiration rate of treatment and control samples observed in the experiment were not statistically different from each other (*p-value* > 0.05). CST did not reduce the magnitude of the respiration rate peaks. This result conflicts with that of Chen et al. (2017) who showed that CST delayed the peak of respiration rate from day 6 to day 8 of storage

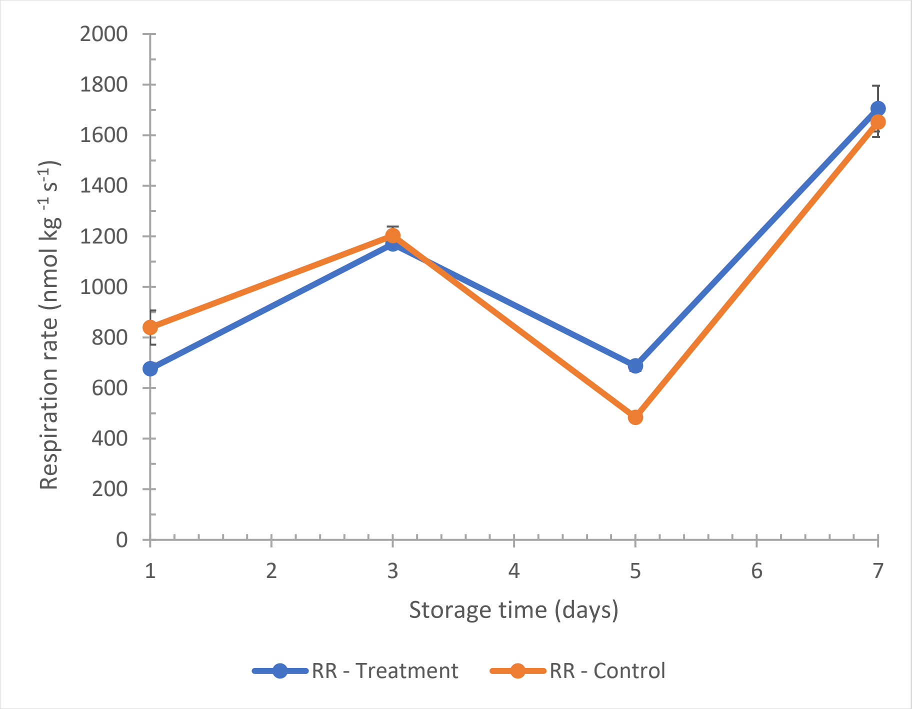


Figure 6.8 Respiration rate (RR) of treatment and control samples after 7 days at 20 °C and 85-90 % RH with an accumulation time of 15 minutes (n = 10)

6.3.5. Ethylene production rate

Figure 6.9 showed that ethylene production rate of control sample increased gradually from day 2 to day 4 and then surged from day 4 to day 5 peaking at 295.00 ±

34.53 pmol kg^{-1} s^{-1} before decreasing to 134.95 ± 15.01 pmol kg^{-1} s^{-1} on day 7. On the other hand, the ethylene production rate of the treatment sample gradually increased to the day 4 peaking at 179.56 ± 24.39 pmol kg^{-1} s^{-1} then dropped to 97.56 ± 11.94 pmol kg^{-1} s^{-1} and maintained on day 7. The ethylene production rate of the control sample peaked one day later than that of the treatment sample, but the magnitude of the peak of the control sample was significantly higher than compared to the treatment sample (*p-value* = 0.015).

The result of this experiment agreed with the result of the previous experiment showing that CST suppressed the ethylene production rate (section 5.3.4). However, this result was opposite to the result from Chen et al. (2017) which showed that CST delayed the ethylene production rate peak of treatment sample from day 6 to day 8, but not significantly reduce the magnitude of the ethylene production rate peak. However, the result of respiration rate did not show the effects of ethylene suppression caused by CST from this experiment.

