EFFECT OF COLD STORAGE TEMPERATURE AND SILICON DIPS ON PHYSICO-CHEMICAL PROPERTIES OF A NEW MANDARIN SELECTION "M37" FRUIT

by

RHULANI BEAUTY SHIBAMBU

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SUPERVISOR: DR N MATHABA (ARC-ITSC)

CO-SUPERVISOR: PROF TP MAFEO (UL)

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TABLE OF CONTENT

DECLARATION

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

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Student: Ms RB Shibambu Date

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Supervisor: Dr N Mathaba Date

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Co-supervisor: Prof TP Mafeo Date

DEDICATION

To my daughter Akelo

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LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Newly developed mandarin selection “M37” has the potential to become a future export soft citrus cultivar. However, the selection is highly susceptible to chilling injury, which normally reduces post-storage quality and marketability of citrus fruit. Silicon (Si), applied at post-harvest as potassium silicate (K_2SiO_3), has potential to mitigate against chilling injury. So far, the effect of Si on “M37” mandarin chilling susceptibility is unknown. Therefore, the aim of the study was to investigate the effect of cold storage temperature and postharvest potassium silicate dips on the chilling susceptibility and physico-chemical properties of new mandarin selection “M37” fruit. The experiment was carried out in a factorial, arranged in a completely randomised design (CRD) with three replicates. Treatment factors were: 2 x cold storage temperature (-0.6±1 and 4.5 ±1°C) and 4 x potassium silicate concentration (0, 50, 100 and 150 mL L^{-1} K_2SiO_3). Fruit were dipped in different potassium silicate solutions (0, 50, 100 and 150 mL L^{-1}) for 30 minutes, air dried, waxed; and thereafter, stored for 28 days at -0.6 and 4.5°C with 85-90% relative humidity. After withdrawal from cold storage, fruit were held at ambient temperature (±23°C) for 7 days (shelf-life); during shelf-life fruit were evaluated for chilling injury, physical properties (weight loss and firmness loss) and biochemical properties (electrolyte leakage, total soluble acids (TSS), titratable acids (TA) and TSS: TA ratio). The results showed that fruit were highly susceptible to chilling injury after storage at -0.6°C when compared with 4.5°C. However, treating fruit with postharvest potassium silicate dips improved their chilling susceptibility, especially with 50 and 100 mL L^{-1} K_2SiO_3 concentrations. Electrolyte leakage was lower for fruit treated with K_2SiO_3 compared to the control across all the storage temperatures. Although, “M37” fruit stored at 4.5°C showed higher weight loss, firmness loss, TSS and TSS: TA ratio when compared with -0.6°C storage. Fruit firmness increased with the increase in potassium silicate concentrations during storage at -0.6 and 4.5°C; with the highest firmness loss occurring on fruit treated with 150 mL L^{-1} than control. Similarly, TSS increased concomitant with K_2SiO_3 concentration. Although, TA decreased with increasing K_2SiO_3 concentrations for fruit stored at 4.5°C; resulting in higher TSS: TA ratio. In conclusion, postharvest silicon dips effectively improved the storability of “M37” mandarin fruit, preserved quality and extended the cold storage period.

Keywords: Biochemical properties; Chilling injury; Firmness loss; Potassium silicate dips; Weight loss

Figure 1: Typical characteristics (internal and external) of “M37” mandarin selection fruit.

CHAPTER 1
GENERAL INTRODUCTION

1.1 Background

The citrus industry is the largest fruit industry in South Africa, and ranks third within the horticultural sector in terms of gross value (Citrus Growers Association, 2014). During the 2013/2014 production season, the industry contributed about R9.69 billion to the total gross value of the South African agricultural production and the horticultural sector, respectively (Department of Agriculture, Fisheries and Forestry, 2015). In terms of global citrus export, the industry is positioned at third place, and only surpassed by countries such as Spain and Turkey (Citrus Growers Association, 2014). Majority of citrus exports are directed to European countries. However, recently there has been increasing interest in high paying export markets such as Japan and United States of America (Mathaba *et al*., 2008; Mathaba and Bertling; 2013). These new export markets have shown high preference for mandarins, largely due to the ease with which they can be eaten when compared with other types of citrus that are more difficult to peel (Obenland *et al*., 2011).

Mandarins are a diverse group of thin-skinned, easy-peeling fruit, which include popular types such as Satsumas, Clementine and Tangerines (Obenland *et al*., 2011). They are well known for their health benefits and consumption is beneficial to human health by providing nutrients, reducing incidence of cardiovascular diseases and improving blood circulation (Ladaniya, 2008). Despite the growing interest for mandarin fruits, South Africa still export less mandarins when compared with oranges and lemons and this has been largely attributed to the lack of new soft citrus cultivars (Jourbert, 2014). Hence, over the past few years, the SA citrus industry, through the Citrus Research International (CRI), Agricultural Research Council and other partners, has actively bred and developed new early mandarin selections aimed at diversifying the available cultivars and increasing mandarin export volumes (Dodd *et al*., 2010).

As with the other citrus types from South Africa, fruits of newly developed mandarin selections or cultivars have to be cold sterilised at sub-zero temperatures, before

being considered for approval by some importing countries (Mmako *et al*., 2015). Fruits should be cold sterilised at -0.6°C for 22-24 successive days when shipped to China, Korea, Thailand and the United States of America (European and Mediterranean Plant Protection Organization, 2007; Mathaba *et al*., 2008; Hordijk; Mathaba and Bertling, 2013) in order to kill eggs and larvae of invader (*Bactrocera dorsalis*) and Mediterranean (*Ceratitis capitata*) fruit flies, prevalent in various citrus production areas of South Africa (Department of Agriculture, Fisheries and Forestry, 2012; Perishable Products Export Board, 2013). However, since citrus fruits originate from subtropical regions, they often develop chilling injury (CI) under cold storage (Ladaniya, 2008; Hordijk, 2013).

Chilling injury is a rind physiological disorder that manifests as small pitting areas and progressively spread out over the fruit surface as collapsed brown cluster (Hordijk, 2013). The severity of chilling injury symptoms increase with storage duration (Sala, 1998; El-Hilali *et al*., 2003) and worsened when fruits are moved to room temperature (Lafuente *et al*., 2005). Therefore, such limits shelf-life, reduces marketability and consumer acceptability of the damaged fruits; and thereby, result in large postharvest losses (González-Aguilar *et al*., 2000). The susceptibility of citrus fruits to chilling injury is the main limiting factor for long-term storage under low temperatures, although such storage serves as an obligatory quarantine treatment (Wang, 2006; Sala *et al*., 2005).

Several methods have been used to minimise chilling injury and extend shelf-life of citrus fruits and impressive responses have been reported. Some of the treatments used on mandarin fruits include; hot water dips (Schirra and D'hallewin, 1997; Gonzalez-Aquilar *et al*., 1997; Ghasamnezhad *et al*., 2008), and high or low temperature conditioning (Martinez-Tellez and Lafuente, 1997; Sala *et al*., 2002). However, some techniques are more effective in alleviating chilling injury in certain cultivars than others (Wang, 2010). Furthermore, the optimum treatment conditions of postharvest treatment vary with different fruit. Silicon (Si) dips is amongst the recently developed technique and has shown ability to mitigate the occurrence of chilling injury symptoms during long-term cold storage (Mditshwa *et al*., 2013; Nasar *et al*., 2013). Silicon applied at postharvest as potassium silicate dips has been reported to reduce chilling injury symptoms on 'Eureka' lemon fruit (Mditshwa *et al*.,

2013) and preserve fruit quality of ‘Hass’ avocado (Kaluwa *et al*., 2010). Although postharvest Si application has yielded positive results in other crops, its use has not been investigated on any mandarin cultivars.

1.2 Problem statement

Newly developed early mandarin selection “M37”, has shown good internal and external qualities. The fruit are easy to peel, contain few seeds and coupled with an excellent taste and have potential to become a major future export soft citrus cultivar. Therefore, increase income for the growers and citrus industry. Similar to other citrus cultivars, the selection needs to be cold sterilized during shipment to meet phytosanitary requirements of importing countries. However, the selection is highly sensitive to cold sterilisation temperatures, and therefore, develops chilling injury (Mmako *et al*., 2015). To date, no postharvest studies were conducted to address and mitigate the susceptibility of this selection to chilling injury, despite that; such disorder may limit its export potential. According to the Agricultural Product Standards Act, 1990 (Act No. 119 of 1990), pre- plus postharvest behaviour of any new or improved plant material should be known prior to their release or registration as cultivars or varieties.

1.3 Rationale

Silicon has the potential to mitigate against chilling injury. The benefits of Si applied pre-harvest have been well documented in plants (Ma and Yamaji, 2006). These include enhanced productivity and tolerance to various biotic and abiotic stresses, such as salinity (Liang *et al*., 2007; Epstein, 2009), drought (Crusciol *et al*., 2009), heat (Agarie *et al*., 1998) and frost (Epstein, 1999; Matichenkov *et al*., 1999). At postharvest, Si has also shown potential ability to maintain fruit quality, extend shelf-life and reduce rind physiological disorders such as chilling injury. Silicon is a plant nutrient which is non-toxic even when absorbed in excess (Currie and Perry, 2007). However, little is known about the effect of Si alleviating CI on new “M37” mandarin fruit. Therefore, the study would provide valuable information about response of the studied selection to postharvest cold storage temperatures and silicon treatment.

1.4 Aim and objectives

1.4.1 Aim

The aim of the study was investigate the effect of postharvest cold storage temperature and potassium silicate dips on the chilling susceptibility and physico-chemical properties of new mandarin selection “M37” fruit.

1.4.2 Objectives

a) To evaluate the effect of cold storage temperature and potassium silicate dips on the chilling susceptibility of new mandarin selection “M37” fruit.

a) To evaluate the effect of cold storage temperature and postharvest potassium silicate dips on new mandarin selection “M37” fruit physico-chemical properties.

1.4.3 Hypotheses

a) Cold storage temperature and potassium silicate dips had no effect on the chilling susceptibility of new mandarin selection “M37” fruit.

b) Cold storage temperature and potassium silicate dips had no effect on the physico- chemical properties of new mandarin selection “M37” fruit.

1.5 Format of the mini–dissertation

This mini–dissertation consists of six chapters. In the first chapter (Chapter 1), the study is justified with the research problem and objectives being identified. The second chapter (Chapter 2) reviews existing knowledge on postharvest factors affecting postharvest quality attributes of mandarin fruit. The third chapter (Chapter 3) describes the material and methods used in conducting this study. The fourth (Chapter 4) and fifth chapter (Chapter 5) include the results and discussion on the interactive effect of cold storage temperature and postharvest potassium silicate dips on chilling susceptibility and physico-chemical properties of “M37” mandarin selection fruit. Finally, chapter six (Chapter 6) summarized the findings of this study and also provided recommendations as well as directions for future research.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Over the past few years, the South African citrus industry has selected and bred new mandarin selections, with an aim of increasing competitiveness, cultivar diversity and access to new high paying markets (Dodd *et al*., 2010). Although preharvest aspects of these new mandarin fruit are known, their postharvest behaviour still needs to be investigated if they are to be registered as export cultivars. A considerable number of studies have been conducted on different mandarin cultivars all over the world. These studies were focused largely on understanding the quality attributes, rind physiological disorders, as well as the response to a number of cold storage temperatures and postharvest treatments (Sala, 1998; Lafuente *et al.,* 2005; Khumalo, 2006; Obenland *et al.,* 2011; Cronje *et al.,* 2011). However, it is widely known that the response of any fruit crop to cold storage temperatures and postharvest treatments is not homogeneous; and therefore varies with cultivars (Prasad, 1996; Tietel *et al.*, 2012). For this reason, new mandarin selections must be evaluated for postharvest quality and storability in order to ensure that high quality fruit are sent to export markets. Therefore, this review was aimed at discussing existing knowledge on postharvest factors affecting postharvest quality attributes of mandarin fruit.

2.2 Postharvest factors affecting citrus fruit quality

2.2.1 Storage temperature and relative humidity

Storage conditions have been shown to affect physical and chemical attributes of citrus fruit. Hence, it is important to control both storage temperature and relative humidity during postharvest handling (Paul, 1990, Henroid, 2005; Henroid, 2006). In fact, temperature management is the most important factor used to maintain fresh horticultural produce quality after harvest (Saltveit and Morris, 1990; Ghasemnezhad *et al*., 2008; Tietel *et al*., 2012; Lado *et al*., 2016). However, relatively few postharvest studies in citrus have examined the effect of relative humidity (RH) on fruit quality, particularly at low temperature and under prolonged storage conditions (Peretz *et al*., 2001; Porat *et al*., 2004; Henriod, 2006). Low temperatures reduce

fruit respiration rate, weight loss, firmness loss as well as general decay (Paul, 1999; El–Otmani and Ait–Oubaho, 2011; Maul *et al*., 2011; Tietel *et al*., 2012). Furthermore, low storage temperature also maintains fruit biochemical properties such as total soluble solids and organic acid (Echeverría and Ismail, 1987; Rab *et al*., 2015; Marcilla *et al*., 2016).

Optimum storage temperatures differ among produce and cultivars. For mandarins, the optimum storage temperature ranges between 5–8°C (Alfarez and Burns, 2005; Obenland *et al*., 2011). However, in order to comply with the quarantine regulations, all the citrus fruit exported by South Africa to high paying markets such as Japan and USA must be cold stored at -0.6°C for 21–24 days during shipment (Hordijk, 2013; Mathaba and Bertling, 2013; Siboza and Bertling; 2013). This cold treatment is highly effective for sterilising insect larvae of major pests in citrus such as false codling moth (*Cryptophlebia leucotreta*) and Mediterranean fruit fly (*Ceratitis capitates*) (White and Elson, 2004; European and Mediterranean Plant Protection Organization, 2007; Hordijk, 2013). Nonetheless, extended storage of horticultural fruit crops at cold sterilising temperature might result in chilling induced physiological rind disorders such as chilling injury (CI). In some instances, chilling injury might develop at temperatures as high as 12°C in highly susceptible cultivars (Schirra and Mulas, 1995; Martínez-Téllez and Lafuente, 1997). So far, the susceptibility to CI appears to be the main factor limiting the storability of horticultural fruit under low temperatures and the most noteworthy cause of postharvest losses in citrus fruit (González–Aguilar *et al*., 2000; Sala *et al*., 2005; Wang, 2006).

