THE POTENTIAL OF POST-HARVEST POTASSIUM SILICATE DIPS TO MITIGATE CHILLING INJURY ON CITRUS FRUIT

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DECLARATION

I, **ASANDA MDITSHWA**, declare that the research reported in this thesis is my own and is expressed in my own words. Any use, and/or reference made within it, of other authors in any form (e.g. ideas, equations, figures, text and tables) are properly acknowledged. A full list of the references employed has been included. I further declare that this thesis has not been submitted for any degree or examination at any other university.

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We certify that the above statement is correct.

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DEDICATION

This thesis is dedicated to the Mditshwa family. My father, Mr E.M Mditshwa, who did not only raise and nurture me, but also taxed himself over the years for my education and intellectual development. It is also dedicated to my late mother, Mrs. M. Mditshwa, who taught me invaluable lessons in life which became a source of motivation and strength in moments of despair.

ABSTRACT

The South African Citrus Industry is the second largest exporter of citrus, after Spain. The industry is under pressure to supply high quality fruit as well as to expand into new, high paying markets. However, high paying markets such as Japan and the USA require cold sterilised fruit as obligatory quarantine treatments against Mediterranean fruit fly (*Ceratitis capitata*) in order to reduce any possible spread of the pest. Citrus fruit originated from tropical climates and hence are chilling susceptible.

Chilling injury symptoms appear as dark brown spots, pitting and/or decay when fruit are transferred to shelf temperatures; thus reducing the marketability of citrus fruit. Therefore, there is need for methods to mitigate chilling injury. Previous studies have shown silicon to mitigate many forms of stress without any hazardous effect on human health. Thus, the aim of the study was to investigate the potential of post-harvest silicon dips in mitigating chilling symptoms in citrus fruit.

Briefly, fruit from two sources (Ukulinga Research Farm and Ithala Farm) were dipped in different silicon concentrations (0, 50, 150, and 250 mg ℓ^{-1}) for 30 minutes and thereafter stored at -0.5 or 2^0C for up to 28 days with weekly evaluation for chilling injury symptoms. Total antioxidants were determined using FRAP, ABTS, and DPPH assays under spectrophotometer. In addition, sugars, ascorbic acid, phenolics and flavonoids were analysed using High Performance Liquid Chromatography (HPLC).

Fruit from Ukulinga Research Farm showed significantly higher total antioxidants (ascorbic acid total phenolics and specific flavonoids hesperidin and naringin) and sugars relative to fruit from Ithala Farm. Low concentrations of silicon dips significantly reduced the appearance of chilling injury symptoms by inducing an enzymatic conversion of glucose to ascorbic acid, thereby increasing the antioxidant capacity of chilling susceptible fruit. Moreover, silicon increased the concentration of total antioxidants, total phenolics and total flavonoids. High silicon concentrations had a negative effect on post-harvest quality of lemons by increasing fruit weight loss and electrolyte leakage, resulting in appearance of chilling symptoms.

In conclusion, the study showed that silicon had potential to reduce chilling injury. However, high silicon concentrations raised concern, in particularly, on fruit appearance.

TABLE OF CONTENTS

DECLARATION i
ACKNOWLEDGEMENTS ii
DEDICATION iv
ABSTRACT v
TABLE OF CONTENTS vii
LIST OF FIGURES xi
LIST OF TABLES xiii

CHAPTER 1: LITERATURE REVIEW 1
1.0 INTRODUCTION 1
1.1 Chilling Injury 5
1.1.1 Economic impact of chilling injury 6
1.1.2 Physiology of chilling injury 7
1.2 Si and abiotic stresses 8
1.3 Antioxidants 11
1.3.1 Effect of Si on non-enzymatic antioxidants 12
1.3.1.1 Phenolics 12
1.3.1.2 Ascorbic Acid 13
1.3.2 Effect of Si on enzymatic-antioxidants 14
1.4 Chilling Injury and Carbohydrates 15
1.4.1 Effect of Si on carbohydrate 16
1.5 Electrolyte Leakage (EL) 16
1.5.1 Effect of Si on Electrolyte Leakage 16
1.5.2 Effect of EL on Chilling injury 17
1.6.1 Chilling Injury and Lipid Peroxidation 18
1.6.2 Si and Lipid Peroxidation 18
1.7 Problem Statement 19

1.8 Hypothesis 20
1.9 Objectives 20
1.10 LITERATURE CITED 22

CHAPTER 2: THE POTENTIAL OF POST-HARVEST SILICON DIPS IN CITRUS FLAVEDO TO MITIGATE CHILLING INJURY IN LEMONS BY REGULATING THE GLUCOSE-ASCORBIC ACID RELATIONSHIP 32
ABSTRACT 32
2.1 INTRODUCTION 33
2.2 MATERIALS AND METHODS 35
2.2.1 Plant material 35
2.2.2 Chemicals 35
2.2.3 Chilling injury and fruit weight loss determination 36
2.2.4 Electrolyte leakage determination 37
2.2.5 Ascorbic acid determination 37
2.2.6 Soluble sugars determination 38
2.2.7 Statistical analysis 38
2.3 RESULTS 39
2.3.1 Chilling injury 39
2.3.2 Effect of fruit source on flavedo fruit weight loss electrolyte leakage, ascorbic acid-glucose relationship, sucrose and fructose concentration 39
2.3.3 Principal component analysis 40
2.3.4 Effect of post-harvest silicon soaks on fruit weight loss, electrolyte leakage, glucose-ascorbic acid relationship, fructose and sucrose in lemon flavedo during cold storage 41
2.4 DISCUSSION AND CONCLUSION 42
2.5 LITERATURE CITED 46

CHAPTER 3: THE POTENTIAL OF POSTHARVEST SILICON DIPS TO REGULATE PHENOLICS IN CITRUS PEEL AS A METHOD TO MITIGATE CHILLING INJURY IN LEMONS 59
ABSTRACT 59

3.1 INTRODUCTION 60
3.2 MATERIALS AND METHODS 62
3.2.1 Chemicals 63
3.2.2 Determination of Electrolyte Leakage 63
3.2.3 Weight loss Percentage Determination 64
3.2.4 Extraction of phenolics 64
3.2.4.1 Free Phenolics 64
3.2.4.2 Conjugated phenolics 65
3.2.5 Determination of phenolics content 65
3.2.6 Determination of flavonoids content 66
3.2.7 Statistical Analysis 66
3.3 RESULTS 67
3.3.1 Chilling injury 67
3.3.2 Weight Loss 67
3.3.3 Electrolyte leakage (EL) 68
3.3.4 Phenolics 69
3.3.4.1 Free Phenolics 69
3.3.4.2 Conjugated phenolics 69
3.3.5 Flavonoids 70
3.3.5.1 Free Flavonoids 70
3.3.5.2 Conjugated Flavonoids 70
3.4 DISCUSSION AND CONCLUSION 71
3.5 LITERATURE CITED 75

CHAPTER 4: AN INVESTIGATION INTO THE EFFICIENCY OF THE TOTAL ANTIOXIDANTS ASSAYS IN SILICON TREATED LEMON 89
ABSTRACT 89
4.1 INTRODUCTION 90
4.2 MATERIALS AND METHODS 93
4.2.1 Chemicals 93

4.2.2 Chilling injury evaluation 94
4.2.3 Total antioxidants capacity (TAC) Assays 94
4.2.3.1 Ferric reducing antioxidant power (FRAP) assay 95
4.2.3.2 Total antioxidant activity (TAOA) 95
4.2.3.3 DPPH assay 96
4.2.4 Total phenolics 96
4.2.5 Lipid peroxidation determination 97
4.2.6 Mineral analysis 98
4.3 RESULTS 99
4.3.0 Antioxidant strength of antioxidant compounds occurring in citrus 99
4.3.1 Effect of silicon on chilling injury symptoms during cold storage 99
4.3.2 Effect of fruit source on total antioxidant capacity and lipid peroxidation in lemon rind 100
4.3.3 Effect of storage temperature on total antioxidants, total phenolics and lipid peroxidation of lemon flavedo during 101
4.3.4 Effect of cold storage period on total antioxidants, total phenolics and lipid peroxidation of lemon flavedo. 101
4.3.6 Principal component analysis 103
4.4 Discussion and Conclusion 104
4.5 LITERATURE CITED 109

CHAPTER 5 121
5.1 General Discussion 121
5.2 Conclusions 126
5.3 Recommendations and Future research 127
5.4 LITERATURE CITED 128

LIST OF FIGURES

CHAPTER 1

Figure 1.1: South Africa's lemon crop distribution between year 2000 and 2009. 6
Figure 1.2: Possible mechanisms for Si-mediated tolerance to chilling injury (modified from Liang *et al*., 2007). 9

CHAPTER 2

Figure 2.1: Lemon fruit without chilling symptoms (A). Chilling injury symptoms increased by high silicon concentration (250 mg ℓ^{-1} K_2SiO_3) (B).....35
Figure 2.2: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 28 days cold storage time plus 5 days shelf-life. 55
Figure 2.3: Effect of fruit source on lemon (A) weight loss after storage (B) weight loss after shelf-life (C) electrolyte leakage (D) glucose and ascorbic acid (E) fructose and (F) sucrose concentration. 55
Figure 2.4: Principal component analysis (PCA) showing correlation loadings (A). Score plot lemon sugars and chilling injury indicators (B). Score plot for the groups of sugars for lemons from different source with difference in chilling susceptibility, PC1 explains 83% and PC2 explains 13% of total variation. 56

CHAPTER 3

Figure 3.1: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 21 days (A) and 28 days (B) storage time. 84
Figure 3.2: Effect of post-harvest K_2SiO_3 dips on electrolyte leakage (A) and weight loss (B) of lemon fruit sourced from Ithala and Ukulinga Farm after 28 days cold storage, plus 5 days at room temperature. 84
Figure 3.3: The effect of K_2SiO_3 concentration and storage temperature production of free phenolics (A) and conjugated phenolics (B) of fruit sourced from Ithala and Ukulinga Farm stored at -0.5^0C and 2^0C during 28 days of cold storage. 85
Figure 3.4: The effect of K_2SiO_3 concentration and storage temperature production of free flavonoids (A) and conjugated flavonoids (B) of fruit sourced from Ithala and Ukulinga Farm stored at -0.5^0C and 2^0C during 28 days of cold storage. 85

CHAPTER 4

Figure 4.1: Mineral analysis as determined by energy dispersive X-ray spectroscopy (EDX)……97
Figure 4.2: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 21 days (A) and 28 days (B) storage time. 116
Figure 4.3: Effect of fruit source on total antioxidants, FRAP (A), DPPH (B), ABTS (C), and silicon (D),lipid peroxidation (MDA) (E) and total phenolics (F). 116
Figure 4.4: Evaluation of antioxidant capacity over different trolox equivalent using FRAP (A), ABTS (B) and DPPH (C) assays. 117
Figure 4.5: Principal component analysis (PCA) showing correlation loadings (A). Score plot lemon total antioxidants (FRAP, DPPH, ABTS), MDA, Silicon, and total phenolics (B). Principal component analysis (PCA) led to variation of 90% with principal component 1 (PC1) explaining majority of variation (80%) and principal component 2 (PC2) explaining 11% of total variation. 118

LIST OF TABLES

CHAPTER 2

Table 2.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage, sugars (glucose, fructose, sucrose) and ascorbic acid under -0.5^{0}C. 57

Table 2.2: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage, sugars (glucose, fructose, sucrose) and ascorbic acid under 2^{0}C. 58

CHAPTER 3

Table 3.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage and chilling injury percentage under -0.5ºC and 2ºC storage temperatures. 86

Table 3.2: Table 1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on production of phenolic compounds on lemon fruit susceptible to chilling injury stored at-0.5ºC and 2ºC storage temperatures. 87

Table 3.3: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on production of flavonoids on lemon fruit susceptible to chilling injury stored at-0.5ºC and 2ºC storage temperatures. 87

CHAPTER 4

Table 4.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3), cold storage time, fruit source on total antioxidants (FRAP, ABTS, DPPH), total phenolics, and lipid peroxidation (MDA) of lemons stored at -0.5^{0}C. 119

Table 4.2: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3), cold storage time, fruit source on total antioxidants (FRAP, ABTS, DPPH), total phenolics, and lipid peroxidation (MDA) of lemons stored at 2^{0}C. 120

CHAPTER 1

THE POTENTIAL OF POST-HARVEST POTASSIUM SILICATE DIPS TO MITIGATE CHILLING INJURY ON CITRUS FRUIT

LITERATURE REVIEW

1.0 INTRODUCTION

Fruit and vegetables contain vitamin A and C, folic acid and dietary fibre, and are an important source of nutrients in the daily diet (Mukherjee, 1997). Nutritionally, fruit contributes 39% of vitamin C, whilst vegetables contribute 50% (Tsaneem, 2004). Citrus fruit, such as lemons contain folic acid and have high antioxidant potential so that their consumption can reduce cardiovascular disease and cancer risks (Gorinstein *et al*., 2001; Sun *et al*., 2002; Tsaneem, 2004). Folic acid also reduces the risk of infant neural tube defects (Brouwer *et al*., 1999). For this reason, consumers often prefer fresh produce as opposed to processed fruit because vitamins and folic acid are partly lost when produce is processed or cooked. The loss of vitamins is caused by removal of pulp from the fibrous part of fruit when juice is filtered hence consumers are often advised to eat fruit rather than to drink juice (Ladaniya, 2008). The world lemon and lime production between 1996-1998 was 9.25 million metric tonnes (MT) with 7.3 million MT used in the fresh market and 1.9 million MT

processed (Spreen, 2003). This confirms the demand for fresh produce harvested at the right time.