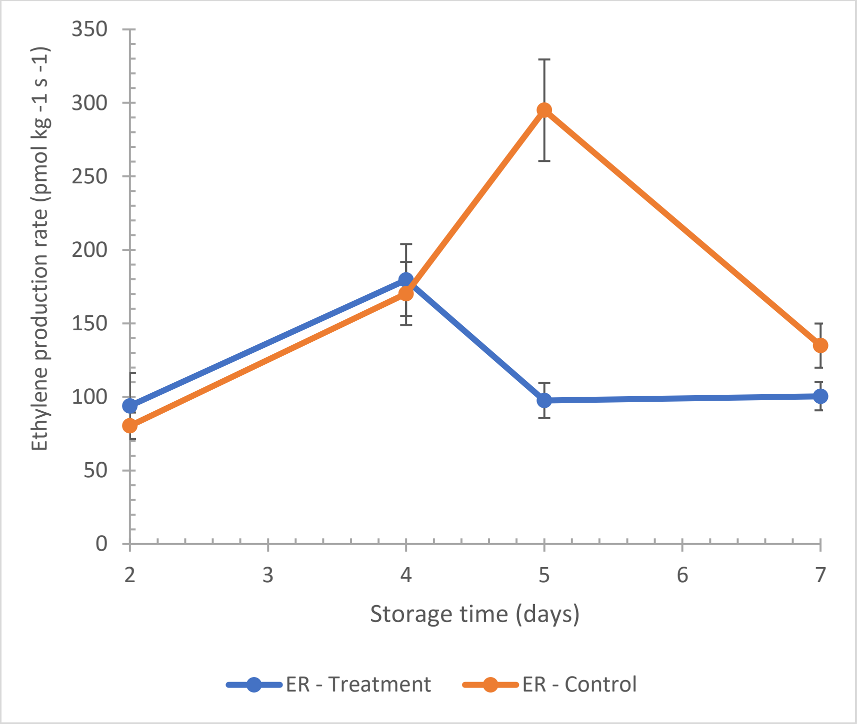


Figure 6.9 Ethylene production rate (ER) of the treatment and control sample after 7 days at 20 °C and 85-90 % RH with an accumulation time of 15 minutes (n=10)

6.3.6. Chilling injury

Symptoms of chilling injury including internal and external chilling injury of avocado fruit did not appear in both sample sets after the storage time of 7 days, stored at 20 °C an 85-90% RH. The result agreed with the study of Chen et al (2017) and the previous experiment (section 5.3.6).

On the other hand, fruit rot including stem-end and body rots appeared after 7 days of storage, as shown in Table 6.3. The statistical analysis using Chi-Square Test reported that there was no statistical difference between the number of rotten fruit in each level of severity of both sample sets (*p-value* > 0.05). The per cent of rotten fruit in the experiment was much lower than that of a study of Hopkirk et al. (1994) which reported the rot incidence of 67 % for avocados stored and ripened at 20 °C. Fungi (*Botryosphaeria spp.*

and *Colletotrichum spp*.) were the major organism causing rot in avocados (Hopkirk et al., 1994).

Table 6.3 The incidence and the severity of rots appearing on the skin and in the flesh of avocados after 7 days at 20 °C and 85-90% RH evaluated according to "The International Avocado Quality Manual" (White and Woolf, 2005)

	Severe Rot (50%)	Medium Rot (25%)	Less Rot (10%)	The total number of rotten fruit	Average point of rot severity
Severity level	3	2	1		
Treatment	14	7	10	31	2.13
Control	13	6	9	28	2.14

6.4. Conclusion

This experiment aimed to replicate the previous experiment with some improvements to reduce the fruit variability and observe the change of physiological properties of avocados during the ripening for 7 days, through the measurements of non-destructive and destructive firmness, skin colour parameters, respiration rate and ethylene production rate. The aim of the experiment to identify the suitable CST for avocados and clarify the experimental results of the work of Chen et al., (2017) which claimed that CST delayed the ripening process of avocados. The results on firmness, skin colour, respiration rate and ethylene production rate suggest that despite the positive results from a previous study, CST did not have significant effects on the ripening of avocados.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS, AND FUTURE WORK

7. General discussion, conclusions, and future work

7.1. Introduction

Avocado ripens rapidly taking 5 – 7 days at 20 °C after harvest (Seymour and Tucker, 1993). CST is believed to extend the storage life of crops such as cucumber (Chen et al., 2015), banana (Zhang et al., 2010) and sweet cherry (Alique et al., 2005, Gu et al., 2020). Chen et al. (2017) reported that immersion of ‘Hass’ avocado in ice water at 0 °C for 30 mins effectively delayed ripening-associated processes such as reducing respiration rate and ethylene production rate and cell-wall enzyme activities.