2.2.2 Effect of storage temperature on chilling injury

Chilling injury is a physiological disorder that manifests when subtropical and tropical fruit are stored at temperature ranging from 0–8°C (Lyons, 1973; Sevillano *et al*., 2009). Chilling injury is expressed as pitting, staining and necrotic areas in the peel that increase in number and size over time (Sanchez–Ballesta *et al*., 2003; Ghasemnezhad *et a*l., 2008; El–Otomani *et al*., 2011; Hordjk, 2013). According to Sanchez–Ballesta *et al*. (2003) and Ghasemnezhad *et al*. (2008), the physical and chemical symptom characteristics of CI vary amongst citrus cultivars. Chilling injury symptoms often become visible when fruit are removed from cold storage to warm temperatures (Lyons, 1973; Saltveit and Morris, 1990; Schirra and Cohen, 1999;

Lafuente *et al*., 2005). Although, rising evidence now suggest that symptoms could also become visible while fruit are still in cold storage. For instance, the report by Ghasemnezhad *et al*. (2008) on 'Satsuma' mandarin showed that fruit stored at 2°C for 8 weeks developed CI symptoms during cold storage which increased with storage time.

According to Saltveit and Morris (1990), the severity of chilling injury is a function of the period for which the fruit are stored under low temperature; in which, an increase in storage time, at a certain storage temperature also increases the incidence of chilling injury. Therefore, the longer the cold storage duration the greater the potential of chilling injury symptoms to develop (Van Rooyen, 2005). Sala (1998) found a drastic increase in chilling injury of 'Fortune' and 'Nova' mandarin stored for 8 weeks at 2.5°C as the time of storage was increased, and suggested that the postharvest handling period be kept to a minimum to avoid excessive storage periods. Several studies have shown that storage temperature and relative humidity as well as storage duration have significant effect on the development of chilling injury on fruit. Concellon *et al*. (2005) studied the effect of storage temperature on 'Money Maker No. 2' eggplant fruit after 6 and 13 days of storage. The author reported that 'Money Maker No. 2' eggplant fruit stored at 0°C were highly susceptible to chilling injury after 6 days compared to fruit stored for more than days 13 days at 10°C. Similarly, Khumalo (2006) found that the development of chilling injury on 'Nules Clementine' mandarin fruit was more pronounced after 12 weeks of storage at -0.5°C than at 7.5°C. Likewise, El-Hilali *et al*. (2003) demonstrated that chilling injury symptoms on 'Fortune' mandarin fruit were considerably higher after 4 weeks of storage at 4°C than at 8°C. In contrast, Siboza and Bertling (2013) reported that chilling injury on 'Eureka' lemon fruit stored for 28 days plus 7 days at 20°C and 85-90% RH was significantly higher at 4.5°C in comparison to -0.5 or 2°C. These findings are similar to those reported by Mditshwa (2012), who observed higher chilling injury for 'Eureka' lemon fruit after storage at 4.5°C for 28 days compared to -0.5 or 2°C. The author associated less chilling injury to higher antioxidant activity observed in fruit stored at 4.5°C. It is well known that a high antioxidant pool is generally associated with low CI incidence (Yang *et al*., 2011). These results suggested that antioxidants play a vital role in reducing chilling injury development during cold storage. Thus, enhancement in the activity of antioxidant enzymes has

been observed in fruit stored at low temperatures (Sala, 1998; Siboza and Bertling, 2013). In addition, the damage induced under such conditions was suggested to be due to the loss of efficient scavenging of cold-induced reactive oxygen species (ROS) such as hydrogen peroxides and hydroxyl radicals (Sala, 1998; Sala and Lafuente, 1999; Ghasemnezhad *et al.,* 2008; Lado *et al*., 2016). Therefore, sustaining high antioxidant levels of treated fruit during cold storage periods may also be important for preserving chilling tolerance.

The physiology of chilling injury

Cell membranes

The cell membrane is considered the primary site at which chilling injury develops (Campos *et al*., 2003) as it consist of lipids, which are capable of undergoing liquid to gel phase transitions (Lyons, 1973; Raison and Orr, 1990; Lado *et al*., 2016). The types of fatty acid present in the membrane lipids play a significant role in reducing chilling stress (Lyons, 1973; Nishida and Murata, 1996). In fact, saturated fatty acid will induce CI susceptibility and unsaturated will induce CI tolerance (Nishida and Murata., 1996; Hordijk, 2013). Subtropical and tropical crops have a higher concentration of saturated fatty acids in their lipid membranes when compared with temperate crops hence; they are susceptible to CI (Wolfe, 1978, Mditshwa, 2012). According to the bulk lipid phase transition theory established by Lyons (1973), when chilling susceptible crops are stored at critically low temperatures, their membrane lipids change from a flexible liquid-crystalline to a solid-gel structure (solidification). As a result of these changes, the ultra-structure of the cell is damaged, leading to the function of membranes being compromised (Lafuente *et al*., 2005).

Membrane permeability increases which allows for water and solutes to be passed through the membrane at an increased rate (Wolfe, 1978; Wang, 2010; Mathaba, 2012). Frequently, solute leakage determines electrolyte leakage and can be used with fruit weight loss as indicators of chilling damage (Cohen *et al*., 1994, Mathaba *et al*., 2008). Increased cell membrane permeability has been found to have an effect on membrane bound enzymes, altering their activation energy (Lyons, 1973). Consequently, when cell membrane permeability is increased, the activation energy for membrane bound enzymes increase leading to suppressed reaction rate. Non-membrane bound enzymes are however not affected; instead membrane

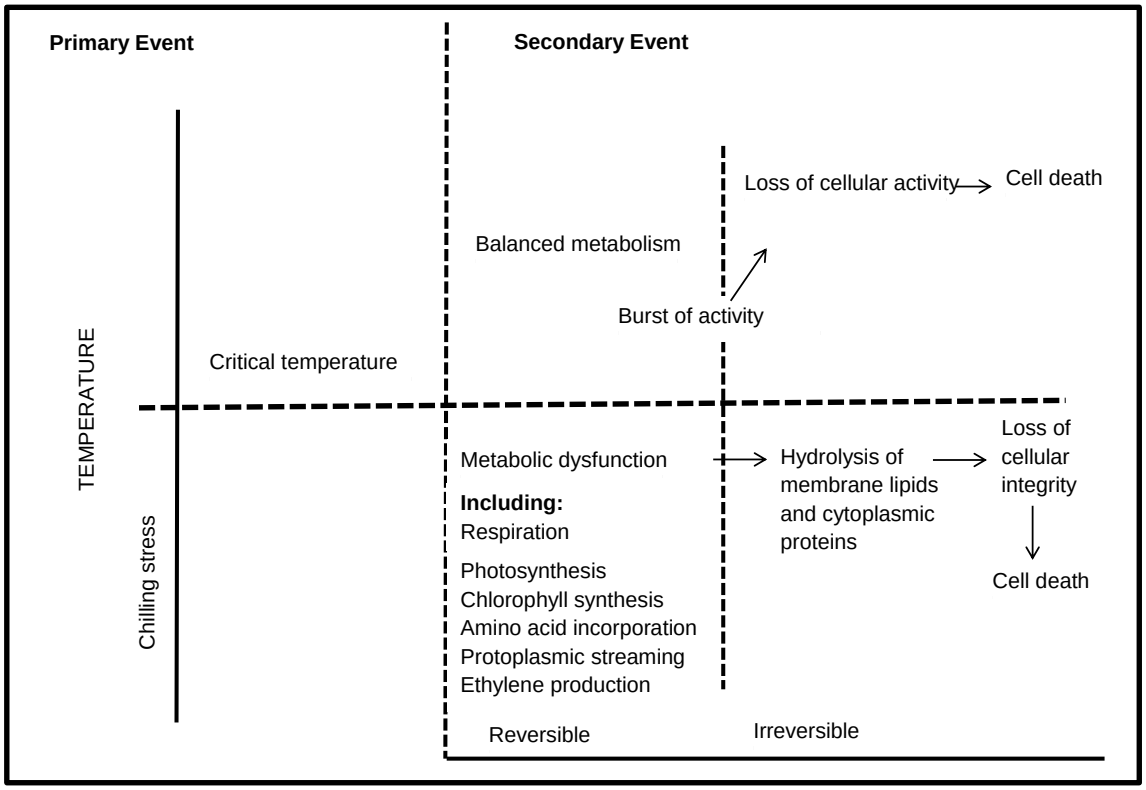
permeability creates an imbalances within critical systems such as mitochondrial respiration and glycolysis, as well as photosynthetic metabolism (Lyons, 1973).

Moreover, another biochemical event that often precedes the expression of symptoms of chilling effects is reactive oxygen species (ROS) accumulation (Sala, 1998; Hodges *et al*., 2004). High levels of ROS concentration in the cell membrane cause lipid peroxidation, protein oxidation, enzyme activity inhibition and damage to nucleic acids, thereby, resulting in cell death (apoptosis) (Huang *et al*., 2007; Mathaba and Bertling, 2013). This process is known as oxidative damage and could cause irreversible damage leading to cell death and CI symptoms. (Lyons, 1973; Mittler *et al*., 2004; Lee *et al*., 2005; Siboza and Bertling, 2013). In general, critical low temperatures modify membrane function and biophysical properties, which result in injury that develop in sensitive tissues. However, it is worth noting that with cold acclimation, the membrane will alter its composition when exposed to low cold temperature, but will not change its biophysical properties and will not develop CI (Hordijk, 2013).

Primary vs. secondary events of chilling injury

Hypothetically, CI can be subdivided into two events; a primary event that is temperature-dependent and is initiated when the temperature falls below a threshold temperature for a specified duration, thereby resulting in metabolic dysfunction (Figure 2.1) (Raison and Orr, 1990; Sharom *et al*., 1994; Kratsch,2000). The secondary event is time-dependent and includes a multitude of metabolic processes that could be adversely affected as a consequence of the primary event, and lead to the development of measurable symptoms characteristic of chilling injury and possibly, cell death (Figure 2.1) (Raison and Orr, 1990, Wills *et al*., 2007). The primary event is commonly assumed to involve changes in the lipid membrane which initiate several secondary events involving metabolic dysfunctions (Figure 2.1). Interestingly, the process is reversible if the exposure to chilling temperature has not been long and cell integrity has not been compromised. The chilling temperatures create an imbalance in metabolism within the cells, but a rise in temperature in the reversible stage, creates an increase in metabolic activity allowing for intermediates accumulated from the chilling stress to be removed, resulting in the restoration of the

metabolic balance within the cells (Lyons, 1973). Separating CI into these two stages helps to describe in details the fundamental molecular mechanisms underlying this phenomenon, which is enormously complex (Luengwilai *et al*., 2012). Therefore, it becomes possible to differentiate the primary 'cause' (i.e., the initial event happening upon chilling) from the secondary 'effect' (i.e., the subsequent events that produce physiological and visual signs of chilling injury) (Raison and Orr, 1990; Wills *et al*., 2007; Luengwilai *et al*., 2012).



2.1 Relationship between the primary and secondary events of chilling injury (Raison and Orr, 1990)

2.2.3 Effect of storage temperature on fruit physical properties

Weight loss

Fruit continue to respire and lose water to the surrounding environment after harvest as a consequence of transpiration (Roongruangsri, 2013; Hassan *et al*., 2014; Rab *et al*., 2015). Moisture loss is one of the main post-harvest phenomena that affect the quality of mandarins and oranges during long-term storage. Moreover, weight loss as low as 5-6% during long-term storage of mandarins and oranges can result in having shrivelled fruit which are unmarketable (Ben-Yoshua, 1969; Prasad, 1996; Hung *et al.,* 2011). The relative humidity and storage temperature at which the produce is kept at are the primary factors that affect weight loss (Maguire *et al*., 2001; Jourbert, 2016). In addition, water loss from stored produce occurs when the RH in the cooling room is below the humidity inside the fruit (Veraverbeke *et al*., 2003; Jourbet, 2016).

Loss in fruit weight especially on the peel is generally high during storage under low-RH condition than high RH (Ladaniya, 2008). However, storage trials conducted by Roongruangsri *et al*. (2013) on ‘Sai Num Phueng’ and ‘See Thong’ tangerine showed that the losses of fruit weight and moisture content of the peel during storage at 5°C, 58±2% RH were lower than at 25°C, 85±2% RH in both tangerine cultivars. The authors concluded that, storage temperature had a greater influence in the control of weight loss and moisture content of the peel than the relative humidity. Similarly, ‘Fortune’ mandarin fruit cold stored at 12°C with at 80–90% RH showed higher weight loss of 2-15% compared to 2-5% lost at 2°C (Gonzalez-Aguilar *et al*., 1997). Mditshwa (2012) demonstrated higher fruit weight loss for ’Eureka’ lemon fruit stored at 4.5°C compared to 2°C after 28 days under 95% RH, which could be as a result of higher transpiration and fruit respiration. Indeed, previous studies in citrus fruit have confirmed that high moisture loss at high temperature and RH was as a result of high respiration (Hassan *et al*., 2014; Siboza and Bertling, 2013). Based on these findings, it is clear that to control RH and storage temperature is important to reduce weight loss on citrus fruit.

Firmness

Firmness has been used widely as a quality index for fruit as it is associated with the fruits physiological maturity, freshness, bruising, texture, compression and damage

(Ladaniya, 2008; Pranamornkith, 2009). Firmness loss could be used as an indicator at the end of shelf-life and a key factor that determine the consumer's product acceptances. The mechanisms involved in fruit firmness maintenance depend primarily on the species and cultivar (Liplap, 2013). The loss of firmness on horticultural fruit involves the change in cell structure, middle lamella and cell wall components such as pectin, cellulose and hemicelluloses (Ali *et al*., 2004; Valero and Serrano, 2010). According to Ali *et al*. (2004), high water loss and the resultant turgor decline contribute to high and rapid fruit firmness loss by accelerating cell wall degradation through relevant enzymes such as cellulose, pectin methylesterase (PME) and hemicellulose.

Several studies have shown that the changes in the textural properties of fruit were primarily reliant on the storage temperature and duration (El-Hilali *et al*., 2003; Ali *et al*., 2004; Pranamornkith, 2009; Khorshidi *et al*., 2010), and to a minor extent, due to relatively humidity. Pranamornkith (2009) observed that storage of Tahiti' lime fruit at 2 and 5°C with 90-95% RH resulted in the decrease in fruit firmness after 8 and 10 weeks, respectively. Based on their results, the authors reported that fruit stored at 5°C with 90-95% RH had higher firmness loss compared to those stored at 2°C. In contrary, El-Hilali *et al*. (2003) showed that fruit stored at a higher temperature (8°C) for 4 weeks experienced lower firmness loss compared to those stored at lowest temperature (4°C) for the same storage period which was associated with weight loss.