Citrus is a non-climateric fruit, thus it is not capable of continuing with ripening once it has been picked off the tree (Kader, 1997). The fruit is harvested when eating quality is at an optimum; hence there is a probability of wastage. Vitamin C is an important antioxidant of citrus fruit. Unfortunately, vitamin C content decreases with fruit maturity in citrus (Ladaniya, 2008) and this consequently puts the harvested fruit at risk of any kind of stress. The postharvest wastage varies with marketing chains (Wills *et al.*, 1981). Generally, deterioration is of little concern when fruit is transferred from Farms to end users; but under more convoluted marketing systems, fruit may take a long time to reach end users. When marketing chains are longer, the fruit reaches end users at a lower quality, thus lowering commodity prices. The relatively short shelf life of fresh produce is a challenge; many Farmers experience economic failure because of produce deterioration. Surveys have also revealed that ample produce is wasted annually due to poor postharvest practices as well as the inability to prolong post-harvest shelf life where facilities and technologies are lacking (Tsaneem, 2004). Thus, postharvest wastage is of global concern.

The growth rate of lemon (*Citrus limon*) is anticipated to be low due to these reasons, even if cooling technologies are used (Spreen, 2001). High quality fruit can be supplied when exporters use improved transportation logistics (Spreen, 2001), particularly, high value crop such as lemon. Among the citrus family, lemon is one of the fruits most affected by deterioration in quality during storage under cool conditions, thus affecting the produce and its price.

Prolonging the postharvest shelf-life of lemon is achieved by shipping the fruit at sub-zero temperatures (-0.6ºC) for 22 days. This is also practised to comply with quarantine protocols of citrus export requirements (Cri, 2008). Amongst the various citrus species exported, lemon develops physiological disorders, such as rind breakdown, puffiness, colour loss, and most importantly chilling injury (Cri, 2008). Cold sterilization seems to be the only effective method to kill insect larvae hence to improve shelf life; however, chilling injury develops (Lyons, 1973; Wolfe, 1978; Spalding and Reeder, 1983; Tsaneem, 2004). Susceptibility to chilling injury is the main limiting factor for long-term lemon storage under low temperatures. Spalding and Reeder (1983) reported that chilling injury appears in the rind as small pitted areas or, in more serious cases, as brown, sunken areas of various size and shape arising from the coalescence of small areas of injury. This leads to large postharvest losses during storage. It is therefore imperative to develop methods that could reduce chilling injury in lemons.

Several methods have been used to reduce chilling injury in fruit and impressive responses have been found. Among these techniques, applied to many horticultural crops including lemons, are hot water dips(Mclauchlan *et al.*, 1997b; Sapitniskaya *et al.*, 2006; Mathaba *et al.*, 2008; Mclauchlan *et al.*, 1997a), intermittent warming (Wang and Baker, 1979; Schirra and Cohen, 1999; Kluge et al., 2003), waxing(Wild, 1993; Petracek et al., 1998; Perez Gago et al., 2002),controlled atmosphere (Wang and Qi, 1997), storage and application of chemicals such as molybdenum (Xue-Cheng et al., 2006; Mathaba et al., 2008), ethylene (Wang, 1990;

Zhou et al., 2001) and methyl jasmonate (Gonzalez-Aguilar et al., 2000; Cao et al., 2009). Silicon (Si) is another promising chemical in this regard.

Silicon has been shown to induce resistance against both abiotic and biotic stresses in several agronomic plants (Matichenkov *et al.*, 1999; Ma and Yamaji, 2006). Several studies reported that Si was effective in controlling many fungal and bacterial diseases. Application of sodium silicate was found capable of reducing postharvest rot in Chinese cantaloupe (*Cucumis melo* L.), ring spot in sugarcane (*Saccharum officinanum* L) and powdery mildew in cucumber (*Cucumis sativus* L.) (Gou *et al.*, 2007; Ma and Yamaji, 2006). Keepings and Reynolds (2009) stated that a variety of abiotic stresses including drought, water logging, frost, salinity and heavy metal toxicity could be alleviated by Si application. Agarie *et al*. (1998) found that structural and functional deterioration of cell membranes could be prevented by Si application. Ma and Yamaji (2006) described several mechanisms by which Si enhanced resistance to various stresses. Provision of physical barrier when Si is deposited underneath the cuticle to form a cuticle-Si double layer, thereby reducing stress susceptibility has been reported. Si can also increase antioxidant defence systems, thus facilitating plant resistance to stress (Agarie *et al.*, 1998; Ma and Yamaji, 2006; Keeping and Reynolds, 2009).

1.1 Chilling Injury

Chilling injury is a physiological disorder that occurs in several horticultural commodities when stored at temperatures below certain threshold temperatures characteristic for the particular species (Lyons, 1973; Wolfe, 1978; Lafuente *et al*., 2005). The disorder was first discovered in 1897 by Molisch, naming it “Erfrieren” meaning “freezing” (Ryall and Lipton, 1979). The term “chilling injury” was first used by Eakes and Morris (1956), referring to physiological damage by low temperatures, and storing commodities at temperatures just above 0ºC.

Temperature is a critical factor in the maintenance of quality produce (Tsaneem, 2004). Generally, low temperatures lessen the rate of metabolic processes, and are an effective method for prolonging postharvest life of horticultural produce (Lyons, 1973). However, tropical and subtropical fruit, as well as vegetables, are unable to withstand low temperatures, hence chilling injury develops. Lyons (1973) mentioned that the threshold temperatures below which chilling-sensitive plant species suffer injury is most often around 10ºC to 12ºC. The region of origin of a species also impacts on chilling sensitivity with tropical and subtropical species being mostly prone to chilling injury than temperate species (Lyons, 1973).

1.1.1 Economic impact of chilling injury

Citrus is one of the largest export fruit commodities of South Africa. In 2009, the total cultivated area under citrus in South Africa was 58 101 hectares (ha), with total production estimated to be 1,000,346 tons/year (CGA, 2010). South Africa's lemon production was estimated to be 214,415 tons in 2009, of which129, 930 tons were exported. Lemons contribute significantly to the South African economy since most of the fruit produced is exported, with only a small portion sold on the local market (Figure 1). This is attributed to the fact that the export market gives higher revenue than the local market.

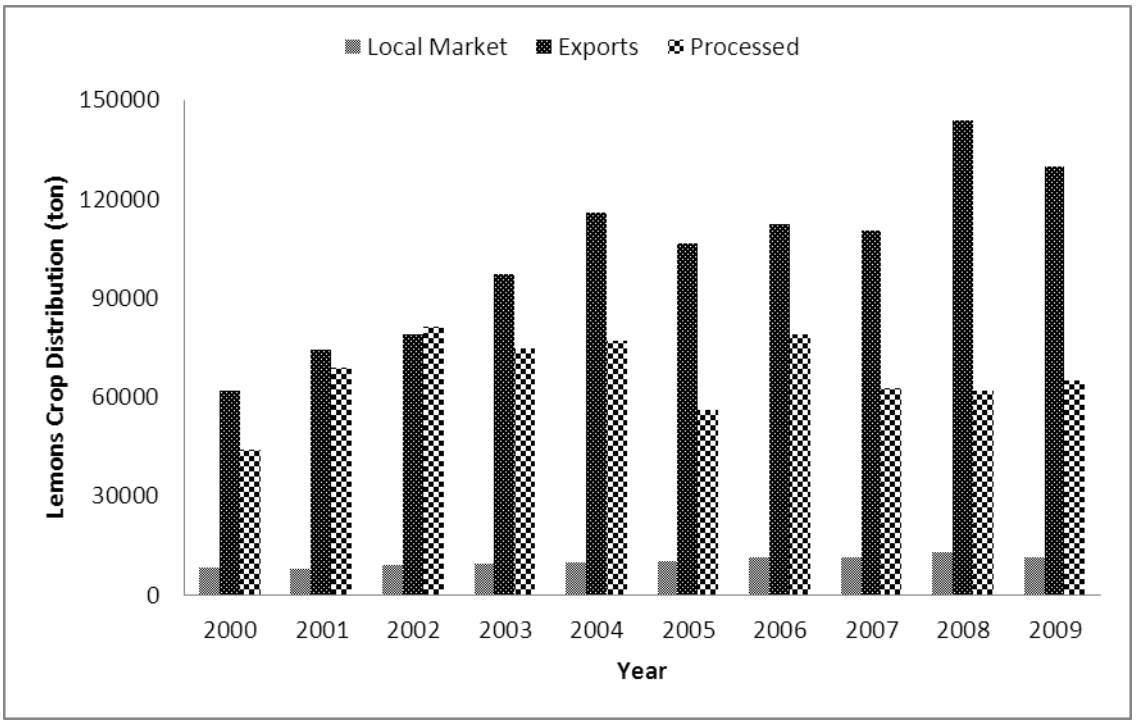

Figure 1.1: South Africa's lemon crop distribution between year 2000 and 2009.

However, the export market has unique challenges that require specialised postharvest procedures. There are terms and conditions of the export market that must be constantly followed for the produce not to be rejected. The presence of the Mediterranean fruit fly (*Ceratitis capitata*) requires cold sterilization quarantine treatments that must be applied to export citrus fruits (Serry, 2010). This often results in the development of chilling injury. Chilling injury manifests in the flavedo in the form of pitting, sunken areas on the scalding or browning; such fruit has low external quality even though internal quality might be excellent (Lyons, 1973; Mathaba *et al*., 2008; Serry, 2010). The resultant poor quality fruit results in low financial returns to the producers.

1.1.2 Physiology of chilling injury

The damage of cell membranes is the main cause of chilling injury. Transformation of membrane lipids from a liquid crystalline to a solid-gel structure has been associated with the damage caused by low temperature (Lafuente *et al.,* 2005). The ratio of saturated to unsaturated fats plays an important role in the sensitivity of produce to chilling temperatures. Generally, horticultural crops originating from tropical and subtropical climates contain more saturated fatty acids in their lipids compared to crops originating from cool climates (Lyons, 1973). When chilling sensitive crops are stored at low temperatures, the membrane lipids solidify and form a solid-gel structure (Lyons, 1973; Lafuente *et al*., 2005). Thereafter, events such as imbalance of metabolism, cell autolysis, and cell death occur, and chilling injury develops (Lyons, 1973; Lafuente *et al.,* 2005). Bio-membranes of horticultural crops from temperate

climates (chilling-resistant) contain more unsaturated fats than those of crops from warm climates (chilling-sensitive) (Wolfe, 1978). The solidification of these saturated lipids is partially responsible for the development of chilling injury (Lyons, 1973, Wolfe, 1978). This is attributed to the fact that the solidification causes the contraction of membrane causing the cracks and consequently increased membrane permeability (Lyons, 1973; Wolfe, 1978; Lafuente *et al*., 2005). The effect of increased membrane permeability is high electrolyte leakage from the cells, resulting in chilling injury.

1.2 Si and abiotic stresses

Pre and post-harvest use of Si has gained attention in recent years. Silicon has been associated with a variety of positive effects on growth of several plant species. It is believed to induce resistance to both abiotic and biotic stresses (Liang *et al.*, 2003; Zhu *et al.*, 2004; Hammerschmidt, 2005; Keeping and Reynolds, 2009; Epstein, 2009). Silicon application has been reported to alleviate an array of environmental stresses such as salinity (Liang et al., 2007a; Epstein, 2009), drought (Crusciol *et al.*, 2009), heat stress (Agarie *et al*., 1998) and frost (Epstein, 1999; Matichenkov et al., 1999) in fruit, vegetables, and ground crops.