The objective of this thesis was to provide better insight into the effects of CST on delaying avocado ripening by observing the influence of CST before storage on post-storage firmness, skin colour, respiration rate and ethylene production rate. The experiments replicated the experimental work of Chen et al. (2017), followed by conducting a storage trial to study the potential effects of CST on post-storage quality.

Experiment 1 was expected to identify the suitable CST in a full matrix of three temperatures (0, 2 and 4 °C) and six durations (15, 30, 45, 60, 90 and 120 minutes) at a 2-day storage interval (Chapter 3). The effects of treatment temperature and duration were not observed on firmness retention in this experiment, however, there was a consistent trend that colder temperatures and shorter treatments resulted in better firmness outcomes although these are not statistically significant, possibly due to the large fruit variability. Experiment 2 was to replicate the experiment 1 with increased sample size to three times to reduce the influence of fruit to fruit variability on the data analysis (Chapter 4). The effects of treatment temperatures and durations on firmness retention were not seen for this experiment. The large fruit variability remained the big issue for this experiment.

Experiment 3 was designed to further increasing the sample size to 10 times compared to the previous experiment to minimise the large fruit variability using a selected single treatment (0 °C and 60 minutes) (Chapter 5). The effect of CST treatment on the ripening was not statistically significant, however, a considerable reduction of ethylene production rate was observed. Experiment 4 was designed to provide more information about the effects of CST on the overall qualities of avocado at 1 and 2-day storage intervals by using 2 different methods for firmness measurements (Chapter 6). The effect of CST on the ripening such as firmness, skin colour, respiration rate was insignificant, however, ethylene production rate significantly reduced with treated fruit. In this work, all post CST storage conditions remained at 20 °C in air.

7.2. The effects of CST on avocado physiology and quality

7.2.1. Firmness and cell-wall enzymes

Firmness is the most popular determinant when avocado fruit is ripe enough to be consumed. Fruit firmness decreases during 6 - 7 days at the storage temperature of 20 °C (Chapter 3 – Chapter 6). The decrease in the fruit firmness is related to the hydrolysis of the peptic compounds present in the cell wall, resulting in the loss of water retention capacity in the membrane of the plant cell (Defilippi et al., 2018; Goulao and Oliveira, 2008; Sierra et al., 2019). The physiological changes during ripening associated with softening involve the modification of the cell wall that requires the activity of hydrolytic enzymes (polygalacturonase, pectin-methylesterases and CMCase) (Awad and Young, 1979; Wakabayashi et al., 2003; Ali et al., 2004).

CST has previously been observed to have variable effects on cell-wall enzymes in various fruits. Chen et al. (2017) reported that CST suppressed the activities of polygalacturonase, pectin-methylesterases in avocados resulting in delayed softening. Zhang et al. (2010) also showed that CST depressed the activities of of hydrolytic enzymes during the ripening of banana fruit.

The puncture test using a penetrometer is one of the most popular methods to assess the firmness of avocados, besides using a firmometer and hand squeezing (White et al., 1999). The puncture test is used in many previous studies of fruit softening (e.g. Harker et al., 2002; Volz et al., 2003; Ma et al., 2011). The puncture probe geometry heavily influences the magnitude of firmness values obtained. In the Experiment 1 and 2, an effort was made to use the same equipment and measurement method (TA.XT.Plus analyser equipped with a P/2N needle probe) as that used by Chen et al. (2017). Chen et al. (2017) subsequently reported firmness values of 112 N (at harvest) and 35 – 68 N (after 6 days) for CST treated samples stored at 20 °C. These values were significantly higher than firmness values of 38 N (at harvest) and 1 – 10 N (after 6 days) obtained in the current study (section 3.3.2).

Chen et al. (2017) showed that CST at treatment durations of 15 and 30 minutes regardless of treatment temperature (0, 2 and 4 °C) effectively delayed the ripening of avocado fruit, and the most effective CST was at 0 °C and 30 minutes. However, the result of Experiments 1 and 2 led to a conclusion that firmness was not statistically influenced by CST temperatures (0, 2 and 4 °C) or time of exposure (15 – 120 minutes). It is possible that the high fruit variability observed can mask the potential effects of the CST (Chapter 3 and 4).

In this work, samples size of 15 fruit was used, which resulted in an SD from 24 to 62 %, in comparison the work of Chen et al. (2017) achieved low variation (SD < 10 %) with the same sample size (n = 15) using fruit sourced from a local market. However, there was a possible trend that colder temperatures and shorter treatments resulted in higher firmness outcomes after storage although the average values were not statistically different. Chen et al. (2017) showed the same trend that shorter treatments (15 and 30 minutes) and lower temperatures (0 °C) resulted in statistically higher firmness values after 6 days at 20 °C.