Likewise, Ali *et al*. (2004) studied the response of 'B10' carambola fruit in relation to fruit firmness and found that fruit stored at 10°C for 25 days with 80-85% RH showed the highest firmness loss compared to those stored at 5°C. Khorshidi *et al*. (2010) also did work on storage temperature and texture using 'Red Delicious' apple fruit. Their report indicated that 'Red Delicious' apple fruit stored at 0°C and 80% RH had lower firmness loss when compared with those kept at 12°C. The results from these studies suggested that the increase in firmness loss at higher storage temperatures could be due to moisture loss from the fruit resulting in hardening and increase in mechanical strength of fruit peel, while chilling injury is responsible for firmness loss at lower temperatures. Therefore, it is important to control moisture loss with high RH conditions at low temperatures, in order to maintain a high degree of rind firmness

(Ben Yehoshua *et al*., 2001; Porat *et al*., 2004). It is worth noting that, the inconsistent reports on changes in firmness of horticultural crops stored at low temperatures could be due to storage duration and methods used to measure firmness

2.2.4 Effect of storage temperature on fruit biochemical properties

Electrolyte leakage

Electrolyte leakage is an effective parameter to assess membrane permeability; and therefore, used as a membrane integrity indicator (Zhang *et al.,* 2011). Chilling affects membrane integrity and increase electrolyte leakage (Campos *et al*., 2003). Several studies have correlated electrolyte leakage with the appearance of chilling injury symptoms in many horticultural crops and have since been used to objectively quantify the severity of chilling injury (González-Aguilar *et al*., 2000; Vicente *et al*., 2005; Zhao *et al*., 2009). Recently, Siboza and Bertling (2013) found electrolyte leakage to be an excellent indicator of cell membrane damage when 'Eureka' lemon fruit held at 4.5°C and 90-95% RH for 28 days.

In general, storage temperature is a major factor that increases the fruit membrane permeability and the subsequent rate of electrolyte leakage (Lyons, 1973; Saltveit and Morris, 1990; Campos *et al*., 2003; Concelleon *et al*., 2005; Siboza and Bertling, 2013). For instance, Concelleon *et al*. (2005) revealed that the electrolyte leakage for 'Money Maker No 2' eggplant fruit stored for 13 days at 0°C was higher than at 10°C. Hussain *et al*. (2015) also showed that 'Blood Red' sweet orange fruit stored at 2°C had higher electrolyte leakage when compared to those kept for 60 days at 10°C. The authors suggested that high electrolyte leakage observed on fruit stored at 0°C was due to increased membrane permeability. According to Lyons (1973) and Mathaba (2012), storage of horticultural fruit at chilling temperature causes membrane lipids solidification leading to the contraction of membranes. As cellular membranes contract, they subsequently crack, thereby, resulting in increased cell permeability. Moreover, the change in the membrane bilayer due to extended chilling temperature is associated with increased ion movement and water molecules through the membrane (Nishida and Murata, 1996). The effect of increased membrane permeability is high electrolyte leakage from the cells, resulting in chilling injury.

Total soluble solids (TSS) and titratable acidity (TA)

Chemical parameters like TSS, TA and TSS/TA have been used to describe taste (flavour) with regards to the sweetness and acidity, and have been used as a quality criterion for the formulation of citrus products and juice (Ladaniya, 2008; Rab *et al*., 2015). During storage at low temperature, TSS often increases, while acidity decreases, therefore, TSS/Acid ratio also increase (Grierson and Ben-Yehoshua, 1986; Ladaniya, 2008; Roongruangsri, 2013; Rab *et al*., 2015). For instance, studies by Marcilla *et al*. (2009) on 'Clemenules' mandarin stored at 5°C with 90% RH for 62 days plus 7 days at 20°C showed that, as the storage period progresses, TSS increase whereas TA decreases.

Various studies have shown that storage temperature has a profound effect on fruit TSS and TA (Obenland *et al*., 2011; Sdiri *et al*., 2012; Khorshidi *et al*., 2010). For instance, Obenland *et al*. (2011) investigated the effect of storage temperature on quality attributes of two mandarin cultivars; 'Owari' and 'W. Murcott' both stored at either 0, 4 or 8°C for 3 or 6 weeks including a week at 20°C to simulate marketing period. Based on their findings, 'W. Murcott' mandarin fruit TSS and TSS/TA content was higher after storage 8°C compared with 0 and 4°C. Furthermore, for 'Owari' mandarin fruit, storage temperature did not have any significant influence TSS and TA at the end of the storage period.

Similarly, Tietel *et al*. (2012) studied the biochemical properties of 'Or' and 'Odem' mandarin fruit stored for 4 weeks at 2, 5 or 8°C followed by 3 days at 20°C. The authors reported no significant effect of temperature on TSS, TA and TSS/TA ratio. Likewise, El-hilali *et al*. (2003) found that total soluble solids and acidity in 'Fortune' mandarin fruit showed no significant differences between treatments after 30 days storage at 4°C and 8°C. Based on these reports, it could be argued that the response of biochemical properties of mandarin fruit to storage temperature is not homogenous. Furthermore, several studies have linked the increase in TSS during cold storage with the reduction of water content and cell wall components including; protein, pectin and hemicelluloses into simple soluble sugars (Ladaniya, 2008; Valero and Serrano, 2010; Roongruangsri, 2013; Rab *et al*., 2015). Ghasamnezhad *et al*. (2008) reported that the total soluble solids for 'Satsuma' mandarin stored at 2°C for 8 weeks showed a slight increase which was consistent with water loss.

2.3 Postharvest treatment

2.3.1 Silicon dips

There is a growing concern by consumers about the use of potentially hazardous chemicals in food production. Such chemicals raise health concerns, and therefore, interest in the use of more nontoxic compounds to mitigate chilling injury in fruit is increasing (Wang, 2006). Unlike other chemical treatments, silicon has less mammal toxicity (Ma and Yamaji, 2006; Curry and Perry, 2007; Tarabih *et al*., 2014. Recent findings have established the potential and efficacy of silicon dips to control chilling injury and prolong the quality of a few horticultural crops (Kaluwa *et al.*, 2010; Mditshwa *et al*., 2013; Nasar *et al.,* 2013; Tarabih *et al*., 2014).

2.3.2 Effect of silicon dips on chilling injury

Mditshwa (2012) investigated the potential of postharvest silicon dips (50, 100, 150 and 250 mg L^{-1}) to mitigate chilling injury of 'Eureka' lemon fruit stored at -0.5, 2 or 4.5ºC with 90% RH for 28 days plus 5 days at 20ºC. The author reported that treatment with 50 mg L^{-1} K_2SiO_3 significantly reduced chilling injury symptoms compared to control and high silicon concentration (100, 150 and 250 mg L^{-1}) after 28 days storage at -0.5°C. In another study, Nasar *et al*. (2013) reported that storage of 'Pioneer' plums treated with 2500 and 500 ppm potassium silicate at 0±1°C for 28 days showed less cell permeability, measured as electrolyte leakage when compared with untreated fruit. These findings suggest that the lowest Si concentrations are most probably likely to mitigate CI on different horticultural crops.

Theoretically, when silicon is deposited into the cell wall, it takes part in cell metabolism and either modify the structure of membrane lipids (from saturated to unsaturated) or reduce the energy requirements needed to activate membrane-bound enzymes (Figure 2.2) (Liang *et al*., 2007). Silicon increases both enzymatic (ascorbate peroxidase) and non-enzymatic (ascorbic acid and phenolic) antioxidants (Zhu *et al*., 2004; Liang *et al*., 2007; Mditshwa *et al*., 2013) which are responsible for lowering membrane lipid peroxidation. Consequently, as antioxidant actions increase membrane lipid peroxidation is lowered; and ultimately, membrane permeability and electrolyte leakage are also reduced (Figure 2.2) (Liang *et al*., 2008). This suggests that, Si is not only deposited into the cell wall, but it is actively involved in

physiological activities that lead to a Si-induced fruit tolerance to chilling injury (Figure 2.2).

Several reports have shown that silicon increase antioxidant defense systems, thus facilitating plant resistance to stress (Agarie *et al*., 1998; Ma and Yamaji, 2006; Keeping and Reynolds, 2009). For instance, Mditshwa (2012) showed that effective reduction of 'Eureka' lemon fruit chilling symptoms was due to higher total antioxidants and total phenolic content which were enhanced through the application of Si. Therefore, the author concluded that Si has potential to increase both phenolic and flavonoid antioxidants of fruit during long-term storage; and therefore, maintain cell membrane integrity (Mditshwa *et al*., 2013). Furthermore, silicon was found to increase the amount of unsaturated to saturated fats in salt tolerant 'Jiang 4' barley crops (Liang *et al*., 2003). In another study, Wang and Galleta (1998) reported that the application of Si enhanced the ratio of unsaturated to saturated lipids, subsequently, inducing membrane stress resistance of 'Earliglow' strawberry fruit. Hence, increased saturation of membrane lipids result in greater membrane rigidity whereas decreased membrane fluidity results in decreased permeability and subsequently low electrolyte leakage (Mditshwa, 2012).

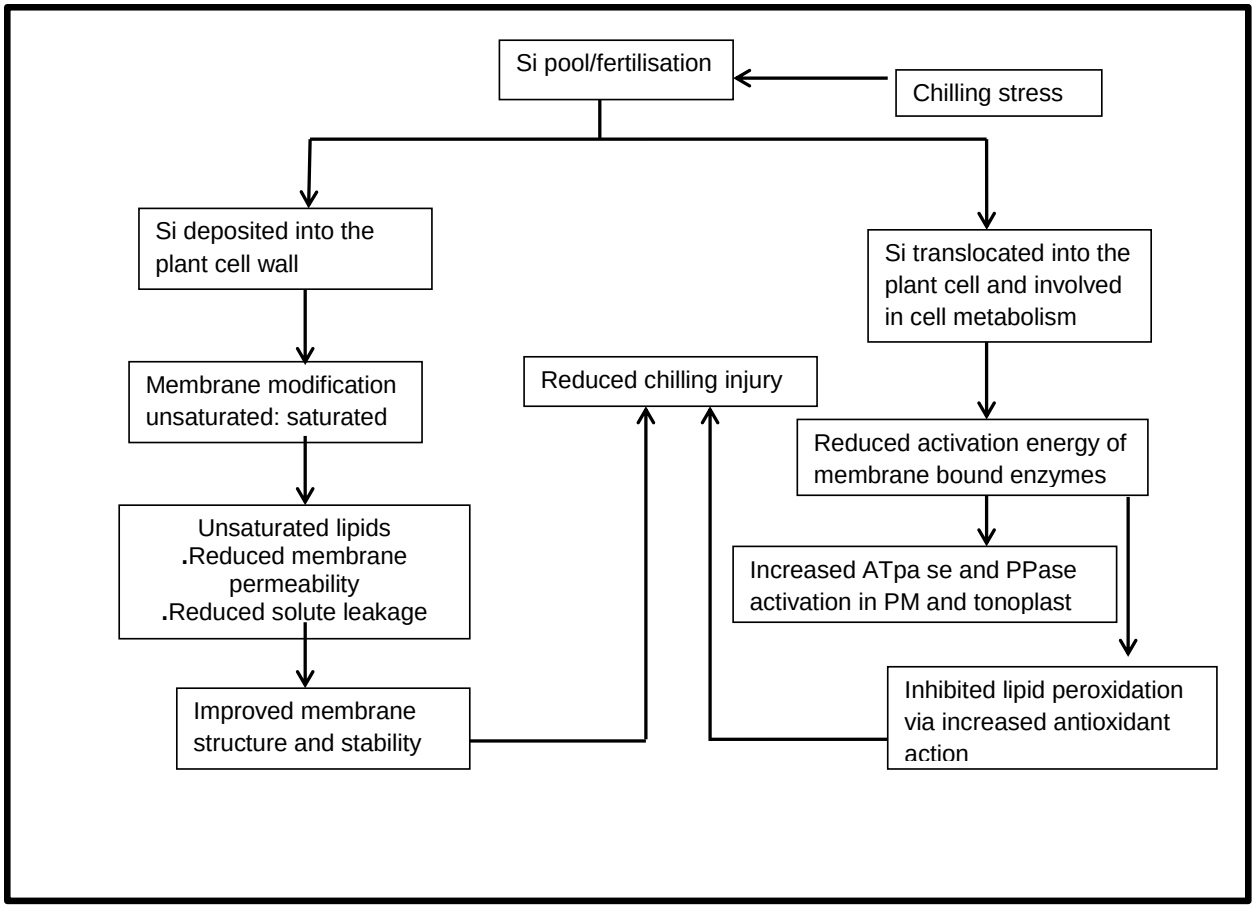


Figure 2.2 Cascade of physiological and biochemical events associated with Si-mediated alleviation of chilling injury (A model developed by Liang *et al.* (2007) and modified by Mditshwa (2012))

2.3.3 Effect of silicon dips on physico-chemical properties

Weight loss

Deposition of silicon into the cell membrane has been largely associated with the prevention of moisture loss through transpiration and provision of mechanical strength and rigidity to plant cell and tissue (Epstein, 2009; Savvas and Ntatsi, 2015). According to Mditshwa *et al*. (2013), treating 'Eureka' lemon fruit with lower silicon concentration (50 mg L^{-1}) significantly reduced weight loss after storage at -0.5°C and 90% RH for 28 days plus 5 days at 20°C. Treatment with silicon (150 and 250 mg L^{-1}) at similar temperature and storage duration resulted in increased weight loss percentage. Similarly, Tarabih *et al*. (2014) indicated that weight loss on 'Anna' apple fruit stored at 0±1°C with 90-95% RH for 60 days treated with different potassium silicate concentration (0.1, 0.2, 0.3% K_2SiO_3) was higher than the non-

treated fruit. In addition, a low weight loss percentage was observed on fruit treated with high K_2SiO_3 concentration (0.3%) after 60 days storage at 0±1°C and 90-95% RH.

These results were, however, contrary to those of Nasar *et al*. (2013), who investigated the effect of potassium silicate dips on 'Pioneer' plums firmness after storage at 0 ±1ºC with 85±85% RH for 28 days. The authors reported that, lower potassium silicate concentrations (2500 and 5000 ppm) reduced fruit weight loss. Similarly, Tesfay *et al*. (2011) demonstrated that for 'Hass' avocado fruit, all the Si treatments (0, 5000, 13.000 or 25.000 mg L^{-1}) reduced fruit weight loss by 20%. The author further noted that, the loss in 'Hass' avocado fruit weight decreased with an increase in Si concentration applied (0, 5000, 13000 or 25000 mg L^{-1}). In contrast, Bertling *et al*. (2009) reported that for 'Hass' avocado stored at 5.5ºC for 16 days, the weight declined significantly in control than in Si treated fruit (5000, 13000 and 25000 ppm), with the highest Si application (25000 ppm) maintaining the highest weight. Therefore, based on these findings, it is safe to suggest that Si dips might possibly induce a thin film of coating substances when applied to the surface of the fruit. These coatings can act as a semi permeable barrier against oxygen, carbon dioxide, moisture and solute movements. Hence they could reduce the rates of the respiration, water loss and oxidation reaction.