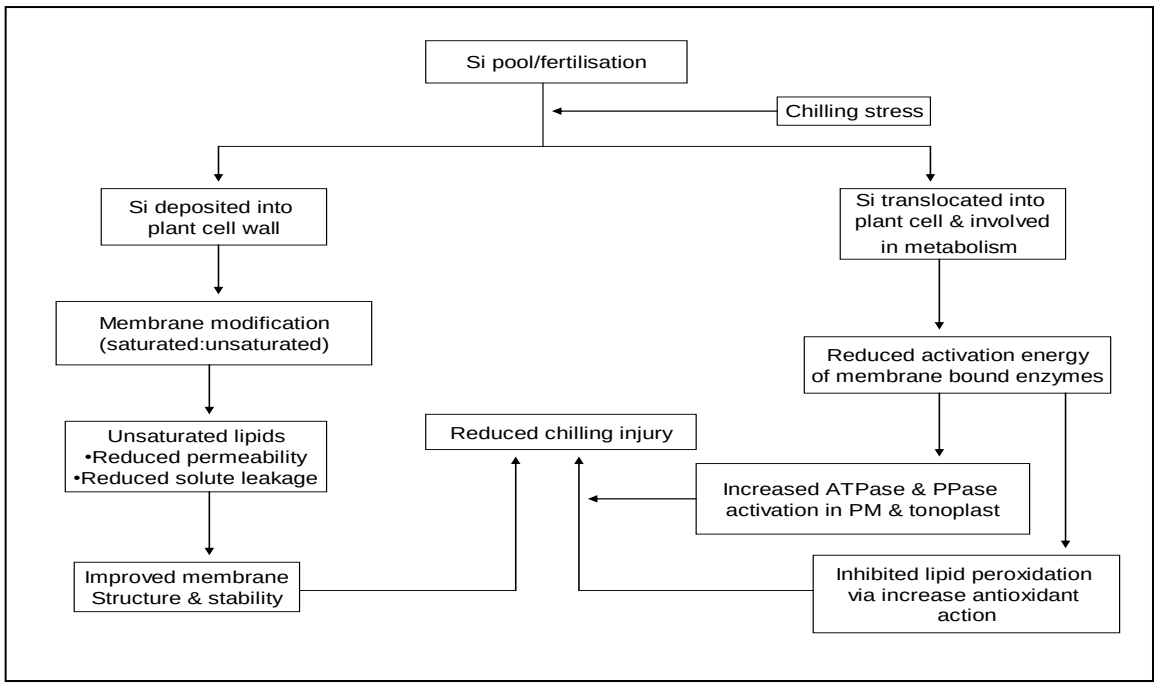

Figure 1.2: Possible mechanisms for Si-mediated tolerance to chilling injury (modified from Liang *et al*., 2007).

Briefly, after potassium silicate has been applied to a plant, Si is deposited in the plant cell wall. Epstein (2009) postulated that Si acts through providing protection by deposition of solid silica into the cell membrane. In addition, membranes are subsequently modified as production of unsaturated fatty acids is favoured over that of saturated fatty acids. Furthermore, increased saturation of membrane lipids results in greater membrane rigidity whilst decreased membrane fluidity results in decreased permeability and subsequently low electrolyte leakage. Membrane stability and integrity is thus improved and subsequently chilling injury is reduced.

Another explanation is that after Si is deposited into the cell wall it takes part in enzyme activities. The first prominent effect of Si is reduced energy requirements for

activation of membrane-bound enzymes, and reduction of membrane lipid peroxidation via stimulating enzymatic and non-enzymatic antioxidants (Liang *et al*., 2007). The incidence of chilling injury is thus reduced. This Si-mediated tolerance mechanism has proved to be effective in lemons grown in frost-prone conditions (Matichenkov *et al*., 1999). Silicon fertilization has been shown to improve salt tolerance in some plants. Zhu *et al*. (2004) found that the growth of salt-stressed cucumber plants improved following Si application. Liang *et al*. (2008) found freezing stress was alleviated in wheat plants following Si application. Longer fruit shelf life and improved maintenance of fruit quality are advantages both associated with Si application (Walter and Rein, 2004). The damage caused by high temperatures under field conditions can also be alleviated by application of Si. Electrolyte leakage caused by high temperatures was found to be lowered in rice leaves treated with Si relative to untreated leaves (Agarie *et al*., 1998). It appears therefore evident that cell membrane deterioration can be prevented by Si application (Liang *et al*., 2007a; Liang *et al*., 2008).

1.3 Antioxidants

One of the most important functions in a cell is to adapt to environmental fluctuations in order to maintain a constant internal environment. Under stress, the production of reactive oxygen species (ROS) is a vital part of physiology (Wong *et al*., 2006). Reactive oxygen species cause oxidative stress that represents an imbalance between production of ROS and the ability of biological systems to detoxify such oxygen species and to repair resultant damage. An antioxidant is a molecule competent of slowing or preventing the oxidation of other molecules (Bravo, 1998; Holetzky, 2003). Reactive oxygen species are detoxified by antioxidant defence systems consisting of non-enzymatic and enzymatic antioxidants (Shalata and Tal, 1998).

Non-enzymatic antioxidants include hydrophilic (water-soluble) antioxidants, such as ascorbic acid and glutathione, and hydrophobic (water-insoluble) antioxidants such as α-tocopherol and carotenoids (Shalata and Tal, 1998). Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Shalata and Tal, 1998; Holetzky, 2003). Amongst the toxic ROS that damage DNA, lipids and proteins causing cellular damage in fruit are the superoxide anions, hydrogen peroxide and hydroxyl radicals (Lafuente *et al.,* 2005). However, plants including citrus fruits have mechanisms of protecting themselves from the damage caused by oxidative molecules.

1.3.1 Effect of Si on non-enzymatic antioxidants

Non-enzymatic antioxidants include hydrophilic molecules such as ascorbic acid (vitamin C) and phenolics (flavonoids and flavanones), and hydrophobic including α-tocopherol and carotenoids (Shalata and Tal, 1998).

1.3.1.1 Phenolics

Phenolics are secondary metabolites that are produced by plants (Michalak, 2006). One aromatic ring with one or more hydroxyl group is a characteristic that distinguishes phenolics from other secondary metabolites (Michalak, 2006; Apak *et al*., 2007). Phenolics are classified into two groups, free and conjugated phenolics. Free phenolics are available in minute quantities, yet very effective in quenching reactive oxygen species (Cowan, 1999; Imeh and Khokhar, 2002a; Ayaz *et al*., 2005). Imeh and Khokhar (2002) highlighted that free phenolics are often found in dead or dying plant tissue, concluding that the presence of conjugated phenolics is more prevalent in living tissue. Free phenolics occurring in plants species include arbutin, catechol, hydroquinone, phloroglucinol and resorcinol (Green, 2007). Conjugated phenolics are bound to organic acids, sugars, lipids and membranes rendering them unavailable for use by the plant (Peleg *et al*., 1991). Phenolics play an important role in stress resistance in plants.

Application of Si is associated with enhanced phenol production resulting in healthy plants(Maksimovic *et al.*, 2007; Cai *et al.*, 2009). Maksimovic *et al*. (2007) found that powdery mildew resistance in cucumber following Si application was due to

enhanced production of phenolic compounds. Increased phenolic compounds in avocado roots are correlated with an induced resistance to biotic and abiotic stresses following Si treatments (Bekker *et al.*, 2007). Cai *et al*. (2009) found flavonoid levels to be enhanced when Si was applied to cucumber plants, with seedlings becoming stress-tolerant; Michalak (2006) reported that ROS were directly scavenged by flavonoids. The application of Si to citrus fruit, particularly postharvest, is expected to increase the phenolic content resulting in cold stress resistance and subsequently low chilling injury.

1.3.1.2 Ascorbic Acid

Ascorbic acid plays a crucial role in plant stress physiology. Ascorbic acid is associated with detoxification of ROS (Conklin, 2001). According to Conklin (2001) ascorbate peroxidase consumes ascorbic acid to make monodehydroascorbate (MDA) in the detoxification of hydrogen peroxide by reduction to water, while ascorbate peroxidase is able to remove hydrogen peroxide. Ascorbic acid prevents brown discolouration of flavedo tissue caused by low temperatures (Hidemi, 1998), and thereafter, also hinders the occurrence of chilling injury. There is little research on the relationship between Si and chilling injury. Silicon fertilization was reported to have increased ascorbic acid concentration in tomato fruit (Stamatakis *et al*., 2003). It is therefore uncertain whether Si has an impact on the production of ascorbic acid which is an antioxidant of great potential against chilling injury, particularly in citrus fruit.

1.3.2 Effect of Si on enzymatic-antioxidants

Silicon is reported to be involved in the production of enzymatic antioxidants that can reduce stress damage in plants. A study conducted by Gong *et al*. (2005) indicated that application of Si improved the water status of drought-stressed plants. The same author also reported that Si-treated plants had increased activities of some antioxidant enzymes including superoxide, catalase and glutathione reductase. On the other hand, non Si-treated plants had a high concentration of hydrogen peroxide and high oxidative stress. Zhu *et al*. (2004) observed that Si significantly reduced electrolyte leakage and hydrogen peroxide, and appreciably enhanced the activities of superoxide dismutase, catalase and glutathione reductase in salt-stressed cucumber plants. Silicon was also found to alleviate freezing stress and to enhance plant growth under freezing stress. According to Liang *et al*. (2008), higher antioxidant defence activity and lower lipid peroxidation and membrane permeability may be a possible mechanism, which is acquired through Si-enhanced water retention in leaf tissues. These findings suggested that Si strengthened the antioxidant defence system to protect cells from ROS. It is evident that Si is directly involved in the synthesis of antioxidants, and the defence activity that plants exhibit when Si is applied helps in controlling abiotic stresses such as chilling injury.

1.4 Chilling Injury and Carbohydrates

The sensitivity of plant tissues to low temperatures is influenced by carbohydrate levels (Tesfay, 2009). The spreading of chilling injury in fruit peel is marked by a significant reduction in soluble sugar content, especially sucrose, glucose, and fructose (Chhatpar *et al.*, 1971; Couee *et al.*, 2006).Wang (1990) found that resistance of grapefruit to chilling injury was due, in part to high levels of reducing sugars. Decreased sensitivity of plant tissue to low temperature is strongly associated with accumulation of sugars. According to Purvis and Shewfelt (1993), sugar accumulation increased the cell osmotic potential as well as the cell water potential, ultimately resulting in a reduced occurrence of chilling injury.

Sugars prevent desiccation by protecting membranes, by modifying the physical properties of membranes to be resistant to any form of stress (Crowe *et al.*, 1992).Soluble sugars have the ability to stabilize membranes and phospholipid vesicles, with trehalose being the most effective. Sucrose also has the ability to stabilize membranes, but relatively more sucrose is required to achieve the same stabilizing effect compared with trehalose (Crowe *et al.*, 1988). Lipid mobilization and fatty acid transfer are highly correlated with sugar starvation in cells. Sugars confer membrane stability and integrity (Couee *et al.*, 2006) thus retarding the advancement of chilling injury.

1.4.1 Effect of Si on carbohydrate

The effect of Si on carbohydrates is not yet clear. Si has been reported to be involved in the synthesis of sugars (Islam and Saha, 1969). Crusciol *et al*. (2009) reported that Si application reduced total soluble sugars and soluble protein concentrations in potato leaves. Similarly, Prabhu *et al*. (2007) working on rice plants, and Ahmed *et al*. (2008) working on wheat grain, found that Si significantly reduced total soluble sugar concentration. These findings suggest that there may be lack of consensus on the effects of silicon on carbohydrate concentration in plants or fruit.

1.5 Electrolyte Leakage (EL)

Electrolyte leakage is defined as leakage of cytoplasmic solutes from plant tissue, a phenomenon often reported following exposure to stress (Agarie *et al*., 1998). A relationship between electrolyte leakage and the extent of damage caused by stress seems to exist, whereby electrolyte leakage tends to be high when plant tissue have been exposed to stress for a long time.

1.5.1 Effect of Si on Electrolyte Leakage

The structural and functional deterioration of cell membranes in plants exposed to environmental stresses, such as chilling, can be prevented by Si (Liang *et al*., 2007). Use of Si in crops has been found to play a significant role in preventing electrolyte

leakage, possibly by enhancing the synthesis of cell walls thereby preventing electrolyte leakage indirectly (Agarie *et al*., 1998). Rice leaves grown with Si had lower electrolyte leakage than those grown without Si; suggesting that Si alters cell membrane composition. In the same study, Agarie *et al*. (1998) also reported that the degree of electrolyte leakage decreased as Si levels increased, and that Si-deficient plant tissue were prone to water stress while their cell membranes were easily damaged by water stress.

1.5.2 Effect of EL on Chilling injury

Chilling injury results in changes in membrane and cell permeability, and this is the result of the solute leakage and ion imbalance (Fuchs *et al.*, 1989). Wilson (1976) found that the incidence of chilling injury in fruits or vegetables increased with increasing electrolyte leakage.

1.6 Lipid Peroxidation

According to Lui *et al*. (1987), the accumulation of ROS in plant cells results in lipid peroxidation through oxidation of unsaturated fatty acids resulting in membrane damage and electrolyte leakage. Chilling injury is accompanied by lipid breakdown (Wongsheree *et al.*, 2009). Peroxidation of membrane lipids is an indication of membrane damage and electrolyte leakage under cold stress conditions (Katsuhara, *et al*., 2005).