Experiment 3 aimed to reduce the influence of fruit to fruit variability within the sample sets, the resulting firmness values still had high variation (standard deviation from 187 to 198 %) with a non-normal distribution. Over 80 % of treatment and control sample had firmness values in the range of 0.23 – 6.73 N without showing any significant CST effects on firmness retention (section 5.3.2).

Experiment 4 aimed to investigate if the firmness measurement being used influenced the ability to separate treatment effects. However, after Experiment 4 the conclusion was again that CST did not affect fruit softening (Chapter 6). Fruit firmness observed by two different measurement methods (puncture test and acoustic test) showed the same trend of firmness change between treated and control samples during storage, and no significant difference in firmness retention was observed. From all experiments, it can only be concluded that CST had no effect on firmness retention for 'Hass' avocados, opposing the conclusion of Chen et al. (2017) claiming CST at 0 °C and 30 minutes delayed the ripening process including pulp softening.

7.2.2. Skin colour parameters

When 'Hass' avocado ripens, the lightness (L^*), chroma (C^*) and hue angle (h^*) values decrease corresponding to a change of skin colour from green to purple or black. The change of skin colour of avocado fruit is due to the degradation of chlorophyll and the synthesis of pigments e.g. cyanidin 3-O-glucoside (Cox et al., 2004; Sierra et al., 2019).

Chen et al. (2017) showed that CST delayed the peel colour change of avocados. However, CST could cause the opposite effect for other fruits. Barry and van Wyk (2006) reported that CST had effects on the colour pigments in the rinds of mandarins treated by two consecutive CSTs at 2 °C for 30 minutes, followed by 4 °C for 6 h; consequently, reduced the level of chlorophyll and increased the level of carotenoid. Similarly, green mature tomatoes treated with CST at 0 °C for 3 h and stored at 20 °C or 10 °C accelerated development of pigments (carotenoids and lycopene) and the degradation of chlorophyll (Zhang et al., 2018b).

In contrast, the results of experiment 3 and 4 in this study (section 5.3.3 & 6.3.3) did not find any effects of delaying in skin colour development, as caused by CST, in which the L-values of 24.48 and 25.61; C^*-values of 14.18 and 12.91; h^*-values of 92.82 and 94.14 were obtained for treated fruit and control respectively (section 5.3.3). Similarly, Barry and Van Wyk (2006) reported that two consecutive CSTs at 2 °C for 30 min and then 4 °C for 6 h did not produce any effect in ‘Navel’ orange rinds although these CSTs had effects on mandarin rinds.

A high variation in skin colour of avocado has been reported in many previous studies, suggesting that more mature fruit has darker skin compared to less mature one (Vuthapanich, 1995; Hofman and Jobin-Decor, 1999). The variation of the skin colour parameters in the current study were observed with standard deviation of L^*-values, C^*-values, and h^*-values (12 %, 32 %, 12.5 %, respectively) when avocados were stored at 20 °C after storage of 6 days. The variation remained visually observable between fruit in the populations (section 5.3.3). Osuna-García et al. (2010) showed that the degree of dark skin did not correlate to firmness decrease of ‘Hass’ avocados. However, DMC increases with the harvest date, as does the degree of the dark skin of 'Hass' avocados. There is potential for the fruit to be dark but still unripe (Cox et al., 2004).

7.2.3. Respiration rate and ethylene production rate

As a climacteric fruit, avocado fruit show a peak of respiration rate and ethylene production rate during its ripening. The significant increase of ethylene level during ripening is induced by enzymes ACS and ACO (Nath et al., 2014). Chen et al. (2017) showed that CST suppressed the activities of the enzymes ACO and ACS resulting in delaying the ethylene rate peak from day 6 to day 8; however, CST did not significantly change the magnitude of the respiration rate and ethylene production rate peaks, with ethylene production rate of 41 and 38 mL kg^{-1} h^{-1} and respiration rate of 79 and 81 mg kg^{-1} h^{-1} for control and treated fruit respectively.