Firmness

Kaluwa *et al*. (2009) investigated the response of 'Hass' avocado fruit treated with different postharvest Si dips sources at concentrations ranging from 80 to 2940 ppm and subsequently stored at either -0.5, 1, 5ºC or room temperature (25°C). Based on their findings, the authors demonstrated that 'Hass' avocado fruit treated with different postharvest silicate sources and stored at 5ºC were firmer than fruit stored at other temperatures 3 days after removal from storage. However, fruit firmness measurement did not show any significant differences between treatments, probably due to severe anthracnose infection of all fruit hindering fruit softening. Interestingly, reports by Tesfay *et al.* (2011) showed an opposite trend, in which 'Hass' avocado fruit treated with different postharvest silicon application (0, 5000, 13000 or 25000 mg L^{-1}) had lower firmness when compared with control after 17 days of storage at 5.5°C followed by 5 days at 20°C. In a similar study on 'Hass' avocado fruit Bertling

et al. (2009), showed that fruit firmness was not affected by the silicon treatments (5000, 13000 and 25000 mg L^{-1}) during and after storage at 5.5°C for 16 days. Similarly, Nasar *et al*. (2013) reported that, the firmness of 'Pioneer' plum fruit was significantly higher with untreated and treated with 1000 ppm potassium silicate when compared with those treated with 2500 and 5000 ppm. Conversely, the author demonstrated that fruit firmness did not vary between fruit treated with 500 or 2500 ppm.

Electrolyte leakage

Studies have shown that electrolyte leakage of fruit exposed to extended storage at ultra-low temperatures is reduced when silicon is applied. For instance, determination of electrolyte leakage of 'Hass' avocado mesocarp tissue, in order to determine the silicon effect on maintenance of cell to cell communication, revealed that fruit treated 25000 ppm Si application had a significantly higher electrolyte leakage than the lower two Silicon treatments (500 and 1000 ppm) (Bertling *et al*., 2009). However, no significant differences were observed between the silicon treatments and the control. In another study, Tesfay *et al*. (2011) also reported that the 'Hass' avocado fruit treated with 25000 mg L^{-1} Si had significantly higher electrolyte leakage when compared with the lower two silicon treatments (5000 and 1300 mg L^{-1}). The authors suggested that low electrolyte leakage on fruit treated with 5000 and 1300 mg L^{-1} Si was possibly due to silicon deposition between cell wall and cell membrane, maintaining a barrier against solute leakage. However, there were no significant differences in electrolyte leakage between the control andSi treated fruit after cold storage (5.5°C) for 17 days followed by 5 days at room temperature (20°C). Similarly, Mditshwa *et al.* (2013) reported that 'Eureka' lemon fruit dipped in Si (50,100,150 and 250 mg L^{-1}) had lower electrolyte leakage than fruit treated with water after 28 days of storage at 0.5°C with 90% RH plus 5 days shelf-life. This was attributed to the decrease in lipid peroxidation, concomitantly, a decrease in membrane permeability, specifically, electrolytes (Liang, 2004; Tuna *et al.,* 2004; Liang *et al.,* 2008).

Total soluble solids and titratable acidity

Very little is known about the effect of postharvest silicon application on fruit biochemical properties such as total soluble solids and titratable acids. However,

Nasar *et al*. (2013) recently investigated the response of 'Pioneer' plums stored at 0±1°C and 80-85% RH for 28 days to different potassium silicate concentrations (0, 250, 500, and 1000 ppm). Based on their findings, TSS was higher on fruit treated with postharvest silicate (250, 500, and 1000 ppm) dips than control after storage at 0±1°C and 80-85% RH. Furthermore, the fruit titratable acids were higher for control than for fruit treated with different potassium silicate concentrations (250, 500, and 1000 ppm). Similarly, Tarabih *et al*. (2014) evaluated the physiological and pathological impacts of potassium silicate on storability of 'Anna' apple stored at 0±1°C and 90-95% RH for 60 days. The authors reported a reduction in TSS, TA and TSS/TA ratio on all Si treated fruit (0.1, 0.2, 0.3% K_2SiO_3) compared to untreated fruit. Therefore, these studies suggest that postharvest Si application could increase fruit TSS, reduce TA and consequently, increase the TSS/TA ratio.

2.4 Conclusion

The review showed that postharvest storage temperature plays a major role in postharvest fruit quality of horticultural fruit crops, and different cultivars behave differently under similar storage conditions. Low temperature was shown to induce chilling injury, which is responsible for external quality loss in many horticultural fruit, although such temperatures are required by export markets to prevent the spread of fruit flies across fruit fly free countries. Evidence from the current review also showed that postharvest silicon treatments reduce chilling injury and maintain fruit quality. However, only few research papers have been published, particularly those evaluating the effect of silicon on postharvest quality of citrus fruit, even though such treatments have potential to be used alongside cold quarantine treatments required by exporting countries. Hence, there is need for intensive research to determine the effect of silicon on chilling injury in mandarin fruit. The mitigation of chilling injury on new mandarin fruit stored at quarantine temperatures will therefore increase the competitiveness of the South African citrus industry.

CHAPTER 3
RESEARCH METHODOLOGY

3.1 Experimental sites

Mandarin (Clementine x (Ellendale x Novelty) selection, “M37”) fruit were harvested from 6-years old trees grown at Addo Citrus Research Cultivar Block in Eastern Cape (33°34'0"S, 25°41'0"E) in July 2016. In the packhouse, fruits were visually graded for appearance and absence of blemishes, drenched in Imazalil (500 mg L^{-1}) and 2.4-dichlorophenoxyacetic acid (125 mL^{-1}). Thereafter, fruit were packaged and transported to the ARC-ITSC postharvest laboratory in Nelspruit (25°28'0"S, 30°58'0"E) for further treatment, cold storage and analysis.

3.2 Experimental design

The experiment was carried out in a factorial, arranged in a completely randomised design (CRD) with three replicates. Treatment factors were: 2 x cold storage temperature (-0.6±1 and 4.5±1°C) and 4 x potassium silicate concentration (0, 50, 100 and 150 mL L^{-1} K_2SiO_3).

3.3 Postharvest treatment and storage

Fruit were first washed with Sporekill® (Hygrotech Pty Ltd) for 5 minutes, allowed to air-dry at room temperature, and randomly allocated to postharvest potassium silicate (K_2SiO_3) treatment. The K_2SiO_3 treatments were applied as postharvest dip bath according to Mditshwa *et al*. (2013). During dipping, fruit were either soaked in 0 (dipped only in de-ionized water), 50, 100 or 150 mL L^{-1} K_2SiO_3 concentration for 30 minutes (Mditshwa, 2012). Following treatments, fruit were allowed to dry at ±23°C for 2 h, thereafter waxed with Citrashine® (Citrashine Pty Ltd, Johannesburg, South Africa) and allowed to dry at ±23°C for 2 h. After storage, fruit were stored either at -0.6 or 4.5°C (air delivery temperature) in cold rooms with a relative humidity of 85-90%. Fruit were sampled at 0 and 28 days into the cold storage. Following removal from cold storage, “M37” mandarin fruit were transferred to ±23°C for 7 days to observe development of CI symptoms (shelf-life).

3.4 Data collection

3.4.1 Estimation of chilling injury

After 28 days of cold storage at -0.6 or 4.5°C plus 7 days shelf-life at room temperature, fruit were evaluated for chilling injury severity. Pitting and external discoloration was evaluated based on a hedonic scale; 0 = normal (no pitting), 1 = slight pitting (a few scattered pits), 2 = moderate pitting (pitting covering up to 30% of the fruit surface), 3 = severe pitting (extensive pitting covering > 30% of the fruit surface) and expressed as CI index (Sala, 1998). The chilling injury index (CII), which expressed the severity of damage was calculated by adding the products of the number of fruit in each category by the value assigned to this category in the rating scale and dividing the sum by the total number of fruit evaluated;

CII = ∑ (number of fruit with chilling injury symptoms x severity score) / total number of fruit evaluated

3.4.2 Determination of physical properties

Weight loss

In postharvest research, the assumption that fruit weight loss refers to fruit water loss is common. Therefore, in the present study, fruit weight was measured using a weighing scale (Model: SBA 61, Scaltec instruments, Heiligenstadt-Germany) (Figure 3.2). Fruit weight loss was expressed as the difference in fruit initial weight before and after postharvest storage, and calculated a percentage of the initial weight of each fruit, using the following formula:

Weight loss % = $((W_0-W_1) \setminus W_0) \times 100$

Where:

W_0 = Weight of fruit before storage

W_1 = Weight of fruit at a sampling day (28 after cold storage and 7 days shelf-life)

Figure 3.1 Weighing scale used to measure fruit weight.

Firmness loss

Fruit firmness was measured before cold storage and after 28 days cold storage plus 7 days shelf-life using hand-held densimeter (Model: Bareiss, Oberdischingen, Germany) with a 5-mm tip (Figure 3.2). A fruit was placed stationary on a flat surface and a compressive force applied on opposite sides of fruit surface and the average of the two sides was taken as the fruit firmness.

Figure 3.2 Densimeter used to measure fruit firmness.

3.4.2 Biochemical properties determination

Electrolyte leakage

Membrane permeability was measured using electrolyte leakage according to Cohen *et al*. (1994). Electrolyte leakage was recorded after 28 days cold storage plus 7 day shelf-life at room temperature. Three fruit from each treatment were sampled and used for determination of electrolyte leakage. Three flavedo discs from each fruit with a diameter of 1 cm were cut and immersed in a test tube containing 10 mL de-ionized water. Prior to this, the peels were washed three times to eliminate the electrolyte leakage at the cut surface and prevent surface contamination. The first electrical conductivity (EC_1) was measured after shaking the sample for 3 hours, with an electrical conductivity (EC) meter (Model HI 9033, Hanna instruments, Johannesburg, RSA) (Figure 3.1). The second electrical conductivity (EC_2) was measured after the samples were placed in a hot water bath controlled at 100°C for 1 hour and allowed to cool at room temperature. Electrolyte leakage was expressed in percentage using the formula:

Total electrolyte leakage = (EC_1/ EC_2) x 100

Where:

EC_1 = Initial electrolyte leakage reading

EC_2 = Final electrolyte leakage reading

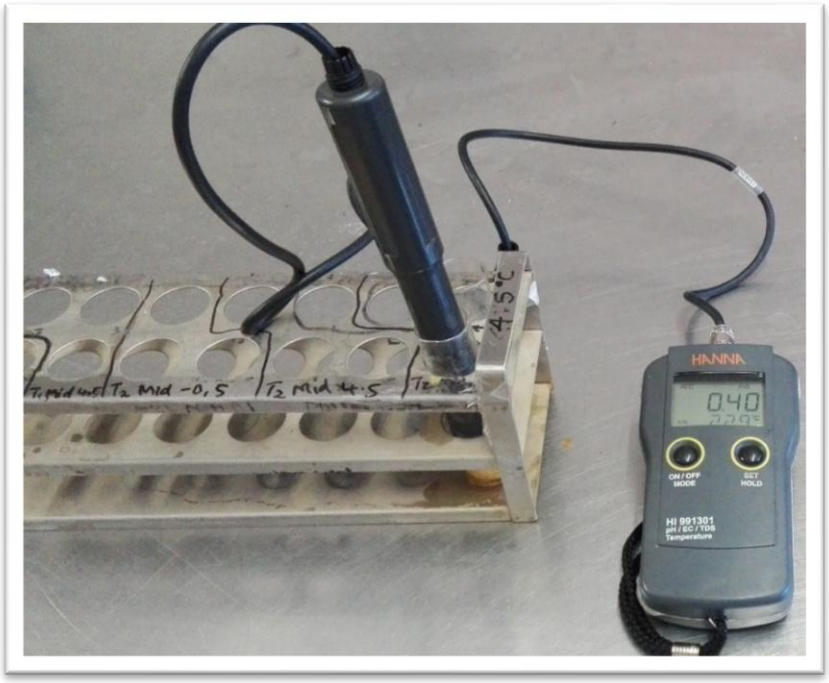


Figure 3.3 Electrical conductivity meter used to measure electrolyte leakage.

Total soluble solids (TSS), Titratable acids (TA) and TSS/TA ratio determination

Total soluble solids were measured from 6 fruit per treatment cold storage and after 7 days shelf-life using digital refractometer (Atago PR-1, Atago Co, Ltd., and Tokyo) (Figure 3.4A). Titratable acidity content was determined by titrating 0.1 N NaOH solution into 10 mL of fruit juice mixed with 3 drops of Phenolphthalein indicator. Titration was complete when the liquid turned pink in colour (Figure 3.4B). The result was therefore converted into citric acid by the equation: Titratable acid content = (mL NaOH/ 10 mL) x (0.1 N NaOH/ 0.1562). TSS/TA was calculated as a ratio between TSS and TA (Marcilla *et al*., 2009)

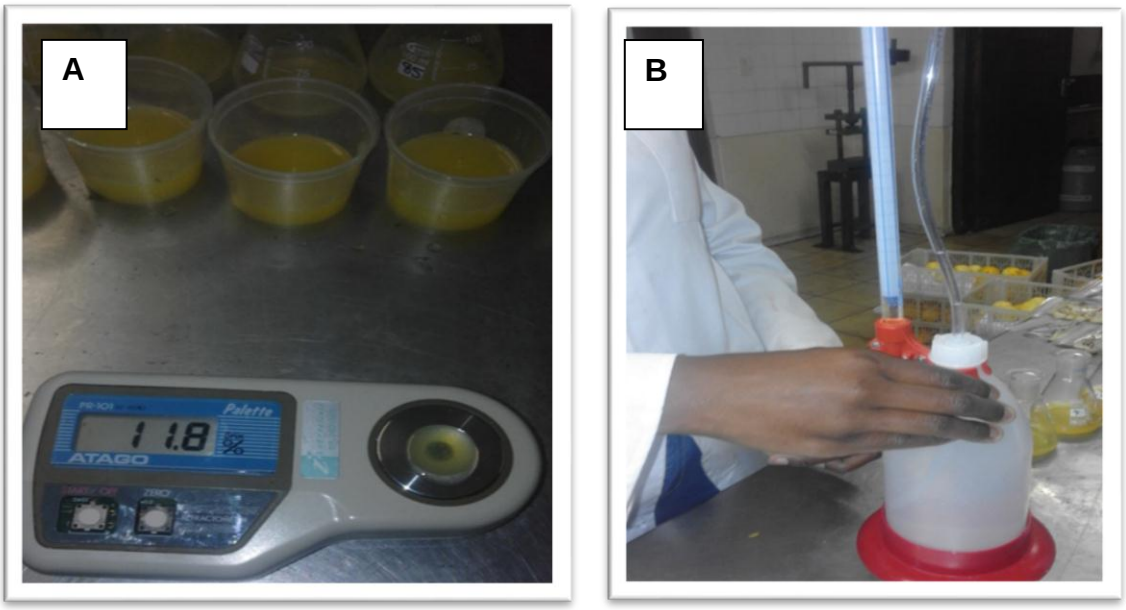


Figure 3.4 (**A**) Refractometer used to measure total soluble solids (°Brix) and (**B**) Burette used to titrate acids (citric acid %).