1.6.1 Chilling Injury and Lipid Peroxidation

Chilling Injury is accompanied by lipid breakdown (Wongsheree *et al.*, 2009). Generally, fruits with high susceptibility to chilling injury show high lipid degradation following cold exposure. Lipid breakdown is associated with high lipoxygenase (LOX) activity, which is enhanced when chilling injury occurs (Wongshereee*t al*., 2009). Linoleic and linolenic acid are common substrates of lipoxygenase. The degradation of these fatty acid results in the production of peroxide ions (O_2^{2-}) (Grechkin, 1998).

1.6.2 Si and Lipid Peroxidation

The Si-levels in plant tissues seem to play an important role in determining whether lipid peroxidation will occur or not. The presence of Si changes the structure and functions of the plasma membrane by influencing stress-dependent peroxidation of membrane lipids (Agarie *et al*., 1998; Liang *et al*., 2007). Liang *et al*. (1998) and Lobalo *et al.* (2009) found that Si fertilization increased the strength of lipid membranes exposed to drought stress, preventing structural and functional deterioration of the cell membrane. Permeability of membranes and lipid peroxidation tend to decrease in response to Si application (Liang *et al*., 1998; Lobalo *et al*., 2009). Wang and Galletta (1998) discovered that application of Si to strawberry improved the ratio of unsaturated to saturated lipids, subsequently inducing stress resistance of membranes. This is attributed to the fact that unsaturated lipids are less chilling susceptible, therefore highly unsaturated to saturated lipid ratios result in low chilling susceptibility of a given tissue.

1.7 Problem Statement and methodology

Export of lemons is a challenge due to their high perishability, often resulting in economic losses. Export lemons must meet the high quality standard for high profitability. Reducing postharvest deterioration is of paramount importance in order to sustain competitiveness on the international market. Refrigeration has long been seen as the best method to lower lemon respiration during shipping thereby reducing perishability. Unfortunately, although low temperature storage prolongs shelf life and maintains high produce quality, it also results in chilling injury. Storing produce below its critical temperature results in damage to cell membranes and eventually, chilling injury.

To minimize the incidence of chilling injury in lemons, certain postharvest treatments are used. However, almost all current methods used to alleviate chilling injury have disadvantages. In this study, Si was applied postharvest to evaluate its performance relative to other treatments previously used to counteract chilling injury.

The fruit was harvested from two Farms. The first harvest was collected from Ukulinga Farm, a region of the KwaZulu-Natal Midlands regularly experiencing frost, while as second location Ithala Farm, a commercial Farm with 'up-to-date' management practices was chosen. Both Farms have a known history of chilling injury with Ukulinga Farm being resistant and Ithala Farm being very sensitive to chilling injury. The fruit was randomly harvested. The fruit was packed in crates and subsequently cold-stored. Fruit was also shuffled within the storage environment and

randomly selected for determination of factors involved in chilling injury. Thirty fruits were used per test with five fruits per replication.

1.8 Hypothesis

This research focused on the evaluation of Si (AgriSil^{TM}K50) and the comparison of different application rates (0, 50, 150, 250 ml/L of AgriSil^{TM}K50 in water) to alleviate the occurrence of chilling injury in lemons stored at low temperatures. The correct temperature at which lemons can be stored after Si application was evaluated. The hypothesis was that postharvest treatment of fruit with Si prior to storage may reduce the incidence of chilling injury and prolong shelf life. It was further hypothesized that postharvest treatment of fruit with Si enhances both external and internal fruit quality of lemons.

1.9 Objectives

The objectives of the study were to:

- Evaluate the potential of postharvest Si applications in decreasing chilling injury,
- Evaluate the optimum Si application rate required to achieve high external and internal quality,
- Determine the optimum storage temperature following Si treatment that would avoid the occurrence of chilling injury symptoms,

- Determine effects of Si application on fruit quality, and
- Understand the possible mode of action of Si in chilling injury, if any.

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CHAPTER 2

THE POTENTIAL OF POST-HARVEST SILICON DIPS IN CITRUS FLAVEDO TO MITIGATE CHILLING INJURY IN LEMONS BY REGULATING THE GLUCOSE-ASCORBIC ACID RELATIONSHIP

ABSTRACT

High value markets such as United States of America and China require cold sterilization of citrus fruit during shipping as a phytosanitary measure. However, cold sterilization may result in appearance of chilling symptoms which reduce the marketability of citrus fruit. Recently, silicon has shown potential to mitigate many forms of stress in agricultural produce and, so far, no research has elucidated the role of silicon on post-harvest cold stress. Therefore, the aim of this study was to investigate the potential of silicon post-harvest dips to mitigate the appearance of chilling symptoms in cold-stored lemon fruit. Fruit were obtained from two different locations differing in susceptibility to chilling injury. Fruit were soaked in 0, 50, 150 and 250 mg ℓ^{-1} K_2SiO_3 for 30 minutes, and thereafter air dried and waxed. Subsequently, fruit were stored at -0.5 or 2^0C and evaluated for chilling injury symptoms 0, 7, 14, 21, or 28 days later. Chilling-susceptible fruit sourced from Ithala Farm had significant lower flavedo glucose and ascorbic acid concentrations compared with chilling-resistance lemons from Ukulinga Farm. Furthermore, dips in lower concentrations of silicon significantly reduced the appearance of chilling injury

symptoms, probably by inducing the conversion of glucose to ascorbic acid, thereby increasing the antioxidant capacity of chilling-susceptible fruit. High silicon concentrations have a negative effect on lemon post-harvest quality as such treatments increase fruit weight loss, electrolyte leakage of exocarp and the appearance of chilling symptoms.

2.1 INTRODUCTION

Cold sterilisation of agricultural produce is a growing phytosanitary requirement against fruit fly for most exporting countries (McLaunchlan *et al*., 1997). However, such cold treatment may result in chilling damage, manifested as sunken lesions and discolouration of the citrus peel, thereby reducing fruit marketability (McLaunchlan *et al*., 1997). Within the citrus family, lemons are the second most chilling-susceptible citrus type after grapefruit (Chalutz *et al*., 1985). Exporting countries need to seek environmentally friendly methods to mitigate chilling symptoms, due to the strong demand for chemical-free agricultural produce (Shellie and Mangan, 2000).

Recently, silicon (Si) has been recognised to have beneficial effects on enhancing plant response to biotic and abiotic stress (Gunes *et al*., 2007; Liang *et al*., 2007). Silicon has been found to mitigate various forms of stress in different crops, reducing boron toxicity in spinach (*Spinacia oleracea* L.) (Gune *et al*., 2007), improving quality of water-stressed sorghum (*Sorghum bicolor* (L.) Moench. cv. Gadambalia) and maintaining quality and post-harvest shelf life of ‘Hass’ avocado fruit (*Persea Americana* Mill.) by maintaining membrane stability and enhancing antioxidant

capacity (Tesfay *et al*., 2010). However, there is little information on the potential role of Si to reduce damage due to postharvest cold stress in agricultural products.

Chilling injury (CI) is partly caused by oxidative stress, which occurs when the production of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), the superoxide radical ($O_2^{\cdot}$) and the hydroxyl radical ($OH^{\cdot}$) exceeds cellular critical threshold levels (Conklin, 2001; Huang *et al*., 2007). These oxygen-free radicals are high in energy and, therefore, rapidly attack cellular biomolecules, such membrane containing phospholipids, DNA, and proteins leading to cell death (Huang *et al*., 2007) following the appearance of chilling injury symptoms. However, certain carbohydrates have been found to play a significant role in reducing the appearance of chilling symptoms, specifically in citrus fruit (Holland *et al*., 2002).

Carbohydrates play multiple roles in plants, serving as energy reserves (Duffus and Duffus, 1984) as well as facilitating hydration of cellular membranes damaged by cold stress (Crowe *et al*., 1998; Back, 1979). Furthermore, glucose has been found to be the dominant carbohydrate in citrus flavedo (Aung *et al*., 2001) and acts through a cascade of enzymatic, rate-limited reactions as the fundamental substrate in the synthesis of ascorbic acid (AA), a potent antioxidant (Smirnoff, 2000). Ascorbic acid is a water-soluble antioxidant (Smirnoff, 2000) and contributing more to the antioxidant capacity of different citrus segments (Abeysinghe *et al*., 2007). Therefore, the objective of this study was to investigate the potential of post-harvest silicon dips to mitigate chilling injury in lemon fruit, and if such mitigation is achieved via conversion of glucose to AA.

2.2 MATERIALS AND METHODS

2.2.1 Plant material

Lemon (cv. Eureka) fruit were harvested from Ukulinga Research Farm (29°40'00'S, 30°24'00''E) in July 2010 and Ithala Farm (29°52'00''S, 30°16'00''E) in April 2011. Fruit was visually graded for appearance and absence of blemishes. Fruit were treated with Spore Kill® and thereafter soaked in various strengths (water, 50, 150 and 250 mg ℓ^{-1}) of potassium silicate (K_2SiO_3) solutions for 30 min (Tesfay *et al*., 2011) and air-dried before waxing with Avoshine® (Citrashine (Pty) Ltd). After treatments, fruit were weighed and stored at -0.5^{0}C or 2^{0}C under 85-90 % relative humidity (RH) for 7, 14, 21 or 28 days. Following storage fruit were weighed, evaluated for CI symptoms immediately after withdrawal from cold storage and five days later. Thereafter, fruit were peeled, the peel was freeze dried, ground using mortar and pestle and stored at -21^{0}C for subsequent AA and sugar analysis.

2.2.2 Chemicals

Oxalic acid, acetic acid, ammonium acetate, octylamine and ethanol were purchased from Sigma-Aldrich Chemical Co (St. Louis, Mo, USA).

2.2.3 Chilling injury and fruit weight loss determination

After 7, 14, 21 and 28 days of cold storage at -0.5^{0}C or 2^{0}C plus five days shelf-life fruit were evaluated for appearance of CI symptoms and weight loss. Fifteen fruits were used per treatment to determine weight loss. The following formulae were used:

Chilling injury (%) = (Number of fruit with chilling symptoms/ total number of fruit evaluated)*100

Fruit weight loss (%) = ((Initial weight loss – final weight loss)/ initial weight loss) * 100)

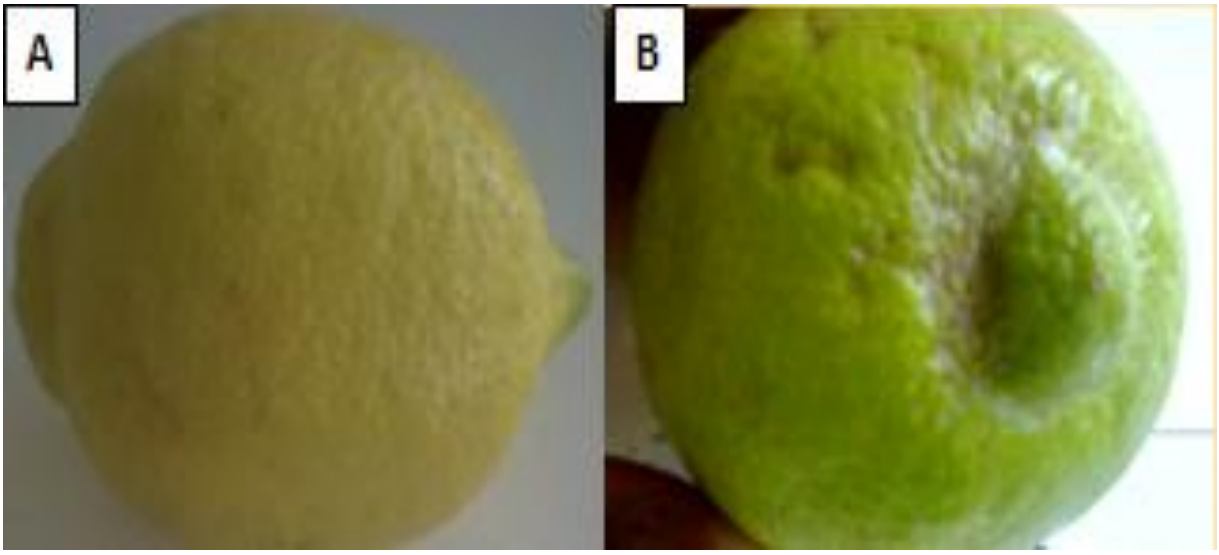

Figure 2.1: Lemon fruit without chilling symptoms (A). Chilling injury symptoms increased by high silicon concentration (250 mg l^{-1} K_2SiO_3) (B)

Chilling injury resulted in pitting, sunken areas and browning, this fruit had low external quality (Figure 2.1).

2.2.4 Electrolyte leakage determination

Membrane permeability following cold storage was evaluated by electrolyte leakage according to Zhu *et al*. (2004) with minor modifications. Three flavedo discs (1 cm diameter) were cut and placed into a test tube containing 10 ml deionized water; after being washed three times to eliminate the electrolyte leakage at the cut surface and surface contamination. After incubation at 25^0C for 3 hours, electrolyte conductivity (EC 1 in µSi x m^{-1}) was measured using an EC meter (HI 9033, Hanna Instruments, Johannesburg, RSA). The second EC reading (EC 2) was taken after the peel sample was placed in a shaking water bath at 100^0C for 1 hour and allowed to cool to room temperature.