By contrast, in the current study, CST did not affect the respiration rate in experiments 3 and 4 (sections 5.3.4 and 6.3.4); however, ethylene production rates were affected by CST application. The ethylene production rates of treatment and control samples were similar from day 1 to day 4 (approx. 80 and 90 pmol kg^{-1} s^{-1} respectively), but were markedly different from day 4 to day 5 (179.56 ± 24.39 and 295.00 ± 34.53 pmol kg^{-1} s^{-1} respectively). Subsequently, no significant difference was observed on day 7 (97.56 ± 11.94 and 134.95 ± 15.01 pmol kg^{-1} s^{-1} for treatment and control samples respectively; section 6.3.5). This result suggested some potential CST suppression of ethylene production of avocados, however, the resulting lower ethylene concentration in fruit was not observed to make a difference in the rate of fruit quality change (softening and colour).

Unlike the experiment with the storage temperature of 20 °C, other studies observed the change of respiration rate and ethylene production rate at low temperature. Zamorano et al. (1994) reported that when storage temperature decreased from 20 °C to 7 °C and 3 °C, respiration rate decreased from 263 mg CO_2 kg^{-1} h^{-1} to 35 mg CO_2 kg^{-1} h^{-1} and 16 mg CO_2 kg^{-1} h^{-1}, respectively; ethylene production rate was significantly low at 1.2 µL kg^{-1} h^{-1} at 7 and 3 °C. Longer storage time at low temperature results in a shorter time for respiration rate and ethylene production rate to peak and lowers the peak's values when avocado fruit is transferred to the ripening temperature at 20 °C. ‘Hass’ avocado stored at 0 or 5 °C for 4 – 6 weeks showed a climacteric peak after being transferred to storage at 20 °C for 2 days (Eaks, 1983).

7.2.4. The variable effects of CST on different fruits

CST has been found to have variable effects on fruit depending on the fruit genotype, treatment temperature, exposure time and storage temperature. CST at 0 °C for 1 h decreased respiration rate, ethylene production rate and the activities of polygalacturonase, pectin-methylesterases and CMCase of banana fruit (Zhang et al., 2010). CST at 0 °C for 4 h reduced chilling injury of mango fruit by enhancing the activities of catalase, ascorbate peroxidase, glutathione, and ascorbic acid which improved the chilling tolerance (Zhao et al., 2006). CST using a hydro-cooling method at 1 °C for 6 minutes delayed the senescence and prolonged the storage life of cherry fruit (maintained the firmness and skin colour), but not RR during the cold storage at 0 °C for 10 days (Alique et al., 2005).

On the other hand, CST has also been reported to have little, and in some cases, adverse effects on post-storage fruit quality. Zhang et al. (2018b) reported that green mature tomato treated with CST at 0 °C for 3 h and stored at 20 °C or 10 °C had accelerated development of senescence including the synthesis of carotenoids and lycopene and the degradation of chlorophyll, additionally, its storage life was shortened by 2 - 4 days. Overall, CST could not consistently affect the post-storage quality of fruit.

7.2.5. Cold stress response of fruits

The effects of CST on fruits may be related to the domain proteins (*CSDPs*) which are responsible for cold stress response of plants and fruits. Gu et al. (2020) reported that CST (0 °C, 10 mins) reduced the expression of *CSDP2*, but induced the expression of *CSDP3* while not having any impacts on the expression of *CSDP1* and *CSDP4* for sweet cherry fruit stored at 0 ± 1 °C for 40 days. The study reported that CST reduced weight loss, improved fruit quality including firmness and skin colour for sweet cheery.

The study of Gu et al. (2020) broadened the knowledge of domain proteins which could be responsible for cold tolerance and this might help to explain the effects of treatment durations on product quality. Nevertheless, the role of *CSDPs* which are responsible for cold stress response on avocado fruit during the CSTs or during the chill storage has not been clarified.

7.3. The variability of avocado fruit and method to reduce the variability

High variability of avocado has previously been reported in several studies. Hofman and Jobin-Decor (1999) reported that the fruit variability could be seen by the remarked difference in DMC of avocado fruit from tree to tree, which was attributed to the influence of direct sunlight (Woolf et al., 1999, Woolf et al., 2000), and the effect of rootstocks which can affect plant nutrition, particularly mineral concentrations (Lahav et al., 2013, Marques et al., 2006).