3.5 Statistical analysis

Statistical analyses were carried out using GenStat® version 14th (VSN International, Hemel Hempstead, UK) ANOVA. Significant differences between treatment means were assessed using Duncan Multiple Range Test at ($P \leq 0.05$). Variations were compared between cold storage temperature and postharvest potassium silicate dips. From the ANOVA focus was emphasized on the interaction of cold storage temperature and postharvest potassium silicate

CHAPTER 4
RESULTS

4.1 Chilling injury index

The results showed that an interaction between cold storage temperature and postharvest potassium silicate dips had no significant effect ($P>0.05$) on fruit chilling injury index after 28 cold storage plus 7 days shelf-life (Appendix 4.1). However, upon removal from cold storage at -0.6 and 4.5°C, fruit showed visible chilling injury (CI) symptoms which manifested as external discolouration on the fruit peel (Figure 4.1). In all the treatments, chilling injury index (CII) was slightly higher on fruit cold stored at -0.6°C when compared with 4.5°C. Moreover, CII increased with the increase in K_2SiO_3 concentration and the symptoms were higher after 7 days shelf-life at room temperature, irrespective of postharvest cold storage temperature (Figure 4.2). Control showed higher CI symptoms than fruit treated with different K_2SiO_3 concentration, irrespective of postharvest cold storage temperature (Figure 4.2). Interestingly, fruit dipped in 50 and 100 mL L^{-1} K_2SiO_3 concentration and cold stored at -0.6 and 4.5°C showed significantly reduced chilling injury when compared with the control and 150 mL L^{-1} K_2SiO_3 concentration. In general, the 50 and 100 mL L^{-1} K_2SiO_3 concentrations were highly effective at 4.5°C than at -0.6°C, with chilling indexes of 0.04 and 0.36 for 50 mL L^{-1} then 0.40 and 0.58 for 100 mL L^{-1}, respectively (Figure 4.2).

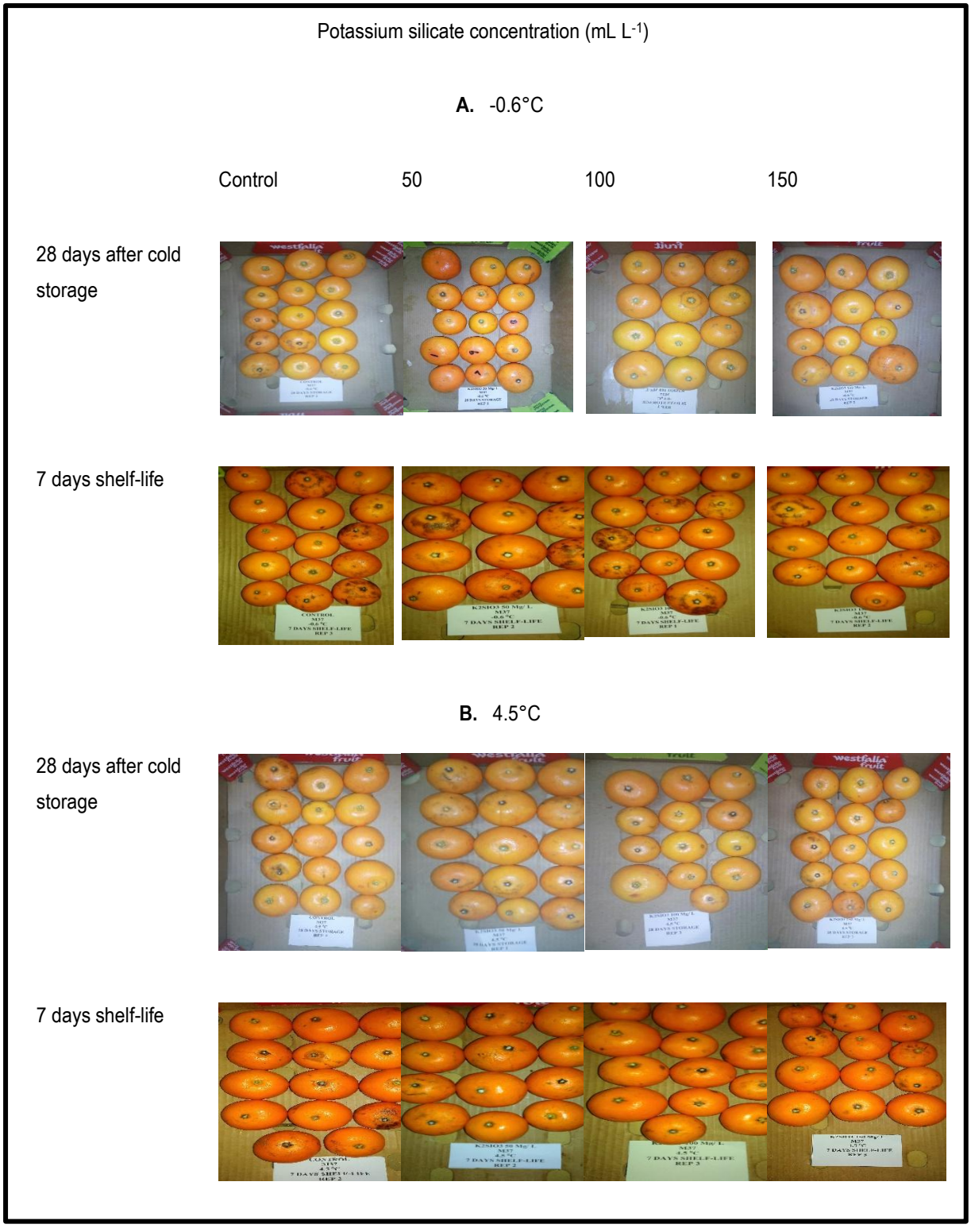


Figure 4.1 Chilling symptoms on “M37” mandarin fruit after withdrawal from storage at (A) -0.6°C and (B) 4.5°C followed by 7 days shelf-life at room temperature (23°C).

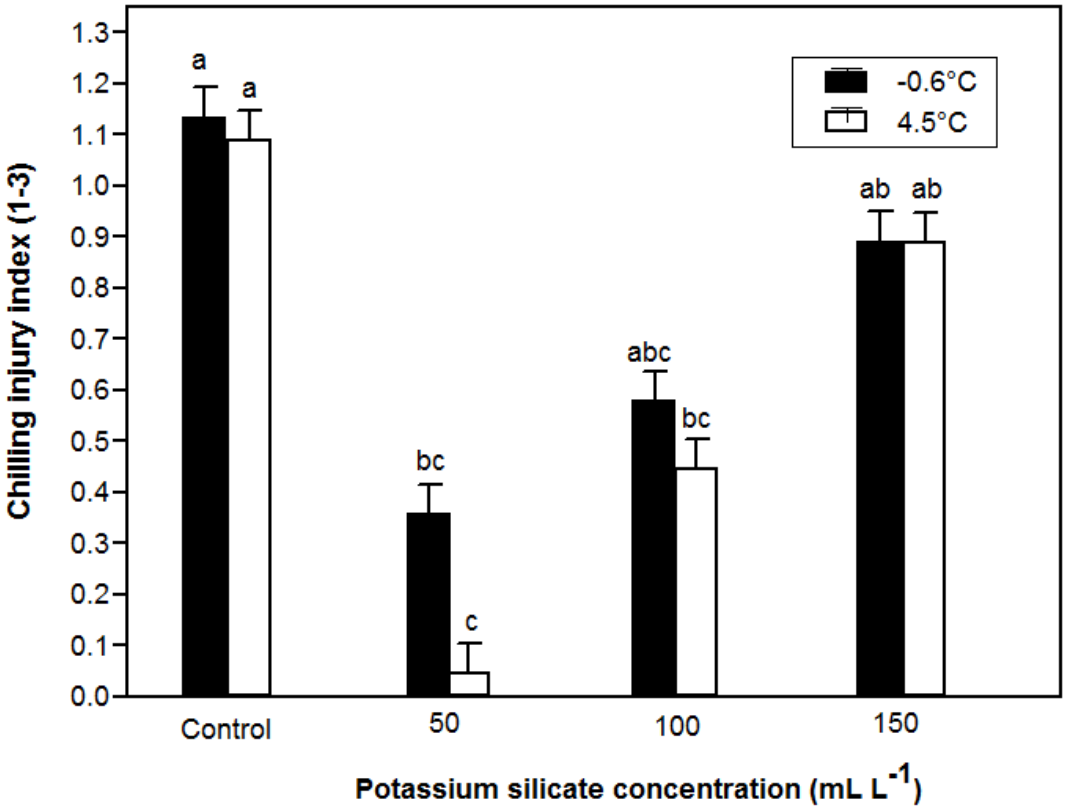


Figure 4.2 The effect of cold storage temperature and postharvest potassium silicate dips on “M37” mandarin fruit chilling injury after 28 days cold storage time plus 7 days shelf-life. Bars with a different letter within each potassium silicate treatment (concentration) were significantly different at, $P<0.05$. Vertical bars indicate the standard error of means.

4.2 Physical properties

Weight loss

The Interaction between cold storage temperature and postharvest potassium silicate dips had a significant effect ($P<0.05$) on fruit weight loss after 28 days cold storage plus 7 days shelf-life (Appendix 4.3). The different cold storage temperatures showed similar trends with respect to weight loss, irrespective of K_2SiO_3 concentration (Figure 4.3). However, fruit cold stored at 4.5°C showed significantly higher weight loss when compared with fruit stored at -0.6°C (Figure 4.3). Interestingly, the study showed that dipping fruit in 50 mL L^{-1} K_2SiO_3 concentration significantly reduced weight loss during cold storage at -0.6°C compared to the control and higher K_2SiO_3 concentrations (100 and 150 mL L^{-1}) (Figure 4.3). However, there were no significant difference on weight loss between the control, 50, and 100 mL L^{-1} K_2SiO_3 concentration when fruit were kept at 4.5°C; although, weight loss mean differences were observed on fruit treated with 150 mL L^{-1} K_2SiO_3 concentration. Furthermore, the higher K_2SiO_3 concentration (150 mL L^{-1}) resulted in slightly higher fruit weight loss than the control and lower K_2SiO_3 concentration (50 and 100 mL L^{-1}) for fruit stored at 4.5°C (Figure 4.3).

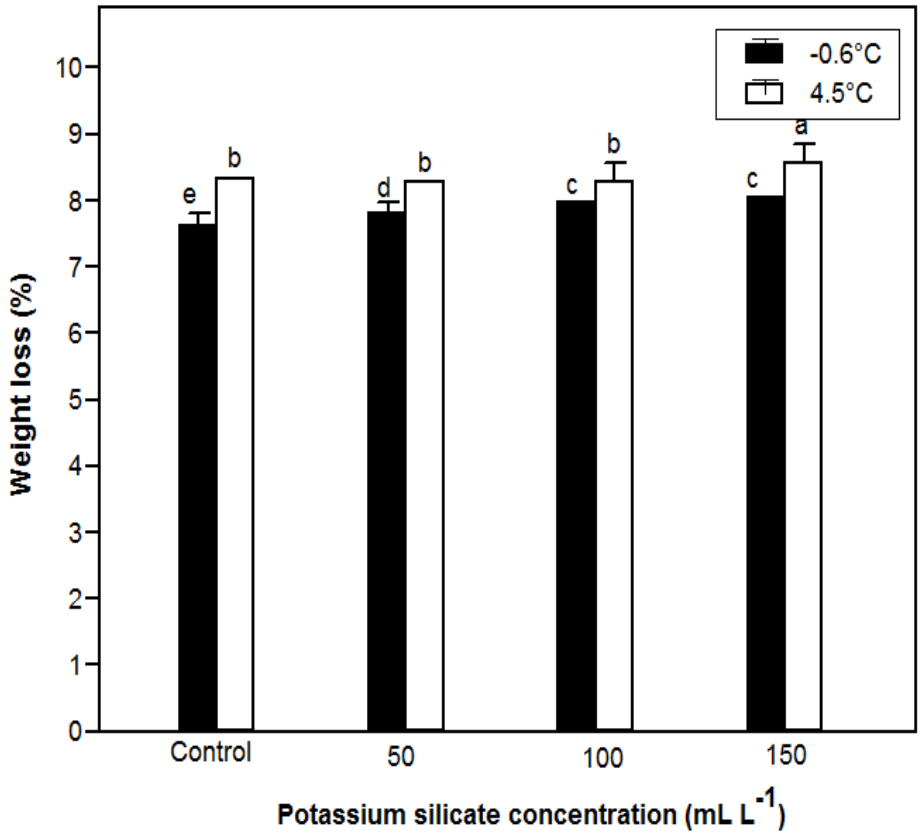


Figure 4.3 Effect of cold storage temperature and postharvest potassium silicate dips on “M37” mandarin fruit weight loss after 28 days of cold storage at -0.6 and 4.5°C plus 7 days shelf-life. Bars with a different letter within each potassium silicate treatment (concentration) were significantly different at P<0.05. Vertical bars indicate the standard error of means.

Firmness loss

An interaction between cold storage temperature and postharvest potassium silicate dips had no significant effect ($P>0.05$) on fruit firmness (Appendix 4.3). In general, the loss in fruit firmness of all the treatments was significantly higher on fruit cold stored at 4.5°C compared to -0.6°C. Furthermore, fruit stored at -0.6°C were firmer when compared with fruit kept at 4.5°C, irrespective of K_2SiO_3 concentrations (Figure 4.4). In general, fruit firmness decreased with an increase in K_2SiO_3 concentration, with the control and 150 mL L^{-1} K_2SiO_3 concentration showing the highest and lowest firmness loss percentage at both cold storage temperatures, respectively (Figure 4.4). Fruit treated with 50 and 100 mL L^{-1} K_2SiO_3 concentrations showed higher fruit firmness loss percentage when compared with those treated with 100 and 150 mL L^{-1} after storage at -0.6 and 4.5°C. However, there were no firmness loss mean significant differences between the control and fruit treated with 50 mL L^{-1} K_2SiO at both cold storage temperatures (Figure 4.4).