The percentage electrolyte leakage was calculated as:

Total leakage (%) = (Initial reading (EC 1)/ final reading (EC 2))*100

2.2.5 Ascorbic acid determination

The method Abeysinghe *et al*. (2007) was used with sight modifications. Ascorbic acid was extracted from 0.1 g sample, using 10 ml 0.1 % oxalic acid. The sample was homogenised for 5 min using an Ultra-Turrax (IKA-T250, Germany) and centrifuged at 30 000 g (2^0C) for 10 min. The resulting supernant was injected into reversed phased HPLC system equipped with a UV detector (PDA-100 Photodiode Array detector) (Spark, Emmen, The Netherlands). The conditions of the system were: ODS C18 column (4.6 x 250 mm) flow rate 1.0 ml/min, injection volume of 20 µl, detector wavelength 251 nm. Ammonium acetate buffer (0.02 M, pH 5.4) with octylamine was

used as the mobile phase. The ascorbic acid concentration was expressed as mg 100 g^{-1} DM.

2.2.6 Soluble sugars determination

Soluble sugars were extracted and determined according to Aung *et al*., (1998). Freeze-dried lemon flavedo powder (0.1 g) was weighed into a glass test tube and 5 ml of 80 % ethanol/water added for extraction. The sample was homogenised for 1 min using Ultra-Turrax (IKA-T250) and incubated for 1 hour in 80^0C shaking hot water bath. After removal from the hot water bath, the test tubes were stored at 4^0C for 24 hours. Thereafter, the extract was filtered through glass wool and dried overnight (12 hrs) in a Savant Vacuum Drier (Savant, Farmingdale, New York, USA). The dried samples were made up to 2 ml using ultra-pure water and centrifuged for 15 min and filtered through 0.4 micron nylon syringe filter into HPLC vials (LC-20AT, Shimadzu Corporation, Kyoto, Japan). Ultra-pure water was used as a mobile phase at a 0.6 ml/min flow rate and sugar extract compared with authentic fructose, glucose and sucrose standards.

2.2.7 Statistical analysis

Data was subjected to analysis of variance (ANOVA) using Genstat, 12^{th} edition. The means were subjected to further analysis using the Duncan test at 5% confidence level and principal component analysis (PCA) using an Unscrambler (Version 9.8).

2.3 RESULTS

2.3.1 Chilling injury

Fruit from Ukulinga Farm did not show any CI symptoms during storage or following the 5 day shelf-life period. However, Ithala fruit showed CI symptoms after 28 days of cold storage plus shelf-life (Fig 2.1). Furthermore, CI symptoms did not appear during cold storage, but only after the shelf-life. Moreover, fruit stored in -0.5^{0}C had less CI symptoms than fruit stored at 2^{0}C (Fig 2.1).

Silicon was effective in reducing CI at 50 mg ℓ^{-1} K_2SiO_3 and stored at -0.5^{0}C for 28 days cold storage compared with control treatment and some treated with higher Si levels (Fig 2.1). At 2^{0}C there was no significant difference between the control and 50 mg ℓ^{-1} K_2SiO_3, while higher Si level increased the appearance of CI symptoms with both cold storage temperatures (Fig 2.1).

2.3.2 Effect of fruit source on flavedo fruit weight loss electrolyte leakage, ascorbic acid-glucose relationship, sucrose and fructose concentration

Ukulinga fruit stored at 2^{0}C showed significantly lower weight loss compared with Ithala fruit stored at -0.5 and 2^{0}C (Fig 2.2A). However, there were no significant differences in weight loss of Ithala fruit between -0.5 and 2ºC storage temperatures and Ukulinga fruit stored at -0.5^{0}C (Fig 2.2A).

Fruit weight loss after 5 day shelf-life was significantly lower in Ukulinga than Ithala fruit. Ukulinga fruit stored at 2^{0}C lost less weight than fruit stored at -0.5^{0}C (Fig 2.2 B).

However, Ukulinga fruit with lower fruit weight loss after shelf-life had a significantly higher electrolyte leakage compared with fruit sourced from Ithala Farm independent of storage temperatures (Fig 2.2 C).

Ithala lemons had significantly lower glucose; however, fruit stored at 2^0C had significantly higher glucose concentration (Fig 2.2 D). Furthermore, Ithala fruit had significantly lower AA levels compared with Ukulinga lemons, with fruit stored at 2^0C having significantly high AA levels than fruit stored at -0.5^0C (Fig 2.2 D). In addition, Ukulinga fruit stored at 2ºC had significantly lower AA concentration than those stored at -0.5^0C (Fig 2.2 D).

Rind fructose and sucrose concentrations were significantly higher in Ukulinga than in Ithala fruit (Fig 2.2 E and F). However, the fructose concentration differed between storage temperatures for both fruit sources (Fig 2.2 E). Moreover, sucrose levels significantly differed between storage temperatures for Ukulinga fruit (Fig 2.2 F).

2.3.3 Principal component analysis

Due to complexity of data, principal component analysis (PCA) was used to extract the important information from the data. Principal component analysis reduced the variation in PCA 1 explaining 83% of total variation which is related to fructose, glucose and sucrose (Fig 2.3A). Principal component analysis (PCA 2) explained 13% of the total variation which was explained by electrolyte leakage (Fig 2.3A).

Ukulinga and Ithala fruit showed a distinct grouping when data subjected to principal component analysis (Fig 2.3B).

2.3.4 Effect of post-harvest silicon soaks on fruit weight loss, electrolyte leakage, glucose-ascorbic acid relationship, fructose and sucrose in lemon flavedo during cold storage

Silicon treatments of 50 mg ℓ^{-1} K_2SiO_3 reduced weight loss during the 28 days cold storage followed by five day shelf life for Ukulinga and Ithala lemons; however, not significantly (Table 2.1). Silicon at 50 mg ℓ^{-1} was effective in reducing fruit weight loss at both storage temperatures. The higher Si concentration (150 and 250 mg ℓ^{-1} K_2SiO_3) significantly increased weight loss of fruit from both sources and storage temperatures (Table 2.1).

The effect of post-harvest silicon application on electrolyte leakage was not clear while the higher silicon concentrations (150 and 250 mg ℓ^{-1}) decreased electrolyte leakage of Ithala fruit stored at 2^0C for 28 days; and at -0.5^0C the control and 50 mg ℓ^{-1} dips significantly reduced electrolyte leakage (Table 2.2). Furthermore, Si at 50 mg ℓ^{-1} significantly reduced electrolyte leakage of Ukulinga lemons at both cold storage temperatures (Table 2.2 and 2.3).

The glucose-ascorbic acid relationship was not evident with chilling-susceptible lemon sourced from Ithala Farm (Table 2.2). There was a variation in flavedo glucose concentration which did not show any associated reduction or synthesis in ascorbic acid concentration during cold storage. This was the same case for both storage

temperatures (Table 2.2 and 2.3). However, a glucose-ascorbic relationship was evident in non-chilling susceptible lemon fruit sourced from Ukulinga Farm (Table 2.2 and 2.3). The rind of fruit treated with 50 or 150 mg ℓ^{-1} Si and stored at 2ºC, showed a significant increase in ascorbic acid concentration as glucose decreased (Table 2.2 and 2.3). In addition, this relationship was also observed in fruit from the same location treated with 150 mg ℓ^{-1} Si and stored at -0.5ºC (Table 2.2).

The effect of Si dips on flavedo fructose concentration during cold storage of Ukulinga and Ithala fruit was not clear (Table 2.2 and 2.3). However, flavedo fructose concentration of Ithala fruit seemed to increase with all Si dips compared with untreated fruit stored at 2^0C (Table 2.2).

Flavedo sucrose significantly decreased independently of Si dips, especially 21 days cold storage independent of fruit sources (Table 2.2 and 2.3). However, sucrose concentration like fructose varied during cold storage (Table 2.2 and 2.3).

2.4 DISCUSSION AND CONCLUSION

Lemon fruit are the second chilling susceptible citrus species, after grapefruit (Chalutz *et al*., 1985). Surprisingly, Ukulinga fruit was completely resistant to cold storage with no appearance of chilling symptoms. Sinclair (1984) associated appearance of chilling symptoms with certain climatic conditions, which determine the morphology and chemical composition of lemon fruit associated with their chilling susceptibility. Furthermore, Mathaba *et al*. (2008) provided evidence that chilling susceptibility could be influenced by orchard management and climatic condition. In addition, chilling

susceptibility of lemon differs between coastal and desert fruit (Aung *et al*., 1999) and within cultivars, as ‘Lisbon’ found to be more chilling susceptible than ‘Eureka’ when fruit was stored at 1°C for 42 days (Underhill *et al*., 1999). Therefore, the chilling susceptibility of Ithala compared with Ukulinga fruit was affirming fruit source, chemical composition and climatic condition as contributing factors to chilling susceptibility of lemon fruit (Fig 2.1).

Chilling injury does not usually appear during cold storage but subsequently, when fruit are transferred to room temperature (McDonald *et al*., 1999). The Ithala fruit lost more weight loss than Ukulinga fruit hence CI was high in such fruit (Table 2.1 and 2.3). The significant increase in fruit weight loss only results in the appearance of chilling symptoms when fruit are kept at room-temperature following cold storage hence, fruit weight loss can be used to as non-destructive parameter to predict the possible appearance of chilling symptoms (Schirra and D’Hallewin, 1997; Nilprapruck *et al*., 2008; Mathaba *et al*., 2008). Therefore, increased fruit weight loss justified the chilling susceptibility of Ithala fruit compared with Ukulinga fruit. According to Rodov *et al*. (1995) chilling resistance fruit have thicker cuticle, thereby reducing weight loss. Furthermore, the assumption is that increased CI will result in increased fruit weight loss as well as electrolyte leakage, however, the assumption did not hold true in this study.

Cellular electrolyte leakage is an important indicator of chilling susceptibility as it is a measure of membrane integrity and, therefore, possible membrane dysfunction occurring during cold storage (Parkin and Kuo, 1989; Zhang *et al*., 2010). In this

study, electrolyte leakage was higher in non-chilled fruit sourced from Ukulinga Farm than from Ithala Farm. Wills *et al*. (2007) stated that the effect of low storage temperature depends on solute concentration of the tissues. This increase in solute concentration lowers the freezing temperature of tissues and, therefore, reduces osmotic stress (Wills *et al*., 2007) and, hence, reduces the potential for occurrence of CI.

Citrus fruit originate from subtropical areas and, therefore, is chilling susceptible to temperature of 10-15^{0}C (Ladaniya, 2007). However, this study showed contradictory results as Ukulinga fruit were completely non-susceptible to storage of 2 or -0.5^{0}C while Ithala fruit were susceptible to 2^{0}C storage (Fig 2.1). Mathaba *et al*. (2008) found 'Eureka' lemon fruit from Ukulinga not chilling susceptible to -0.5^{0}C. Zhang *et al*. (2010) confirmed these findings with peach fruit stored at 0 or 5^{0}C and found no chilling injury at 0^{0}C. Moreover, Cronje *et al*. (2011) found higher rind breakdown in mandarins stored at 7.5^{0}C than at -0.5^{0}C, even when stored at low temperature for up to five weeks. In addition, post-harvest Si dips at low concentrations seem to further enhance chilling resistance of lemon fruit to chilling during storage.

Lower Si concentrations seem to have a potential of mitigating chilling injury in lemon fruit especially at 2^{0}C storage. Silicon dips seem to induce a cascade of physiological events which lead to reduced CI. The reduced fruit weight loss and electrolyte leakage of Si-treated fruit (Table 2.1 and 2.2) may 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 (Lafuente *et al*., 1997). In addition, Si reduces electrolyte leakage in rice supplied with Si compared with no Si addition (Agarie *et al*., 1998).

Smirnoff and Wheeler (2000) found that ascorbic acid is a potent antioxidant, with high demands of this antioxidant during oxidative stress. Biochemically, glucose is the precursor to ascorbic acid. Recently, a glucose-ascorbic relationship has been proven in lemon stored at -0.5^{0}C for up to 28 days (Bower *et al*., In press). However, this relationship seems to exist only in fruit with high glucose concentration before storage, same scenario with Ukulinga lemons (Table 2.1 and 2.2). Furthermore, in this study low Si concentrations also enhance this relationship to increase antioxidant capacity and improving chilling resistance.

The effect of post-harvest Si dips was not clear on fructose and sucrose during cold storage. Citrus flavedo contains only small amounts of fructose and sucrose, the main dominant sugar being glucose (Mathaba *et al*., 2008; Bower *et al*., In press).