Avocado fruit only ripens when it is picked off the tree, consequently, if physiologically mature avocado fruit are left on the trees for later harvest, they gain a higher DMC and oil content over time. Avocado with higher DMC and less water content ripen faster (Bower et al., 2007). Villa-Rodríguez et al. (2011) reported significant differences in firmness, DMC and oil content amongst avocados with different maturity stages. This may be a reason for fruit variability. Firmness is also affected by harvest time and the fruit DMC at that time. Avocados harvested later had higher DMC, less weight loss and ripened more rapidly (Osuna-García et al., 2010). However, Hernández et al. (2016) argued that DMC does not correlate on a fruit-to-fruit basis with time to reach ready-to-eat firmness due to the highly heterogeneous postharvest ripening.

The large biological variability of fruit induced by pre-harvest conditions contributes to undesirable variations in postharvest quality and storability. Pre-harvest factors such as environment temperature, humidity, irrigation and soil nutrient can influence the behaviour of avocado ripening (Rivera et al., 2017). The fruit samples from the first three experiments were sourced from the same orchard in Bay of Plenty (New Zealand), and from the last experiment, the fruit were harvested at a different orchard, graded at the packhouse to reduce the inherent variability and achieve more consistent at-harvest quality.

Fruit size can be a contributor to the variation of fruit quality which may increase the variability in the experimental data. Hofman and Jobin-Decor (1999) reported that smaller fruit harvested at the same time had a higher DMC, was more mature and ripened more quickly than the medium and larger fruit. To manage this variation, at-harvest sorting and classification based on maturity are necessary, while increased sample sizes allow improved descriptions of populations.

In the Experiments 1 and 2, CSTs observed had no effects on fruit quality. The quality variability in the population possibly attributed to the inability to detect differences. In Experiment 3, attempts were made to remove the effects of the fruit to fruit variability to observe the reported effects of CST by Chen et al., (2017). A sample size of 150 (10 times larger than that of the experiments 1 and 2) and controlled fruit weight range (250 – 350 g) were used with the expectation of being able to describe smaller differences between treatments. However, the firmness data sets on day 6 had a high and unhelpful variation (CV of 190 %). Later in Experiment 4, fruit samples sourced and sorted at harvest in the packhouse, showed a smaller variation (CV of 20 %) on day 6, when using the same puncture test as Experiment 3. It may be concluded that the better way to reduce fruit variability for avocado's study is grading and classification at harvest with adequate number of fruit which is over the number of fruit.

Avocado variability causes many problems in the industry, for example, logistics management and the difficulty in ripening date prediction (Bower et al., 2007). There are several methods to reduce the fruit variability in industry. Huber et al. (2002) reported that avocados treated with ethylene (100 ppm) at 13 °C for 24 h ripened more uniformly with less variability at the full-ripe stage and had a lower incidence of rots. Many recently developed technologies such as near-infrared spectroscopy (NIRS), ultrasonic system, ultrasound imaging, hyperspectral imaging, magnetic resonance imaging, and fluorescence imaging were reported to be able to use for avocado fruit maturity determination (Magwaza and Tesfay, 2015).

Specifically, NIRS was reported as a potential tool for estimating the maturity of ‘Hass’ avocado (Clark et al., 2003; Wedding et al., 2011, Zhang et al., 2018a). On-line sorting in consignments of avocado by using NIRS based on time to ripen could be a possible solution to achieve less ripening variation (Blakey et al., 2009). The reduction of avocado variability within harvested batches improves the shelf life and provides better storage practices to suit the fruit quality and to remove fruit at the extremities of the population (such as low DMC) or to classify fruit with high DMC for oil processing (Clark et al., 2007).

7.4. Rots and chilling injury

In all 4 experiments in this current study, chilling injury was not observed. These results are in agreement with the results of Chen et al. (2017). It is possible that the treatment time was not long enough to cause chilling injury. On the other hand, rots appeared when the fruit ripened at 20 °C after a storage time of 6 days. Stem-end and body rots were observed in both treatment and control samples without any significant difference in occurrence.

According to Woolf et al. (2003), chilling injury appears in avocados stored at below 3 °C with the optimal storage temperature of 4 – 6 °C. Mazhar et al. (2018) recommended that avocados stored under 5 °C reduce the incidence and severity of rots. Low-temperature conditioning before cold storage could reduce the development of chilling injury and rots of avocado fruit. Low-temperature conditioning at 6 or 8 °C for 3 – 5 days reduced the incidence and severity of chilling injury when the fruit were stored at 0 °C for 4 weeks (Woolf et al., 2003).