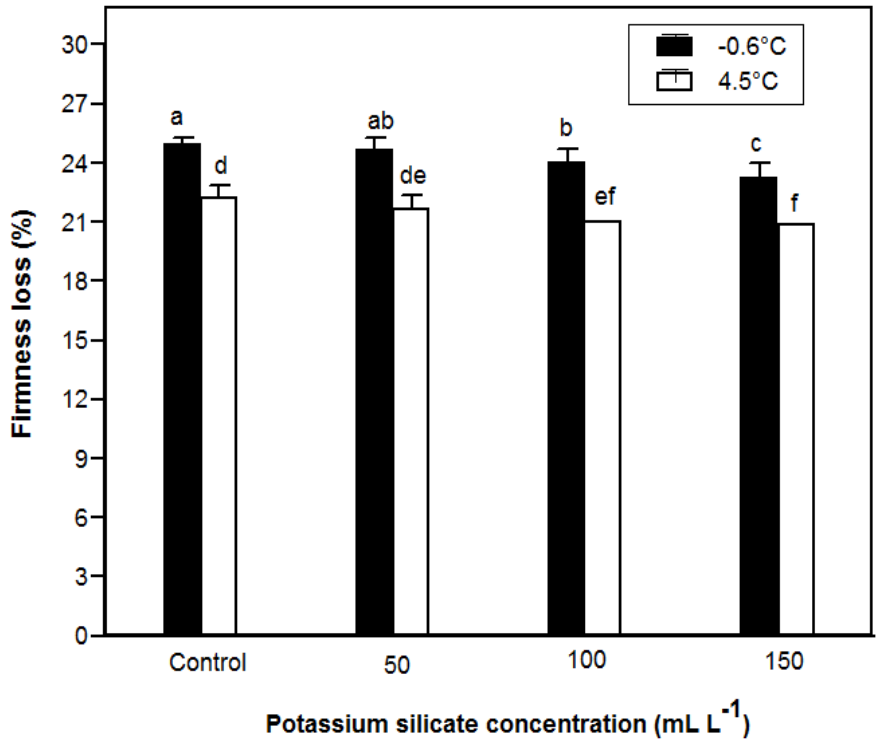


Figure 4.4 Effect of postharvest cold storage temperature and potassium silicate dips on “M37” mandarin fruit firmness loss after 28 days cold storage at -0.6 and 4.5°C plus 7 days shelf-life at room temperature. Bars with a different letter within each potassium silicate treatment (concentration) were significantly different at, $P<0.05$. Vertical bars indicate the standard error of means.

4.3 Biochemical properties

Electrolyte leakage

The results showed that an interaction between cold storage temperature and postharvest potassium silicate dips had no significant effect ($P>0.05$) on fruit electrolyte leakage after 28 days cold storage plus 7 days shelf-life (Appendix 4.4). Fruit electrolyte leakage at lower cold storage temperature (-0.6°C) was slightly higher when compared with higher cold storage temperature (4.5°C), irrespective of potassium silicate concentration (Figure 4.5). Furthermore, fruit treated with potassium silicate dips and cold stored at either -0.6°C or 4.5°C showed lower electrolyte leakage when compared with the control (Figure 4.5). Fruit dipped in 50 and 100 mL L^{-1} K_2SiO_3 concentrations and cold stored at -0.6 and 4.5°C showed lower electrolyte leakage when compared with the control and higher K_2SiO_3 concentration (150 mL L^{-1}). However, no significant mean differences were observed between fruit treated with lower (50 mL L^{-1}) and higher (100 and 150 mL L^{-1}) K_2SiO_3 concentrations (Figure 4.5). A similar trend was recorded at 4.5°C, in which, the electrolyte leakage of control fruit was higher when compared with fruit treated with different potassium silicate concentrations (50, 100, and 150 mL L^{-1} K_2SiO_3), however, mean differences were not statistically significant (Figure 4.5).

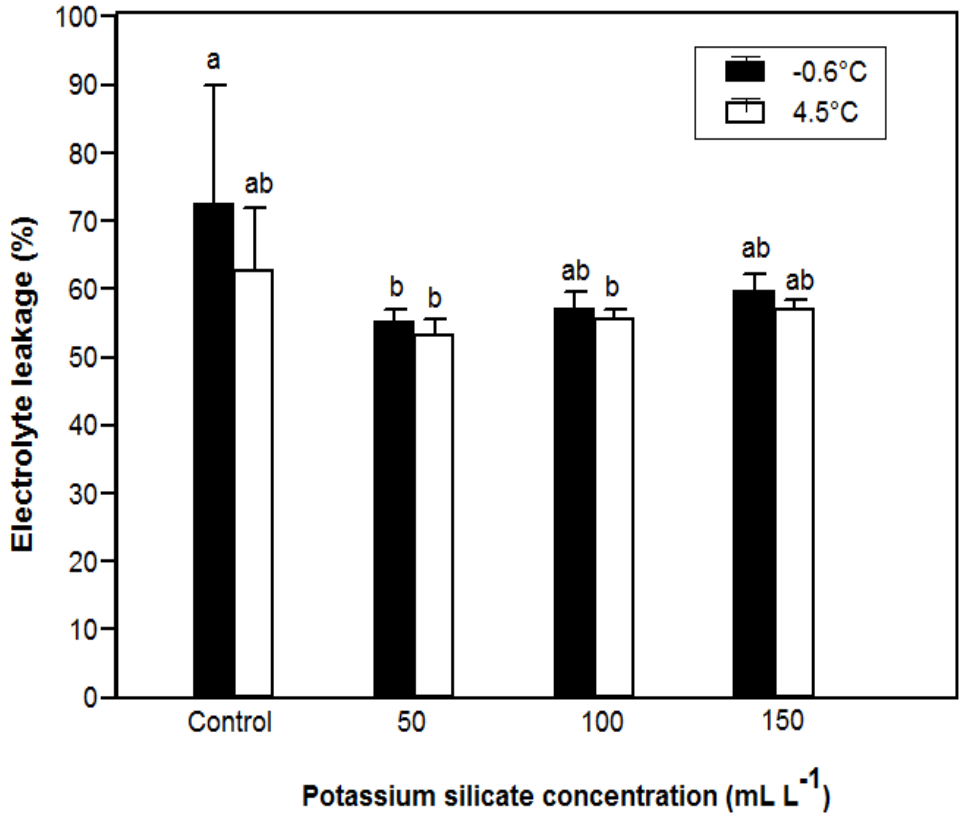


Figure 4.5 Effect of cold storage temperature and postharvest potassium silicate (K_2SiO_3) dips on "M37" mandarin fruit electrolyte leakage after 28 days cold storage at -0.6 and 4.5°C plus 7 days shelf-life at room temperature. Bars with a different letter within each potassium silicate treatment (concentration) were significantly different at, $P<0.05$. Vertical bars indicate the standard error of means.

Total soluble solids (TSS, °Brix)

The interactions between cold storage temperature and potassium silicate dips had significant ($P<0.05$) effect on fruit total soluble solids after 28 days cold storage plus 7 days shelf-life (Appendix 4.5). TSS was higher on fruit stored at 4.5°C when compared with -0.6°C, apart from control which showed higher TSS content at -0.6°C when compared with 4.5°C (Table 4.1). Moreover, TSS increased with an increase in K_2SiO_3 concentration on fruit cold stored at 4.5°C. Furthermore, control fruit showed the lowest TSS of 13.63 °Brix followed by 50 (13.73 °Brix), 100 (14.43 °Brix) and 150 mL L^{-1} (15.27 °Brix), respectively (Table 4.1). This increasing TSS response was also observed on fruit stored at 0.6°C, whereby, the control showed the lowest TSS content (14.7 °Brix) compared to 50 (13.57 °Brix), 100 (13.57 °Brix), and 150 (13.10 °Brix) mL L^{-1} K_2SiO_3 concentrations (Table 4.1).

Titratable acids (TA, %)

The interactive effect of cold storage temperature and postharvest potassium silicate dips on fruit titratable acids was significant ($P<0.05$) after 28 days cold storage plus 7 days shelf-life (Appendix 4.6). Fruit TA % was higher on fruit cold stored 4.5°C when compared with -0.6°C across all the treatments. TA decreased with the increase in postharvest K_2SiO_3 concentration after cold storage (-0.6°C), and the highest TA percentage was found on control (0.91%) followed by 50 (0.70%), 100 (0.84 %) and 150 (0.71 %) mL L^{-1} K_2SiO_3 concentration (Table 4.1). Furthermore, a similar trend was observed on fruit kept at 4.5°C, whereby, control and higher K_2SiO_3 (150 mL $L^{-1)}$ concentration showed the lowest and highest TA values of 0.72 and 1.03%, respectively. TA values of fruit treated with 50 (0.78%) and 100 (0.8%) mL L^{-1} were significantly lower when compared with the control (0.72%) and 150 (1.03%) mL L^{-1} K_2SiO_3 concentration at 4.5°C; although, there were no significant mean differences in TA between fruit treated with 50 and 100 mL L^{-1} K_2SiO_3 concentrations (Table 4.1).

TSS/TA ratio

Cold storage temperature and potassium silicate dips exerted a significant interactive effect ($P<0.05$) on fruit TSS/TA ratio after 28 days cold storage plus 7 days shelf-life

(Appendix 4.6). In general, fruit kept at 4.5°C showed higher TSS/TA ratio compared to those at -0.6°C. Control fruit had the highest TSS/TA ratio than all the potassium silicate treatments, after removal from either -0.6 and 4.5°C (Table 4.1). Furthermore, the results of the study showed that, fruit TSS/TA ratio decreased with increasing K_2SiO_3 concentration, at all the storage temperatures.

Table 4.1: Biochemical properties of “M37” mandarin selection fruit after 28 days of storage at -0.6 and 4.5°C plus 7 days at room temperature (±23°C).

	-0.6 ºC			4.5 ºC		
K_2SiO_3 (mL L^{-1})	*TSS	**TA	***TSS/TA ratio	*TSS	**TA	***TSS/TA ratio
0	13.1 ± 1.13bc	0.74±0.04bc	17.70± 0.32 a	13.63±0.1bc	0.72±0.02c	18.93±1.33a
50	13.57± 0bc	0.78±0.06ab	17.38 ± 0.84a	13.73±0.7bc	0.70±0.01bc	17.60±0.21ab
100	13.57± 0.47bc	0.82±0.19ab	16.54± 2.27ab	14.43±0.84abc	0.81±0.11bc	17.81±0.92abc
150	14.7.1± 0.47bc	1.03±0.19a	14.27± 2.29bc	15.37±0.84a	0.91±0.11ab	16.89±0.84bc
P value	0.025	0.005	0.022	0.025	0.005	0.025

Values are presented as means $\pm$ standard error

Means followed by different letters in each column are significant at Duncan’s Multiple Range Tests, P <0.05.

*TSS (Total soluble solids)

**TA (Titratable acids)

*** TSS/TA ratio (Total soluble solids: Titratable acids ratio)

CHAPTER 5
DISCUSSION

5.1 Chilling injury index

South African citrus fruit must be cold sterilised at sub-zero temperatures (-0.6ºC) for 21-22 days during exportation to certain lucrative markets as a phytosanitary treatment against Mediterranean fruit fly (*Ceratitis capitata*) (Wiedemann) (Diptera: Tephritidae) (White and Elson, 2004; EPPO, 2007; Hordijk, 2013) and invader fruit fly (*Bactocera dorsalis*) *(*Mathaba and Bertling, 2013). However, citrus fruit could develop symptoms of chilling injury (CI) at temperatures below 8°C (El-hilali *et al*., 2003). The findings in this study showed that CI developed when fruit were stored at either -0.6 or 4.5°C, and symptoms were visible as external discolouration on the fruit peel. The chilling injury symptoms became more severe, especially after the fruit were transferred to room temperature for 7 days (shelf-life), which was in agreement with the findings of Lafuente *et al*. (2005) who previously reported that CI symptoms develop during cold storage and become severe during shelf-life. Higher storage temperatures and weight loss during shelf-life enhance the severity of chilling injury (Schirra and D'hallewin, 1997). Therefore, this explain, higher occurrence of weight loss observed on fruit kept at 4.5°C when compared with fruit stored at -0.6°C.

Storage temperature played a role in the chilling susceptibility of "M37" mandarin fruit and symptom severity varied with storage temperatures. On all the treatments, chilling injury was slightly higher on fruit stored at -0.6°C compared to those stored at 4.5°C. These findings were in agreement with Concellon *et al*. (2005), who observed higher chilling injury on 'Money Maker No. 2' eggplant fruit stored at 0°C for 6 days compared to fruit stored for more than 13 days at 10°C. However, in 'Eureka' lemon fruit, storage at lower temperature (-0.5°C) for 28 days under 85-90% RH plus 7 days at 20°C resulted in less chilling injury compared with when fruit were stored at relatively higher temperature (4.5°C) for the same storage period (Siboza and Bertling, 2013). According to Siboza and Bertling (2013), the manner in which a fruit evade chilling injury during cold storage is associated with a high pool of antioxidants accumulated during growth and development. Whereas, chilling susceptibility is associated with higher levels of reactive oxygen species (ROS) and lipid peroxidation. Therefore, prolonged storage of fruit at a lower temperature (-0.6°C) possibly increased the ROS level and membrane permeability leading to the leakage

of electrolytes (Mao *et al*., 2007), and consequently led to the development of visible chilling symptoms. In addition, these results further suggest that antioxidants, although not quantified in the present study, could had played a vital role in reducing the development of chilling injury on fruit kept at a higher storage temperature when compared with sub-zero temperature.

Recently, postharvest studies have established the potential of silicon dips as a possible treatment that could be used to mitigate chilling injury during long-term cold storage of horticultural fruit crops (Mditshwa *et al*., 2013; Nasar *et al.,* 2013). Likewise, the results of this study indicated that dipping fruit in 50 and 100 mL L^{-1} K_2SiO_3 concentration significantly reduce CI symptoms after cold storage (-0.6 and 4.5°C) and shelf-life compared with control and 150 mL L^{-1} K_2SiO_3 concentration. The 50 and 100 mL L^{-1} K_2SiO_3 concentrations were highly effective at 4.5°C when compared with -0.6°C. These results were in agreement with Mditshwa *et al.* (2013), who found that lower Si concentrations (50 mg L^{-1}) reduced chilling symptoms on 'Eureka' lemon fruit after 28 days of storage at -0.5°C under 90% RH plus 5 days shelf-life. According to Mditshwa (2012), treating 'Eureka' lemon fruit with Si increased flavonoid and phenolic accumulation which is responsible for mitigating CI. Moreover, there is increasing evidence suggesting that the antioxidant pool in citrus fruits flavedo plays a pivotal role in the resistance to CI. For instance, the reduction of heat stress, drought and salinity in Si-treated horticultural crops had been previously associated with increased activation of stress-induced antioxidant systems that quench reactive oxygen species responsible for oxidative stress (Maksimovic *et al.*, 2007; Cai *et al.*, 2009). Therefore, the efficacy of 50 and 100 mL L^{-1} K_2SiO_3 concentration in reducing CI on our study might be attributed to its ability to activate the expression of antioxidant defence genes.