In conclusion, chilling susceptibility of lemon fruit depends to a great extent on the growing environment, orchard practises and fruit source. These growing conditions determine chemical composition of the flavedo and, therefore, the potential storage life and susceptibility to CI. Post-harvest Si applied at low concentrations can potentially increase the flavedo ascorbic acid concentration by inducing the enzymatic conversion of glucose to ascorbic acid. However, Si seems not to significantly affect other rind sugar concentration as these sugars decrease during cold storage. Rind glucose could be possible increased through the application of Si in postharvest dip

treatment. Furthermore, high endogenous Si and CI resistant of Ukulinga fruit lemon could indicate the possibility of pre-harvest Si application as a method to mitigate CI.

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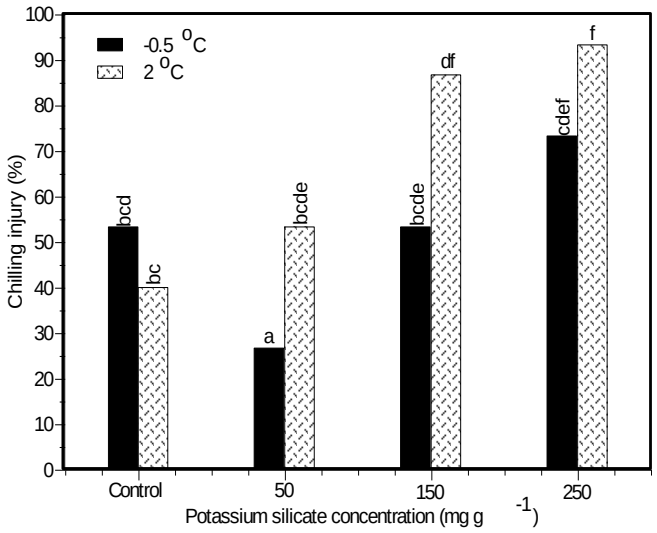

Figure 2.2: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 28 days cold storage time plus 5 days shelf-life.

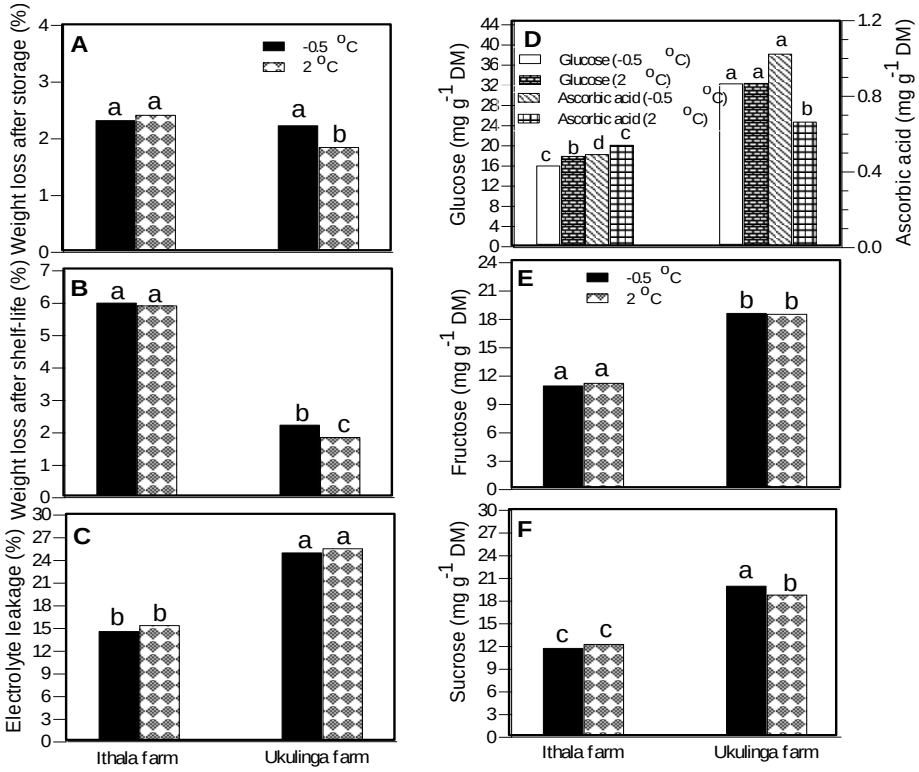

Figure 2.3: Effect of fruit source on lemon (A) weight loss after storage (B) weight loss after shelf-life (C) electrolyte leakage (D) glucose and ascorbic acid (E) fructose and (F) sucrose concentration.

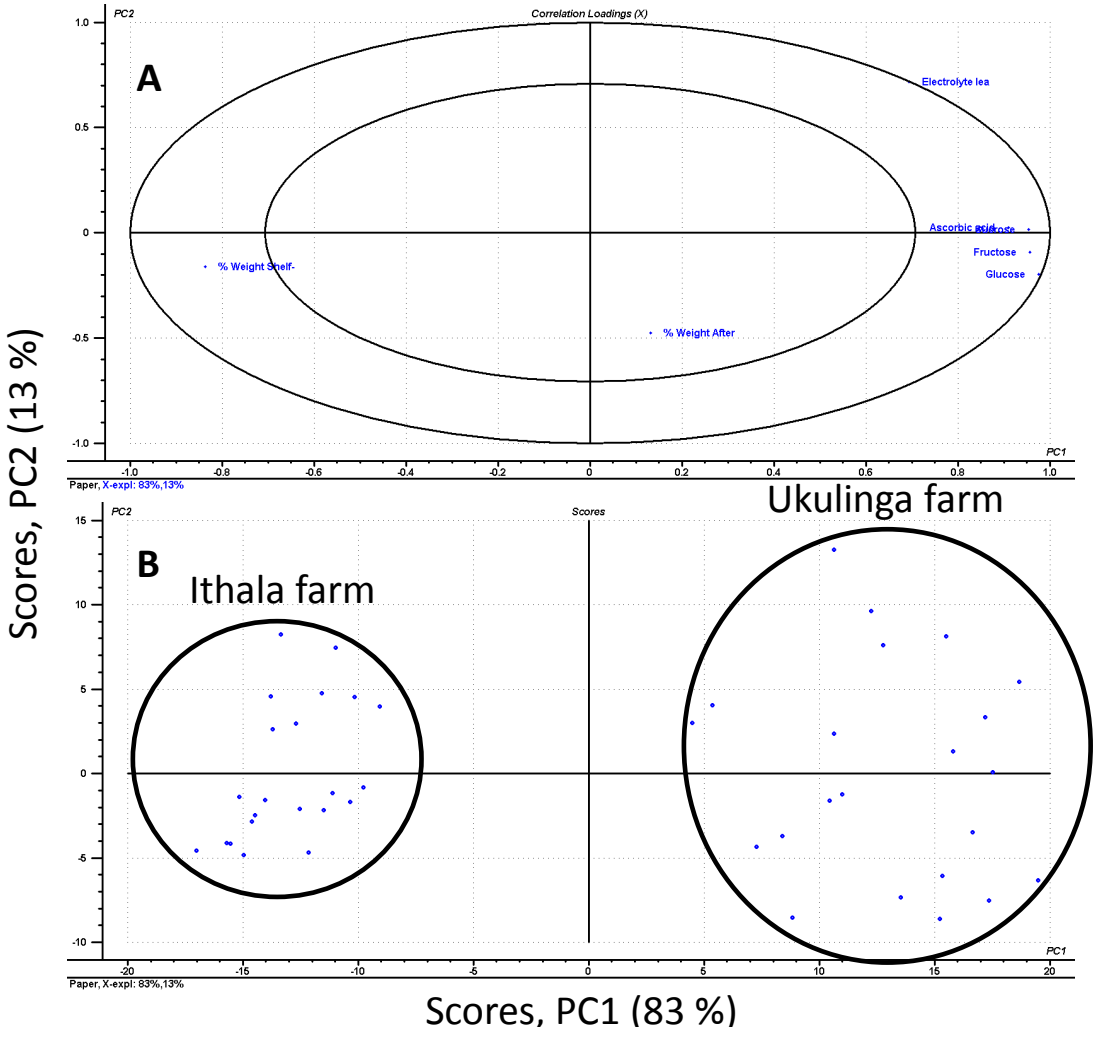

Figure 2.4: Principal component analysis (PCA) showing correlation loadings (A). Score plot lemon sugars and chilling injury indicators (B). Score plot for the groups of sugars for lemons from different sources with difference in chilling susceptibility, PC1 explains 83% and PC2 explains 13% of total variation.

Table 2.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage, sugars (glucose, fructose, sucrose) and ascorbic acid under -0.5^{0}C.

		Ukulinga Research Farm							Ithala Farm						
Cold storage time (days)	Treatments K_2SiO_3 (mg/ℓ)	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)	Glucose (mg l-1 DM)	Ascorbic acid(mg 100 g^{-1} DW)	Fructose(mg l-1 DM)	sucrose(mg l-1 DM)	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)	Glucose (mg l-1 DW)	Ascorbic acid(mg 100 g^{-1} DW)	Fructose (mg l-1 DW)	Sucrose (mg l-1 DW)
0	Control	0.00±0.00	0.00±0.00	24.06±2.0	32.36±6.02	1.50±0.29	17.73±2.59	20.21±1.78	0.00±0.00	0.00±0.00	15.30±1.12	14.07±0.85	0.55±0.03	10.43±0.19	13.36±2.12
	50	0.00±0.00	0.00±0.00	18.70±1.12	26.41±6.20	1.24±0.05	15.21±2.23	17.02±4.22	0.00±0.00	0.00±0.00	16.55±3.78	13.58±2.71	0.57±0.03	11.55±0.41	15.72±3.47
	150	0.00±0.00	0.00±0.00	20.43±2.56	35.93±0.83	1.11±0.04	20.51±1.18	22.67±0.87	0.00±0.00	0.00±0.00	11.71±1.92	18.06±1.57	0.53±0.02	10.04±0.26	13.36±1.88
	250	0.00±0.00	0.00±0.00	22.5 ±0.98	40.05±0.78	1.09±0.03	21.99±0.32	22.02±0.76	0.00±0.00	0.00±0.00	16.04±1.39	13.67±2.72	0.51±0.02	10.56±0.37	14.37±1.83
7	Control	0.89±0.09	4.02±0.20	39.34±3.60	32.01±6.23	1.13±0.01	17.10±2.05	19.30±1.64	1.26±0.04	5.68±0.08	12.2±0.34	16.34±0.25	0.51±0.03	11.83±0.24	13.84±2.00
	50	0.95±0.06	4.13±0.30	27.59±3.18	30.76±3.04	1.39±0.05	18.19±1.78	20.40±0.80	1.29±0.01	5.39±0.23	8.28±0.56	15.44±1.47	0.47±0.00	09.98±0.11	09.80±0.34
	150	1.02±0.02	4.14±0.11	27.62±1.99	31.98±1.67	1.22±0.06	20.95±0.58	22.00±0.86	1.72±0.03	7.32±0.26	10.95±0.33	14.72±0.39	0.50±0.00	08.74±0.04	12.03±0.06
	250	1.37±0.03	5.38±0.30	26.09±2.16	35.49±2.41	1.13±0.11	18.93±0.63	20.98±1.34	2.41±0.15	8.44±0.41	11.03±2.46	14.88±0.41	0.48±0.01	10.12±0.25	10.25±0.19
14	Control	2.75±0.13	5.54±0.23	19.23±1.38	29.96±2.15	1.12±0.05	18.97±1.04	20.93±0.43	1.73±0.07	6.08±0.24	20.65±1.50	12.22±0.96	0.47±0.01	12.40±0.24	10.90±0.13
	50	2.93±0.12	5.69±0.29	16.42±2.21	37.15±1.58	1.09±0.33	21.94±0.52	20.07±0.97	1.87±0.09	5.92±0.34	17.74±3.60	17.36±2.21	0.47±0.00	11.44±0.32	12.27±1.54
	150	3.14±0.05	6.39±0.08	17.40±0.90	38.51±3.73	1.18±0.05	21.34±1.01	21.65±0.82	2.71±0.08	8.77±0.36	18.11±4.53	13.80±0.70	0.46±0.00	10.07±0.34	10.16±0.08
	250	4.44±0.20	8.50±0.55	18.00±1.24	37.09±3.23	1.43±0.08	22.35±1.80	21.22±0.19	3.48±0.21	9.65±0.66	17.50±2.33	16.41±1.34	0.48±0.00	10.49±0.16	10.49±0.44
21	Control	1.58±0.05	5.30±0.11	30.09±0.39	35.65±1.49	1.37±0.03	20.00±0.11	21.40±0.41	2.12±0.11	5.28±0.28	12.77±3.03	16.02±1.12	0.47±0.00	10.94±0.33	10.90±0.26
	50	2.19±0.12	5.51±0.42	32.81±1.57	36.57±1.10	0.98±0.26	19.82±0.29	20.37±0.32	2.87±0.23	6.17±0.48	10.23±2.09	15.10±1.52	0.48±0.01	10.08±0.54	10.33±1.06
	150	3.04±0.13	7.22±0.44	32.39±3.06	36.67±2.48	0.62±0.08	20.37±0.95	22.46±0.66	3.94±0.19	9.40±0.34	10.41±1.64	16.12±1.11	0.47±0.01	11.69±0.43	11.28±0.24
	250	2.95±0.12	6.99±0.31	34.38±3.52	34.86±2.65	0.55±0.00	18.22±0.67	21.81±0.59	4.96±0.13	10.3±0.08	18.47±2.49	17.85±2.36	0.46±0.00	12.07±0.29	10.12±0.86
28	Control	3.19±0.11	6.64±0.20	18.88±1.39	24.69±0.86	0.55±0.00	16.25±0.47	16.87±0.34	2.77±0.02	6.20±0.05	14.04±2.80	16.22±1.62	0.46±0.00	10.79±0.48	11.53±0.82
	50	3.79±0.09	7.48±0.15	21.29±2.62	22.12±3.20	0.54±0.00	13.78±2.14	16.01±0.68	2.89±0.07	5.95±0.16	10.00±1.41	19.85±1.26	0.46±0.00	12.11±0.07	11.24±0.11
	150	4.27±0.48	8.76±0.42	26.02±2.71	21.56±1.37	0.56±0.01	14.01±2.29	13.42±0.80	4.33±0.22	8.55±0.49	15.17±1.61	16.14±1.29	0.47±0.00	10.99±0.25	10.68±0.09
	250	5.76±0.14	10.8±0.03	24.69±1.11	22.99±1.68	0.56±0.01	13.45±0.97	16.74±0.37	5.75±0.34	10.5±0.53	23.05±6.45	19.45±0.59	0.47±0.00	11.42±0.18	11.09±0.18
LSD (P=0.05)		**P<0.05 1.506**	**P<0.05 1.078**	**Ns 6.104**	**P<0.05 8.016**	**P<0.05 0.202**	**Ns 4.02**	**Ns 4.979**	**P<0.05 1.432**	**P<0.05 1.788**	**Ns 7.039**	**P<0.05 8.016**	**P<0.05 0.202**	**Ns 4.021**	**Ns 4.979**