7.5. Recommendations and future work

For any postharvest study, fresh fruit tends to have inherent high variability, especially for those crops in which maturity is difficult to individually ascertain (e.g. avocado). In these circumstances, it is recommended that the sample size must be large enough so that the variability does not cause variation in measured data to a point where it masks treatment effects. One mean to reduce variability is for the initial fruit samples to be pre-sorted (e.g on size or maturity) using non-destructive techniques such as NIRS, so that at-harvest fruit quality is likely to be more homogenous in term of the maturity.

Moreover, during the CST, the water temperature increases by 0.5 – 1 °C. It is important to stabilise the water temperature during the CST because it directly influences the heat transfer speed between fruit and cold water. From the current study, it was unavoidable that water temperature fluctuated after the fruit had immersed underwater during the first 30 minutes. The action of regularly adding and mixing the ice applied in the current experiment might be effective to control the water temperature in the barrels, but also made the fruit circulate leading to the possibility of physical damage. It is recommended that an automatic heat-controlling water bath should be applied for the CST.

Additionally, the treatment sample and control sample should be kept separately in the different storage room to reduce the cross-contamination of gases (such as CO_2 and ethylene). The accumulation of ethylene in the storage environment may accelerate the ripening of avocado, while CO_2 has the opposite effect which inhibits the ripening.

The study of Chen et al. (2017) did not explain the reasons why CSTs at higher treatment durations (45, 60, 90, 120 mins) did not show effects on avocados. The effects of treatment duration were not studied in other experimental work of CSTs. Gu et al. (2020) proposed a new theory about domain proteins being responsible for cold tolerance which may explain the effects of CST on physiological qualities. Therefore, future study should investigate the effects of treatment durations and treatment temperatures on domain proteins to explain the mechanism of CST. These domain proteins may prevent avocado fruit from chilling injury. Zhao et al. (2006) reported that CST reduced chilling injury for cold-stored mango fruit, and increased the activities of catalase, ascorbate peroxidase, glutathione reductase, superoxide dismutase which involved in the protection of mango fruit from cold stress.

On the other hand, instead of using firmness values to determine the effects of CST that the firmness values have a large variation in data sets, the ripening time which is the number of days from harvest time to the point which fruit are ready-to-eat ripe with firmness of 4.4 – 6.7 N could be useful. The method described in the study of Blakey et al., (2009). The CST for graded fruit might show less fruit variability in the results as demonstrated in Experiment 4 (section 6.3.2).

7.6. Final Conclusion

Chen et al. (2017) reported that CST had positive effects on avocado fruit quality, such as delaying pulp softening and skin discolouration, reducing respiration rate and ethylene production rate without showing any symptoms of chilling injury. As a result, the method is potential for extending the storage life of avocados with a relatively low cost and low-sophisticated technology.

The objective of this thesis was to provide further insight into the effectiveness of CST application on delaying the ripening of avocado by replicating the experimental work of Chen et al., (2017). The work aimed to identify the optimal CST conditions and validate the findings of Chen et al., (2017). Unlike the experiment results of Chen et al. (2017), in the current study, CST was not found to have any pronounced effects on fruit quality in Experiment 1 and 2 although the treatments, measurement methods and the sample size replicated the experiment of Chen et al. (2017). High fruit variability was considered a major reason that restricted the ability to replicate the results. To reduce the variation, Experiments 3 and 4 used considerably more fruit than that used by Chen et al., (2017), still this work found that CST did not have any effects for avocados.

A replication (or a reproducibility) of previous experimental work is essential and important in science so that the other experimental work is evaluated and validated. Replication helps to verify and disconfirm false experimental findings in academical literature. Due to the small sample size of some experiments, replication would expand and generalize the concepts or theories of the previous findings and should be performed by various labs (Lamal, 1990, Muma, 1993, Valentine et al., 2011, Simons, 2014). However, a large number of experimental works could not be replicated, which is the so-called “replication crisis”. Measurement errors and/or other uncontrolled variation such as fruit variability add noises into the measurement results leading to unreliable data sets, especially when the sample size is small. If the effect of the treatment is little, the small sample size with a noisy measurement method may make the observed effects larger than the original. As a result, future work might be destined to fail with a larger sample size (Loken and Gelman, 2017).

CHAPTER 8

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8. References

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