5.2 Physical properties

Weight loss

Fruit continue to respire and lose water to the surrounding environment after harvest as a consequence of transpiration (Roongruangsri, 2013; Rab *et al*., 2015). In fact, weight loss as low as 5-6% during long-term storage of mandarins and oranges

could result in fruit having a shrivelled appearance rendering them unmarketable (Ben-Yehooshua, 1969; Prasad, 1996; Hung *et al*., 2011). The relative humidity and storage temperature at which the produce is kept at are the primary factors that affect weigh loss (Maguire *et al*., 2001; Jourbert, 2016). For instance, in the present study, fruit stored at 4.5°C for 28 days plus 7 days shelf-life lost more weight when compared with those kept -0.6°C for the same storage period. Similarly, 'Fortune' mandarin fruit cold stored at 12°C with 80–90% RH for 45 days showed higher weight loss of 2-15% compared to 2-5% lost at 2°C (Gonzalez-Aguilar *et al*., 1997). Likewise, Storage trials conducted by Roongruangsri *et al*. (2013) on 'Sai Num Phueng' and 'See Thong' tangerine showed that the losses of fruit weight and moisture content of the peel during storage at 5°C, 58±2% RH were lower than at 25°C, 85±2% RH in both tangerine cultivars. This could be due to high respiration and transpiration rate as a result of storing fruit under high temperatures (Aferez *et al*., 2005; Rab *et al*., 2015). Based on these findings, it was clear that it is important to control RH and temperature, to reduce weight loss on "M37" mandarin fruit.

In general, the postharvest treatment with different potassium silicate concentrations (50, 100 and 150 mL L^{-1}) increased "M37" mandarin fruit weight loss during cold storage at -0.6 and 4.5°C (85-90% RH), with fruit treated with the highest Si concentration (150 mL L^{-1}) showing the highest weight loss. Similar results were reported by Mditshwa (2012), who stated that 'Eureka' lemon fruit weight loss increased with K_2SiO_3 concentrations during storage at -0.5 or 2°C and 90% RH for 28 days plus 5 days at 20°C. In contrast, Bertling *et al*. (2009) reported a rapid decline in weight loss of untreated 'Hass' avocado fruit when compared with silicon dips treated fruit (5000, 13000 and 25000 ppm) after 16 days of storage at 5.5ºC , with the highest silicon dip concentration (25000 ppm) maintaining the highest weight. Nonetheless, Mditshwa *et al*. (2013) advised that high silicon concentrations (150 and 250 mg L^{-1}), might cause cell membrane damage; an event associated with cracks, consequently, increased moisture and weight loss. In this study, such findings could be used to elucidate higher weight loss observed on "M37" mandarin fruit treated with 150 mL L^{-1} K_2SiO_3 dips when compared with the control, 50 and 100 mL L^{-1} K_2SiO_3 dips. Consequently, this suggests that Si is not an effective treatment for weight loss prevention during postharvest handling of mandarins.

Firmness loss

Firmness has been used widely as a fruit quality index parameter as it is associated with physiological maturity, freshness, bruising, texture, compression and damage of fruit (Ladaniya, 2008; Pranamornkith, 2009). Several studies have previously shown that changes in the firmness properties of a fruit were primarily reliant on storage temperature and duration (El-Hilali *et al*., 2003; Ali *et al*., 2004; Pranamornkith, 2009; Khorshidi *et al.,* 2010) and to a minor extent, due to relatively humidity. In the present study, cold storage temperature had a significant effect on "M37" mandarin fruit firmness after withdrawal from 28 days cold storage plus 7 days shelf-life at room temperature. Furthermore, at a higher temperature (4.5°C), fruit firmness loss was higher than at a lower temperature (-0.6°C), irrespective of postharvest potassium silicate concentration. These findings were similar to Pranamornkith (2009), who found higher firmness loss for Tahiti' lime fruit kept at higher temperature compared to those stored at a lower temperature after 8 and 10 weeks, respectively. In 'Tahiti' lime fruit, firmness reduction during cold storage at 2 and 5°C for 10 weeks was attributed to high weight loss and chilling injury manifestation, respectively (Pranamornkith, 2009). This was also observed on the current study, whereby, fruit firmness loss was high on fruit stored at higher temperature due to increased weight loss when fruit were held for additional 7 days at room temperature after withdrawal from cold storage. In general, fruit firmness reduction is enhanced by rapid water loss from the peel and degradation of cell wall enzymes such as cellulose, pectin methylesterase (PME) and hemicelluloses (Ali *et al*., 2004) The degradation of PME and hemicellulose enzymes in the cell wall increases at a higher rate under higher temperatures when compared with lower temperatures, thereby resulting in rapid firmness loss (Valero and Serrano, 2010).

The results of this study showed that the firmness of "M37" mandarin fruit stored at -0.6 and 4.5°C with 80-95% RH for 28 days plus 7 days shelf-life decreased with increase in K_2SiO_3 dips concentration, with the control and 150 mL L^{-1} K_2SiO_3 concentration showing the highest and lowest firmness loss percentage at both cold storage temperatures, respectively. In contrast, Tesfay *et al. (*2011) reported an opposite trend, whereby, 'Hass' avocado fruit treated with different postharvest silicon application (0, 5000, 13.000 or 25000 mg L^{-1}) had lower firmness when

compared with control after 17 days of storage at 5.5°C followed by 5 days at 20°C. Similarly, Mditshwa (2012) found that dipping 'Eureka' lemon fruit in 150 and 250 mg L^{-1} K_2SiO_3 reduced firmness loss after 28 days of storage at 0.6 and 4.5°C with 90% RH. Bertling *et al.* (2009) showed that Si is deposited into the cell wall of 'Hass' avocado fruit immediately after application. According to Dehghanipoodeh *et al*. (2015), when silicon entered the cell wall of 'Camarosa' strawberry fruit, it formed a strong silica bond with the cellulose structure, a process facilitated by enzymes. This prevented cell wall degradation and maintained fruit firmness. Therefore, in the present study, when "M37" mandarin fruit were treated with low Si dips concentration (50 and 100 mL L^{-1}), Si might have translocated into the cell wall, and accumulated in the middle lamella where it interacted with cell wall pectin, and subsequently, maintained fruit firmness. However, such a hypothesis still needs to be tested. Furthermore, high firmness reduction on "M37" mandarin fruit treated with high Si concentration (150 mL L^{-1}) could be attributed to high weight loss observed on such fruit.

5.3 Biochemical properties

Electrolyte leakage

Electrolyte leakage is an effective parameter to assess membrane permeability, therefore, used as an indicator of chilling injury (Saltveit and Morris, 1990; Zhang *et al.,* 2011). According to Nishida and Murata (1996), cold storage temperature plays a significant role in cell membrane damage. For instance, Hussain *et al*. (2015) reported that storing 'Blood Red' sweet orange fruit at 2°C resulted in a higher electrolyte leakage compared to those kept for 60 days at 10°C. In addition, high electrolyte leakage on 'Blood Red' sweet orange stored at low temperature was associated with increased membrane permeability (Hussain *et al*., 2015). In the current study, fruit kept at -0.6°C and 85-90% RH for 28 days plus 7 days shelf-life had higher electrolyte leakage than those stored at 4.5°C. These results were not necessarily unexpected since chilling injury is associated with the increase in cell membrane permeability that results in higher electrolytes leakage (Marangoni *et al*., 1996; Mao *et al*., 2007). In chilling sensitive citrus cultivars, low storage temperatures cause membrane lipids to solidify. Such modification from a fluid to a gel phase causes contraction of the cell membrane, over time, the cell membrane

lipids cracks, leading to increased permeability (Lyons, 1973, Mathaba, 2012). Therefore, this suggests that the higher occurrence of electrolyte leakage observed on “M37” mandarin fruit cold stored at -0.6°C when compared with 4.5°C storage was due to the physical changes in membrane lipids. Moreover, the cell membranes possibly performed its functions more at 4.5°C and to lesser extent at -0.6°C, which then better explains the severity of chilling injury observed at low storage temperature.

However, treating fruit with different concentrations of potassium silicate dips effectively maintained membrane integrity, as lower electrolyte leakage was obtained with fruit treated with potassium silicate dips (50,100 and 150 mL L^{-1}) compared to control fruit at both cold storage temperatures. This was in agreement with results of Mditshwa *et al.* (2013), who demonstrated that Si treated fruit had lower electrolyte leakage when compared with fruit treated with water after 28 days of storage at 0.5°C with 90% RH plus 7 days shelf-life. This was attributed to the decrease in lipid peroxidation and membrane permeability as a consequence of Si altering the membrane lipids to increase chilling tolerance (Liang, 2004; Tuna *et al.,* 2004; Liang *et al.,* 2008). Physiologically, the decrease in plasma cell membrane permeability, cell membrane lipid peroxidation and integrity are amongst some of the process that leads to reduced electrolyte leakage (Zhu *et al*., 2004). The reduced electrolyte leakage of Si dips treated fruit might indicate a change in membrane lipids from saturated to unsaturated fatty acids, as a high unsaturated to saturated fatty acid ratio has been found to play an essential role in mitigating chilling injury in chilling sensitive mandarins (Nishida and Murata, 1996; Lafuente *et al*., 2007; Mditshwa, 2012; Hordijk, 2013). On the other hand, Mditshwa *et al*. (2013) reported that high silicon concentrations (150 and 250 mg L^{-1}) increased electrolyte leakage on Eureka’ lemon fruit that were showing chilling injury symptoms after 28 days of storage at -0.5°C and 90% RH followed by 5 days at 20°C. Likewise, in this study potassium silicate concentration of 150 and the control showed higher electrolyte leakage than the 50 and 100 mL L^{-1} K_2SiO_3 concentrations when fruit were stored at 0.6 and 4.5°C cold storage temperatures, respectively. Therefore, results obtained in this study suggest that low potassium silicate concentrations reduce electrolyte leakage in chilled fruit tissues thereby, reducing “M37” mandarin fruit susceptibility to chilling injury.

Total soluble solids (TSS) and Titratable acids

Chemical parameters like TSS, TA and TSS/TA have been frequently used in the citrus fruit to describe taste (flavour) with regards to the sweetness and acidity (Ladaniya, 2008). According to Hassain and Rab (2015), cold storage temperature slows down the rate of metabolic activities such as, respiration; and therefore, making the fruit less acidic and acceptable for consumption. In this study, fruit stored at a higher temperature (4.5°C) had the highest TSS and TSS/TA ratio when compared to those stored at lower temperature (-0.6°C). These results were in contrast with Tietel *et al.* (2012), wherein it was reported that for 'Or' or 'Odem' mandarin, storage at 2, 5 or 8°C followed by 3 days at 20°C showed no significant effect on TSS, TA and TSS/TA ratio of the fruit.

Likewise, Obenland *et al*. (2013) reported that, 'W. Murcott' mandarin fruit stored at 8°C had higher TSS and TSS/TA ratio when compared with those stored at 0 and 4°C, possibly, due to higher rate of weight loss. These results suggest that the higher TSS/TA ratio on fruit kept at -0.6°C could be linked to lower consumption of sugars as a result of metabolic activities reduction. However, higher TSS and lower TA on fruit stored at 4.5°C could be attributed to a mechanism known as *De novo* synthesis of organic acids as previously suggested by Echeverria and Valich (1989). This mechanism involves the breakdown complex carbohydrates during storage (Echeverria and Valich, 1989; Roongruangsri *et al*., 2013); a process more prevalent at higher temperatures than at low temperatures due to increased metabolic activities (Hassan *et al*., 2014; Rab *et al*., 2015).

Postharvest silicon dips have been shown to have had minimal effect on fruit total soluble solids, titratable acids and TSS/TA ratio. For instance, Tarabih *et al*. (2014) reported lower TSS, TA and TSS/TA ratio for 'Anna' apple fruit treated with potassium silicate dips (0.1, 0.2, 0.3%) when compared with control after 60 days of storage at 0±1°C and 90-95% RH. In contrast, the present study revealed that, potassium silicate dips had a significant influence on the TSS, TA and TSS/TA ratio of "M37" mandarin fruit. Furthermore, it was observed that fruit treated with different potassium silicate concentrations (50, 100 and 150 mL L^{-1}) had a higher TSS and

TSS/TA ratio when compared with the control after 28 days storage, at either -0.6 or 4.5°C, which was in agreement with results of Nasar *et al*. (2013). Storage at low temperature combined with potassium silicate treatment perhaps increased the accumulation of respiratory metabolites which resulted in slow decrease in fruit titratable acids, as previously suggested by Nasar *et al*. (2013).

Based on these findings, high TSS for fruit treated with potassium silicate dips, was possibly due to the high weight loss as observed in the present study, since, TSS percentage is a function of total dissolved solids and moisture content of the fruit However, further studies are necessary to test this hypothesis. According to Mditshwa *et a*l. (2013), at high concentrations, Si dips might cause cell membrane damage which is accompanied by cracks and water loss, subsequently, high fruit weight loss. Therefore, the occurrence of such a phenomena could better explain the higher TSS observed on fruit treated with 150 mL L^{-1} K_2SiO_3 dips when compared with the control and those treated with lower potassium silicate concentrations (50 and 100 mL L^{-1}) after storage for 28 days plus 7 days shelf-life.

CHAPTER 6
SUMMARY, CONCLUSIONS AND FUTURE RESEARCH

6.1 Summary and conclusions

This study investigated the effect of cold storage temperature and postharvest potassium silicate dips on the chilling susceptibility and physico-chemical properties of new mandarin selection “M37” fruit. The interaction between the two treatment factors showed no significant effect on fruit chilling injury, electrolyte leakage and firmness loss. However, showed a significant interactive effects were recorded on fruit weight loss, total soluble solid (TSS) and titratable acids (TA) and TSS/TA ratio. The chilling susceptibility and postharvest quality of “M37” mandarin fruit depended primarily on cold storage temperature.

Cold storage of “M37” mandarin fruit results in the development of CI symptoms, leading to reduced quality. It was also shown that storing fruit at 4.5°C resulted in reduced incidence of chilling injury compared to storing fruit at -0.6°C. Chilling susceptible “M37” mandarin fruit were found to have a high electrolyte leakage, total soluble solids and lower levels of titratable acids. These factors may serve as indicators of CI in “M37” mandarin fruit. Storing fruit at 4.5°C resulted in high weight loss coupled with severe firmness loss compared with storage at -0.6°CThis suggested that -0.6°C was an effective storage temperature with regard to reducing fruit weight and firmness loss although this temperature resulted in higher chilling injury symptoms and electrolyte leakage. It can therefore be concluded that expression of antioxidants involved in metabolic pathways although not quantified in the current study was possibly not highly affected when fruit were stored at 4.5 compared to -0.6°C. Moreover, the fruit membranes possibly performed its functions more at 4.5°C storage and to a lesser extent at -0.6°C.