Note: Values are means± SE

Ns=not significant

DW= Dry weight

Table 2.2: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage, sugars (glucose, fructose, sucrose) and ascorbic acid under 2^0C.

		Ukulinga University Research Farm							Ithala Farm						
Cold storage time (days)	Treatments K_2SiO_3 (mg/ℓ)	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)	Glucose (mg l-1 DM)	Ascorbic acid(mg 100 g^{-1} DM)	Fructose(mg l-1 DM)	sucrose(mg l-1 DM)	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)	Glucose (mg l-1 DM)	Ascorbic acid(mg 100 g^{-1} DM)	Fructose (mg l-1 DM)	Sucrose (mg l-1 DM)
0	Control	0.00±0.00	0.00±0.00	24.06±0.76	32.34±6.02	1.50±0.29	17.73±2.59	20.21±1.78	0.00±0.00	0.00±0.00	15.30±1.12	14.07±0.85	0.55±0.00	10.43±0.19	13.36±2.12
	50	0.00±0.00	0.00±0.00	18.70±0.32	26.41±6.20	1.24±0.05	15.21±2.23	17.02±4.22	0.00±0.00	0.00±0.00	16.55±3.78	13.58±2.71	0.57±0.03	11.55±0.41	15.72±3.47
	150	0.00±0.00	0.00±0.00	20.43±1.28	35.93±0.83	1.11±0.04	20.51±1.19	22.67±0.86	0.00±0.00	0.00±0.00	11.71±1.92	18.06±1.56	0.53±0.03	10.04±0.27	13.36±1.88
	250	0.00±0.00	0.00±0.00	22.50±0.68	40.05±0.78	1.09±0.03	21.99±0.32	22.02±0.76	0.00±0.00	0.00±0.00	16.04±1.39	13.67±2.72	0.51±0.02	10.56±0.37	14.37±1.83
7	Control	1.02±0.03	3.57±0.06	29.63±5.11	30.34±6.35	0.69±0.12	16.14±0.23	16.72±0.89	1.60±0.02	5.23±0.05	9.59±2.19	14.59±1.57	0.55±0.02	10.76±0.19	09.07±0.09
	50	1.23±0.02	4.68±0.15	28.74±1.31	33.52±1.34	0.54±0.00	16.76±0.85	17.91±0.45	1.98±0.06	5.86±0.18	8.93±0.48	23.10±0.62	0.57±0.03	11.70±0.27	12.17±0.65
	150	1.63±0.03	5.60±0.18	30.13±2.03	23.34±2.11	0.53±0.00	14.77±0.76	14.47±1.50	2.51±0.27	7.69±1.01	16.98±3.68	20.55±0.43	0.49±0.05	11.54±0.38	12.46±1.13
	250	1.51±0.01	5.65±0.15	32.41±3.42	26.90±1.58	0.51±0.01	15.10±0.34	16.02±0.34	2.42±0.21	7.12±0.44	8.66±0.20	16.16±2.87	0.48±0.01	11.38±1.06	16.09±2.26
14	Control	1.16±0.05	3.70±0.12	19.56±0.75	32.18±3.05	0.50±0.01	20.71±1.59	19.83±0.89	1.84±0.04	5.52±0.05	28.7±1.78	15.96±2.32	0.48±0.01	10.99±0.21	13.60±2.82
	50	1.63±0.06	4.43±0.19	19.18±0.57	35.96±6.85	0.52±0.02	19.92±1.67	18.08±0.38	2.04±0.06	5.68±0.13	19.5±1.48	19.19±0.77	0.52±0.00	12.29±0.66	13.38±1.63
	150	1.98±0.07	5.61±0.12	23.30±0.67	33.72±2.60	0.51±0.03	18.03±1.16	19.13±0.98	2.96±0.29	9.11±0.87	20.6±2.20	19.93±0.70	0.51±0.04	11.39±0.28	11.09±0.34
	250	1.93±0.04	5.08±0.20	22.21±2.25	39.77±3.10	0.49±0.00	19.90±0.85	19.75±0.34	5.83±2.64	12.3±2.27	20.7±3.10	17.33±3.05	0.58±0.01	10.58±0.83	11.06±0.11
21	Control	1.53±0.07	5.30±0.11	29.65±2.62	37.74±8.32	0.49±0.00	28.36±8.59	28.75±9.58	2.22±0.07	5.63±0.53	10.81±1.07	18.45±1.45	0.56±0.04	11.86±0.39	10.40±0.31
	50	2.19±0.12	5.51±0.42	34.50±3.48	39.07±2.63	0.50±0.01	20.28±0.56	18.43±0.73	2.53±0.06	5.72±0.05	8.44±0.38	15.27±1.88	0.58±0.04	10.69±0.36	10.23±0.41
	150	3.04±0.13	7.22±0.44	31.29±2.27	32.02±0.07	0.49±0.01	17.94±0.91	17.46±0.29	3.04±0.45	8.87±0.28	12.9±1.01	16.16±1.27	0.54±0.04	09.49±0.26	10.29±0.39
	250	2.95±0.12	6.99±0.31	31.91±1.74	25.63±3.46	0.49±0.00	17.40±2.23	16.67±3.23	4.15±0.33	10.2±0.52	14.28±4.15	17.59±0.49	0.57±0.05	10.49±0.46	10.06±0.63
28	Control	1.92±0.08	4.71±0.23	23.06±2.33	31.52±3.40	0.49±0.01	15.71±0.87	17.54±0.98	2.75±0.04	5.31±0.09	17.83±3.11	21.49±1.53	0.55±0.03	12.57±0.81	10.90±0.20
	50	2.71±0.06	6.53±0.03	18.42±1.71	29.60±4.34	0.49±0.01	18.43±2.93	16.64±1.23	3.00±0.09	5.44±0.23	17.02±2.31	21.21±0.72	0.53±0.01	12.15±0.09	14.91±1.78
	150	6.44±2.94	6.67±1.57	24.38±0.75	24.77±5.17	0.50±0.01	14.67±1.99	15.21±2.66	4.42±0.33	9.29±1.75	15.58±2.74	21.82±1.08	0.57±0.02	12.13±0.34	10.54±0.17
	250	3.74±0.09	8.37±0.37	27.79±1.12	33.96±2.67	0.50±0.02	19.67±1.53	19.58±1.53	4.71±0.22	8.90±0.45	15.49±0.89	16.77±1.77	0.51±0.01	10.69±0.70	10.69±0.22
LSD (P=0.05)		**P<0.05 1.506**	**P<0.05 1.078**	**Ns 6.104**	**P<0.05 8.016**	**P<0.05 0.202**	**Ns 4.02**	**Ns 4.979**	**P<0.05 1.432**	**P<0.05 1.788**	**Ns 7.039**	**P<0.05 8.016**	**P<0.05 0.202**	**Ns 4.021**	**Ns 4.979**

Note: Values are means± SE

Ns=not significant

DW= Dry weight

CHAPTER 3

THE POTENTIAL OF POSTHARVEST SILICON DIPS TO REGULATE PHENOLICS IN CITRUS PEEL AS A METHOD TO MITIGATE CHILLING INJURY IN LEMONS

ABSTRACT

The objective of this study was to investigate the ability of silicon dips to enhance the phenolic content in order to reduce the incidence of chilling injury in lemon fruit. Fruit were obtained from two Farms and dipped in 0, 50, 150 and 250 mg ℓ^{-1} solutions of K_2SiO_3 for 30 minutes, afterward fruit were air dried and waxed. Thereafter fruit were stored at -0.5 and 2^0C and sampled after 0, 7, 14, 21, and 28 days for evaluation of chilling injury symptoms. Chilling susceptible fruit sourced from Ithala Farm had significantly lower phenolics concentration compared with chilling resistant lemons from Ukulinga Farm. Phenolic content was improved by dipping fruit in 250 mg ℓ^{-1} of silicon. Moreover, 50 mg ℓ^{-1} reduced the occurrence of chilling injury symptoms whilst high silicon concentrations increased chilling injury. In conclusion, silicon dips have an ability to reduce chilling injury symptoms in lemons however, low concentrations should be used.

3.1 INTRODUCTION

Citrus fruit production is an important industry in South African agriculture. More than one million tonnes of citrus are exported internationally every year (Philp, 2006). However, the export market has unique challenges that require specialised postharvest procedures. The export market has stringent quality requirements that must be continuously adhered to in order for the produce not to be rejected. It is not always easy to meet these quality requirements; this is due to more convoluted marketing systems before the fruits reach end users.

The presence of Mediterranean fruit fly (*Ceratitis capitata*) requires cold sterilization as a quarantine treatment that must be applied to export citrus fruits (Serry, 2010). Cold sterilization requires that citrus fruit be shipped at -0.5ºC for 22 days in order to meet quarantine requirements (CRI, 2008). However, tropical and subtropical fruit are susceptible to chilling injury at temperatures below 12ºC (Lafuente *et al*., 2005).

Chilling injury manifests in the flavedo in the form of pitting, sunken areas on the scalding or browning. Such fruit has low external quality even though internal quality might be excellent (Lyons, 1973; Mathaba *et al*., 2008; Serry, 2010). Fruit quality is always of major concern to consumers. Low external quality leads to downgrading of fruit resulting in low financial returns to the producers.

Several methods have been used to reduce chilling injury in order to extend fruit shelf life and impressive responses have been reported. Among these techniques applied to many horticultural crops, including lemons, are hot water dips

(Mclauchlan *et al.*, 1997b; Sapitniskaya *et al.*, 2006; Mathaba *et al.*, 2008), intermittent warming (Wang and Baker, 1979; Schirra and Cohen, 1999; Kluge et al., 2003), waxing (Wild, 1993; Petracek *et al*., 1998; Perez Gago *et al*., 2002), controlled atmospheres (Wang and Qi, 1997), and application of chemicals such as molybdenum (Xue-Cheng *et a*l., 2006; Mathaba *et al*., 2008), ethylene (Wang, 1990; Zhou *et al*., 2001) and methyl jasmonate (Gonzalez-Aguilar *et al*., 2000; Cao *et al*., 2009).

These chemicals are applied to reduce chilling injury, possibly by reinforcing bioactive compounds that detoxify reactive oxygen species (ROS) (Van Breusegem *et al.*, 1999; Sato *et al.*, 2001; CRI, 2008). Most secondary compounds are bioactive compounds; however, some have antioxidant capacity. One of the effective bioactive compounds in protecting the fruit from oxidative damage are phenolics. Phenolics are bioactive compounds that possess antioxidant properties. Phenolics are often available in free and conjugated forms, with free phenolics often available in minute quantities (Imeh and Khokhar, 2002b; Zuo *et al*., 2002; Robbins, 2003). Flavonoids are a sub-group of phenolics in plants, also with antioxidants capacity to deactivate (ROS) thereby reducing lipid peroxidation and cellular damage (Medina, 2006). Their ability to quench ROS is attributed to their hydrogen-donating ability (Tripoli *et al*., 2007). Flavonoids have highly available H-atoms which makes them more effective than other phenolics (Tripoli *et al*., 2007). Many chemicals and methods have proven to be very effective in maintaining strong defensive mechanisms against ROS through stimulating production of antioxidants such as phenolics and flavonoids. However, most chemicals used to alleviate chilling injury pose health concerns to consumers.