Postharvest treatments using potassium silicate dips can be effective in improving the storage of “M37” mandarin fruit, preserve quality and extend the cold storage period. Treating “M37” mandarin fruit with potassium silicate enhance chilling tolerance in “M37” mandarin fruit during quarantine treatment at low temperatures. It is recommended that the use of -0.6°C as a quarantine treatment temperature is beneficial for slowing metabolism of “M37” mandarin fruit. Whereas, the 4.5°C is

recommend on “M37” mandarin when the occurrence of chilling injury is a concern. Treatment with 50 and 100 mL L^{-1} K_2SiO_3 were more effective in alleviating CI when compared with the control and 150 mL L^{-1} K_2SiO_3. The effect of 50 and 100 mL L^{-1} K_2SiO_3 concentrations on “M37” mandarin fruit chilling tolerance was assumed to be associated with the ability of Si to increase antioxidant pool.

In general, antioxidants are believed to be involved in the defence mechanisms against ROS damage, therefore, decreasing CI. Furthermore, 50 mL L^{-1} K_2SiO_3 treatment seemed to positively affect “M37” mandarin internal fruit quality by increasing membrane integrity and reducing water loss. The possible mode of action of silicon may be through the regulation of bioactive compounds involved in mitigating stress. The study also showed that silicon may be a suitable postharvest treatment for use by the South African citrus industry. However, the issue of appropriate concentrations to be used, particularly on “M37” mandarin fruit, requires further study.

6.2 Future research

- Pre-harvest factors which may predispose fruit to subsequent physiological disorder development should be investigated. Pre-harvest factors such as; environmental conditions, fertiliser application and crop load increase carbohydrate availability in developing fruit. Previously, studies postulated that pre-harvest practice increased antioxidant pool.
- Antioxidants maintain postharvest fruit quality due to their antagonistic power over reactive oxygen species. Therefore, future research should consider pre-harvest silicon application on “M37” mandarin fruit chilling injury susceptibility and quality.
- An understanding on the relationships that may exist if any antioxidants that may be triggered in response to the application of Si postharvest treatment and whether these antioxidants are implicated in the enhancement of chilling tolerance in new mandarin selection “M37” fruit deserves further detailed investigation.

- To mitigate chilling injury on different citrus fruits, the optimum postharvest potassium silicate concentrations should be investigated, these concentrations may range between 40 and 90 mL L^{-1}.

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APPENDICES

Appendix 4.1: ANOVA table for the effect of postharvest cold storage temperature and potassium silicate dips on fruit chilling injury index

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	0.17300	0.08650	0.97	
Temperature (T)	1	0.01889	0.01889	0.21	0.653
Silicon (S)	3	2.92859	0.97620	10.89	<.001
T x S	3	0.15696	0.05232	0.58	0.635
Residual	14	1.25459	0.08961		
Total	23	4.53204			

Appendix 4.2: ANOVA table for the effect of cold storage temperature and postharvest potassium silicate dips on fruit weight loss

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.024197	0.012099	1.43	
Temperature (T)	1	1.580839	1.580839	186.61	<.001
Silicon (S)	3	0.358122	0.119374	14.09	<.001
T x S	3	0.128155	0.042718	5.04	0.014
Residual	14	0.118601	0.008472		
Total	23	2.209914			

Appendix 4.3: ANOVA table for the effect of cold storage temperature and postharvest potassium silicate dips on fruit firmness

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	1.2321	0.6160	3.47	
Temperature (T)	1	45.0247	45.0247	253.77	<.001
Silicon (S)	3	8.5545	2.8515	16.07	<.001
T x S	3	0.4213	0.1404	0.79	0.519
Residual	14	2.4839	0.1774		
Total	23	57.7164			

Appendix 4.4: ANOVA table for the effect of postharvest cold storage temperature and potassium silicate dip on fruit electrolyte leakage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Replication	2	1.32	0.66	0.01	
Temperature (T)	1	22.64	22.64	0.30	0.594
Silicon (S)	3	615.23	205.08	2.70	0.086
S x T	3	155.45	51.82	0.68	0.578
Residual	14	1064.30	76.02		
Total	23	1858.93			

Appendix 4.5: ANOVA table for the effect of cold storage temperature and postharvest potassium silicate dips on fruit total soluble solids (TSS)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	2.8308	1.4154	2.71	
Temperature (T)	1	1.5000	1.5000	2.88	0.112
Silicon (S)	3	2.6233	0.8744	1.68	0.218
T x S	3	6.5967	2.1989	4.22	0.025
Residual	14	7.3025	0.5216		
Total	23	20.8533			

Appendix 4:6 ANOVA table for the effect of cold storage temperature and postharvest potassium silicate dips on fruit titratable acids (TA).

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.062633	0.031317	4.71	
Temperature (T)	1	0.001667	0.001667	0.25	0.625
Silicon (S)	3	0.098400	0.032800	4.93	0.015
T x S	3	0.133667	0.044556	6.70	0.005
Residual	14	0.093167	0.006655		
Total	23	0.389533			

Appendix 4.7: ANOVA table for the effect of cold storage temperature and postharvest potassium silicate dips on fruit TSS/TA ratio

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	8.250	4.125	1.79	
Temperature (T)	1	0.664	0.664	0.29	0.600
Silicon (S)	3	22.789	7.596	3.29	0.052
T x S	3	30.417	10.139	4.39	0.022
Residual	14	32.304	2.307		
Total	23	94.424			

Appendix 4.8: Abstract submitted and presented as poster at the Citrus Symposium, Champaign sport Resort, Drakensberg, KwaZulu–Natal, South Africa, 21–25 August 2016

Effect of cold storage temperature and postharvest silicon dips on chilling susceptibility of a new mandarin cultivar "M37"

Shibambu R.B.[1, 2]*, Mathaba N[2], Mafeo T. P[1]

[1]University of Limpopo, Department of Plant Production, Soil Science and Agricultural Engineering, Private Bag X1106, Sovenga 0727

[2]Agricultural Research Council–Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit, 1200

*rb.shibambu@gmail.com

The aim of this study was to evaluate the potential of postharvest silicon dips to mitigate chilling injury on the new mandarin cultivar "M37". Matured "M37" fruit were harvested from Eastern Cape, where after it was transported to Nelspruit for treatment, storage and analysis. Fruit were dipped into different silicon concentrations (0, 50, 100 and 150 mL L^{-1}) for 30 minutes, air dried and waxed. Afterwards, fruit were packed into smaller boxes and stored at -0.6 and $4,5^0$ C for 28 days. Evaluations were carried out before storage, immediately after storage (28 days) and after 7 days shelf life. Postharvest silicon dips (50 and 100 mL L^{-1}) significantly reduced chilling injury on fruit stored at 4.5^0 C when compared with fruit stored at -0.6^0 C. Fruit treated with 50 and 100 mL L^{-1} silicon concentration showed higher weight loss percentage at -0.6^0 C when compared with fruit stored at 4.5^0 C. Subsequently, after storage and 7 days shelf-life control fruit showed a higher percentage of fruit firmness lost when compared with silicon treated fruit. Silicon dips and storage temperature had no significant effect on TSS and TA. Furthermore, silicon dipped fruit stored at -0.6^0 C showed a higher electrolyte leakage percentage. Increased electrolyte leakage was associated with higher chilling symptoms observed in silicon treated fruit stored at -0.6^0 C. In conclusion, silicon alleviated chilling injury at low concentrations when fruit where stored at a higher temperature.

Die invloed van opbergings temperatuur en silikon-doopbehandeling op die voorkoms van koueskade van "M37" mandaryn vrugte

Die doel van hierdie studie was om te bepaal of na-oes silikon doop behandeling koueskade van die nuwe mandaryn kultivar M37 kan verminder. M37 vrugte was geoes in die Oos-Kaap, waarna dit na Nelspruit vervoer was vir behandeling, opberging en analise. Vrugte was gedoop met verskillende silikon konsentrasies (0, 50, 100 en 150 mL L^{-1}) vir 30 minute, gewinddroog en gewaks. Daarna was vrugte verpak in kleiner kartonne en opgeberg by -0.6 en 4.5^{0} C vir 28 dae. Vrugte was geëvalueer voor, direk na en 7 dae na koelopberging. Na-oes silikon doopbehandeling (50 en 100 mL L^{-1}) het koueskade beduidend verminder op vrugte wat by 4.5°C opgeberg was in vergelyking met vrugte wat by -0.5°C opgeberg was. Vrugte wat met 50 en 100 mg·L^{-1}silikon behandel was en teen -0.5°C opgeberg was, het meer gewig verloor as die wat by 4,5°C opgeberg was. Gevolglik het kontrole vrugte by 7 dae raklewe meer fermheid verloor as silikon behandelde vrugte. Die verskillende silikon dips en opbergings temperature het geen beduidende effek gehad op TOV en TA nie. Vrugte van die silikon doopbehandelings wat teen -0.6°C opgeberg was het hoër elektroliet lekkasie getoon. Verhoogde elektroliet lekkasie van silikon behandelde vrugte opgeberg teen -0.6°C het verband gehou met die hoër voorkoms van koueskade simptome. Die gevolgtrekking was dat laer konsentrasies silikon koueskade kan verminder wanneer vrugte by hoër temperature opgeberg word.

Appendix 4.9: Abstract submitted and presented as oral at the Faculty of Science and Agriculture research day, Bolivia Lodge, Polokwane, South Africa, 24-25 October 2016

EFFECT OF COLD STORAGE TEMPERATURES AND POST-HARVEST SILICON DIPS ON CHILLING SUSCEPTIBILITY OF A NEW MANDARIN SELECTION "M37"

Rhulani B. Shibambu[1], Tieho P. Mafeo[1], Nhlanhla Mathaba[2]

[1]University of Limpopo, Plant Production, Soil Science & Agric. Eng. (PSAE), Private Bag x1106, Sovenga, 0727

[2] Agricultural Research Council-Institute for Tropical and Subtropical Crops (ARC-ITSC), P/Bag X11208, Nelspruit, 1200

Author email address: rb.shibambu@gmail.com

Citrus fruit exported from South Africa to USA and China requires cold treatment of negative 0.6°C for 22-24 days during shipment to sterilize any insect eggs and larvae in the fruit. However, this treatment often causes chilling injury. The new citrus selection "M37" developed by the ARC-ITSC has shown good internal qualities and has potential to become an export cultivar. However, it is highly susceptible to cold sterilization temperatures. Thus, the objective this study was to evaluate the potential of post-harvest silicon dips to mitigate chilling injury on "M37" selection. Fruit were dipped into different silicon concentrations (0, 50, 100 and 150 mL L^{-1}) for 30 minutes, air-dried and waxed. Afterwards, fruit were packed into smaller boxes and stored at -0.6 and 4.5°C for 28 days. Evaluations were carried out before and after 21 days storage. Silicon treatment of 50 and 100 mL L^{-1} reduced chilling injury on fruit stored at -0.6^0 C when compared with fruit treated with water and 150 mL L^{-1} but the effect was not significant. Interestingly, only fruit treated with 50 mL L^{-1} silicon reduced chilling damage after 21 days storage when compared with water and other higher concentration. Weight loss increased with an increase in silicon concentration at both -0.6 and 4.5°C. Untreated fruit showed higher electrolyte leakage at -0.6 and 4.5^0 C. Moreover, storing fruit at -0.6 and 4.5^0 C with or without silicon treatment

showed high and low firmness reduction, respectively. Silicon reduced chilling symptoms at both sub-zero and high storage temperatures. In conclusion, silicon treatment alleviated chilling injuries at both storage temperatures.

Keywords: Mandarin fruit (*Citrus reticulata*); chilling injury; firmness; weight loss; electrolyte leakage.

Appendix 4.10: Abstract submitted and presented as oral at the Combined Congress, ATKV Klein-Kariba Holiday Resort, Bela-Bela, South Africa, 23-26 January 2017

EFFICACY OF POST-HARVEST SILICON DIPS ON THE MANAGEMENT OF CHILLING SUSCEPTIBILITY OF "M37" MANDARIN SELECTION

R.B Shibambu[1, 2], N Mathaba[2], T.P. Mafeo[1]

[1]University of Limpopo-SAES, P/Bag X1106, Sovenga, 0727

[2] ARC-ITSC, P/Bag X11208, Nelspruit, 1200

INTRODUCTION

Citrus fruit exported from South Africa to USA and China require cold treatment of -0.6°C for 22-24 days during shipment to sterilise any insect eggs and larvae in the fruit. However, this treatment often causes chilling injury (Hordjik, 2013). Furthermore, sufficient empirical results showed that silicon induces resistance to both abiotic and biotic stress. Therefore, silicon could be applied to reduce chilling injury (Mditshwa, 2012). "M37", a new selection developed by the ARC-ITSC has shown good internal qualities, with export potential. However, it is highly susceptible to cold damage under sterilization temperatures. Thus, the aim of this study was to evaluate the potential of post-harvest silicon dips to mitigate chilling injury on "M37" selection.

MATERIALS AND METHODS

Mandarin fruit “M37” were dipped into different silicon concentrations (0, 50, 100 and 150 ml/L) K_2SiO_3 for 30 minutes, air dried and waxed with citrishine®. Afterwards, fruit were packed into smaller boxes and stored at -0.6 and 4.5°C for 28 days. Fruits were evaluated after beginning of the experiment just after treatment application, at 21, 28 plus 7 days shelf-life. Fruits were evaluated for firmness, weight, electrolyte leakage, Total soluble solid (TSS), Titrable (TA) acidity and chilling injury.

RESULTS AND DISCUSSION

Treatment with 50 and 100 ml/L K_2SiO_3 reduced chilling injury on fruit stored at -0.6 and 4.5°C when compared with fruit water treated and higher concentration. Moreover, treatment with water or 100 and 150ml/L resulted in higher fruit weight loss and firmness during storage at -0.6 and 4.5°C and shelf-life. Furthermore, water treated fruit stored at -0.6 and 4.5°C showed higher electrolyte leakage compared to potassium silicate treatment of 50 ml/L. As expected, TSS increased and TA decreased with storage duration at -0.6 and 4.5 °C, irrespective of potassium silicate concentration. Contrary, on “M37” treated with 50 ml/L K_2SiO_3, TA increased with an increase in TSS.

CONCLUSION

In conclusion, lower concentration of potassium silicate dips alleviates chilling injury at both -0.6 and 4. °C.

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Keywords: electrolyte leakage, fruit weight loss, fruit firmness, total soluble solids, cold sterilization

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