Recently, Si has been discovered to induce resistance to both biotic and abiotic stresses. There is ample experimental evidence suggesting that Si affects the activity of major antioxidant enzymes involved in plant stress defense systems (Agarie *et al.*, 1998; Liang *et al.*, 2003; Hammerschmidt, 2005; Gong *et al.*, 2005; Gunes *et al.*, 2007a; Liang *et al.*, 2007b; Cai *et al.*, 2009; Crusciol *et al.*, 2009; Epstein, 2009; Keeping and Reynolds, 2009). However, the physiological role of Si and its mode of action are unclear. The aim of this work was to (a) investigate how silicon improves the antioxidant capacity, in particular, phenolics; (b) determine the total phenolics including both soluble and conjugated forms in fruits; and (c) determine the total flavonoids.

3.2 MATERIALS AND METHODS

Lemon fruit were harvested in July 2010 from the University of KwaZulu-Natal's Ukulinga Research Farm (29º40'00''S, 30º24'00''E) and Ithala Farm in May 2011 (29º52'00''S, 30º16'00''E), located in the Midlands of KwaZulu-Natal. Fruit were transported to the laboratory, where it was selected based on visual appearance and absence of blemishes. Fruit were dipped in Sporekill® solution. Thereafter, fruit were dipped in different concentrations (0, 50, 150, 250 mg ℓ^{-1}) of potassium silicate (K_2SiO_3) for 30 minutes according to Tesfay *et al*. (2011). Fruit were later waxed with Avoshine® (Citrashine Pty Ltd) weighed and subsequently stored at -0.5°C and 2°C at 85-90% relative humidity (RH) for 7, 14, 21 and 28 days. After storage fruit were evaluated for weight loss and kept at room temperature for five days before the

second weight loss. The percentage of chilling injury was evaluated. Following evaluation, fruit were peeled, freeze dried, milled and stored at -21ºC for further physiological analyses.

3.2.1 Chemicals

The chemicals that were used in this study were: Potassium silicate (K_2SiO_3), hydrochloric acid (HCl), methanol, Folic-Ciocalteu, sodium carbonate, chlorogenic acid, ethanol, diethylene glycol, sodium hydroxide and rutin were purchased from Sigma-Aldrich Chemical Co (St. Louis, Mo, USA).

3.2.2 Determination of Electrolyte Leakage

Membrane permeability after cold storage was determined using electrolyte leakage. The method of Zhu *et al.* (2004) was used with minor modifications. Three discs of the sample peel were cut and immersed in a test tube containing 10 ml of deionized water. Prior to this, the peel was washed three times to eliminate the electrolyte leakage at the cut surface and prevent surface contamination. After incubation at 25°C for 3 hours, electrolyte conductivity (EC 1) was measured using an electrical conductivity (EC) meter. The second electrolyte leakage (EC 2) was measured after the lemon peel samples were placed in a thermostatic shaking bath at 100°C for 1 hour and allowed to cool to room temperature.

The percentage of electrolyte leakage was calculated as:

$$\%total\ leakage = \frac{initial\ reading\ (EC1)}{final\ reading\ (EC2)} X100$$

3.2.3 Weight loss Percentage Determination

Weight of the fruit in each replicate was recorded initially and after different treatments and storage durations and the difference was used to calculate percentage weight loss.

$$\%\ Weight\ Loss\ 1 = \frac{[\text{Mass before storage} - \text{Mass (immediately after storage}]}{\text{Mass (before storage)}}\ X\ 100$$

$$\%\ Weight\ Loss\ 2 = \frac{[\text{Mass before storage} - \text{Mass (after storage} + 5\text{days at room temperature}]}{\text{Mass (before storage)}}\ X\ 100$$

3.2.4 Extraction of phenolics

3.2.4.1 Free Phenolics

Free phenolic content was determined according to the method of Abeysinghe *et al*. (2007), with some modification. Pulverized lemon peel (0.5 g) was weighed into a

screw-capped test tube. The phyto-chemicals were extracted with 5 ml of 50% dimethyl sulfoxide (DMSO): 50% of 1.2 M hydrochloric acid (HCl) in 80% methanol/water and vortexed for 1 minute and centrifuged at 10 000 rpm for 10 minutes to remove the solid fraction. The resultant supernatant was used for determination of free phenolics, flavonoids.

3.2.4.2 Conjugated phenolics

Five milliliters of 50% 1.2 M HCl in 80% methanol/water was added in the solid fraction left after extraction of free phenolics and the samples were heated at 90°C for 3 hours, with vortexing every 30 minutes. Thereafter, samples were allowed to cool down to room temperature, following which they were diluted to 10 ml with methanol and centrifuged at 10 000 rpm for 10 minutes to remove the solid fraction. The supernatant was used for determination of total conjugated phenolics, total flavonoids.

3.2.5 Determination of phenolics content

Free and conjugated phenolics of flavedo extract were measured using a modified colorimetric Folin-Ciocalteu method (Abeysinghe *et al*., 2007). Four milliliters of distilled water and 0.5 ml of properly diluted flavedo extract were placed in a glass test tube. Folin-Ciocalteu reagent (0.5 ml) was added to the solution and allowed to react for 3 minutes. The reaction was neutralized with 1 ml of saturated sodium carbonate. Absorbance was read at 760 nm after 3 hours using a spectrophotometer

(Beckman Coulter DU-800, USA). Chlorogenic acid was used as a standard and data were expressed as mg chlorogenic acid equivalents (CAE)/100 g DW.

3.2.6 Determination of flavonoids content

The total flavonoid content was determined as described by Abeysinghe *et al*. (2007), with some modifications(Abeysinghe *et al.*, 2007). Properly diluted flavedo extract (0.5 ml) was added to a glass test tube containing 3.5 ml of absolute ethanol. After addition of 4 ml of 90% di-ethylene glycol and thorough mixing, the reaction was initiated by adding 0.1 ml of 4 M sodium hydroxide. Absorbance at 420 nm was read after 10 minutes of incubation at 40°C using a spectrophotometer (Beckman Coulter DU-800, USA). Rutin was used as a standard and total flavonoid content was expressed as mg rutin equivalent (RE/100 g DW).

3.2.7 Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using GenStat® 12th edition (VSN International, UK). Means were separated using Duncan's Multiple Range Test at the 5% level of significance.

3.3 RESULTS

3.3.1 Chilling injury

Fruit from Ukulinga Farm did not show chilling injury symptoms during cold storage and shelf-life (Data not shown). However, fruits from Ithala Farm showed chilling injury symptoms after 21 and 28 days of cold storage (Figures 3.1A & B). Storage temperature had an effect on percentage chilling injury. However, in the present study, storing fruit at -0.5^{0}C consistently showed less chilling injury relative to storage at 2^{0}C. There were significant differences ($P<0.05$) between treatments (Figures 3.1A & B), with 50 mg ℓ^{-1} K_2SiO_3 significantly ($P<0.05$) reducing chilling injury compared with control at 28 days cold storage at -0.5^{0}C.It was only 27% of the fruit stored -0.5ºC treated with 50 mg ℓ^{-1} K_2SiO_3 that had chilling injury compared with more than 60% chilling injury at 2ºC. However, control, 150 mg ℓ^{-1} and 250 mg ℓ^{-1} K_2SiO_3 treatments had 40-97% chilling injury after 28 days of cold storage at -0.5ºC. It was observed that the chilling symptoms increased with K_2SiO_3 concentration (Figure 3.1). Furthermore, fruit treated with 150 and 250 mg ℓ^{-1} K_2SiO_3 had reduced firmness and whitish patches of Si.

3.3.2 Weight Loss

Fruit weight loss increased significantly ($P<0.05$) with storage time (Table 3.1). Storage temperature had no significant effect on fruit weight loss. Fruit sourced from

Ithala Farm showed significantly ($P<0.05$) higher fruit weight loss compared with Ukulinga fruit (Figure 3.2B).

Fruit weight loss increased with increasing K_2SiO_3 concentration; however, the control had significantly less weight loss. Furthermore, control and 50 mg ℓ^{-1} K_2SiO_3 had significantly ($P<0.05$) low fruit weight loss for Ithala fruit and significant differences ($P<0.05$) were observed between withdrawal of cold storage and 5 days shelf-life.

3.3.3 Electrolyte leakage (EL)

In this study, storage temperatures proved to have an effect on the severity of EL, with -0.5ºC having lower EL than 2ºC even though the differences were not significant (Figure 3.2A). Electrolyte leakage also differed ($P<0.05$) between fruit sources; Ukulinga fruit showed higher EL compared with fruit sourced Ithala fruit (Figure 3.2). However, despite having higher EL, Ukulinga fruit did not show any symptoms of chilling injury. There were significant differences ($P<0.05$) between treatments, with respect to EL (Table 3.1). Both Ithala and Ukulinga fruit had significantly lower EL at 50 and 150 mg ℓ^{-1} K_2SiO_3 compared with control and 250 mg ℓ^{-1} K_2SiO_3 (Table 3.1).

3.3.4 Phenolics

3.3.4.1 Free Phenolics

Fruit sourced from Ukulinga Farm had significantly ($P<0.05$) higher free phenolics compared with Ithala fruit (Figure 3.3A). Ithala fruit had significantly ($P<0.05$) lower free phenolics at -0.5ºC compared with Ukulinga fruit. Free flavonoids were not affected by cold storage time. Furthermore, storage temperature had an impact on phenolic content for fruit sourced from Ithala at -0.5ºC. There were no significant differences in free phenolic content between treatments (Table 3.2).

3.3.4.2 Conjugated phenolics

Conjugated phenolic content was significantly ($P<0.05$) higher in fruit sourced from Ukulinga compared with fruit from Ithala Farm (Figure 3.3B). Storage temperature had no effect on the level of conjugated phenolics (Figure 3.3B). There were significant differences ($P<0.05$) in conjugated phenolics between treatments (Table 2). For both storage temperatures, fruit sourced from Ukulinga showed a significant increase in conjugated phenolics in response to treatment with 250 mg ℓ^{-1} K_2SiO_3; Ithala fruit showed no response to the treatment (Table 3.2). In general, the amount of conjugated phenolics was shown to increase with increasing concentrations of K_2SiO_3.

3.3.5 Flavonoids

3.3.5.1 Free Flavonoids

Fruit source had a significant effect ($P<0.05$) on concentration of free flavonoids. Ukulinga fruit showed significantly ($P<0.05$) higher free flavonoid concentration compared with Ithala fruit (Figure 3.4A). Cold storage time had no effect on the amount of free flavonoids. The effect of storage temperature on levels of free flavonoids was clearly observed in Ukulinga fruit; storing fruit at -0.5ºC resulted in increased of free flavonoids compared to storage at 2ºC. There were no significant differences between treatments with respect to free flavonoids content (Table 3.3). However, treatment with 250 mg ℓ^{-1} K_2SiO_3 resulted in higher levels of free flavonoids content in Ukulinga fruit compared with Ithala fruit (Table 3.3).

3.3.5.2 Conjugated Flavonoids

Fruit source had a significant effect ($P<0.05$) on the amount of conjugated flavonoids in the flavedo. Ukulinga fruit had significantly higher conjugated flavonoids compared with Ithala fruit (Figure 3.4B). Conjugated flavonoids were not affected by cold storage time. Cold storage temperature had no significant effect on conjugated flavonoids. Significant differences in conjugated flavonoids were observed between treatments (Table 3.3). Ukulinga fruit showed higher conjugated flavonoids at 150 and 250 mg ℓ^{-1} K_2SiO_3 relative to the control and 50 mg ℓ^{-1} K_2SiO_3 ($P<0.05$). However, Ithala fruit did not respond to treatments as there was no significant difference observed.

3.4 DISCUSSION AND CONCLUSION

The difference in chilling susceptibility of Ithala lemon fruit compared with Ukulinga fruit was evidence that environmental or growing conditions may determine fruit susceptibility to chilling injury (Mclauchlan *et al.*, 1997b; Mathaba *et al.*, 2008). Fruit from Ithala Farm were found to be highly susceptible to chilling injury (Figure 3.1) as compared to fruit from Ukulinga Farm which was chilling resistant. There is a possibility that Ukulinga lemons are high in endogenous Si thus conferring them resistance to chilling injury.

The possibility of endogenous Si being an important player in determining fruit susceptibility to chilling injury was observed in fruit weight loss. Fruit from Ukulinga Farm showed less fruit weight loss compared with Ithala fruit (Figure 3.2). The increased fruit weight loss for Ithala Farm may be connected to loss of membrane integrity, cellular breakdown and perhaps the removal of epicuticular waxes known to reduce water loss through the rind (Schirra and D'hallewin, 1997; González-Aguilar *et al.*, 2000). In addition, low fruit weight loss in Ukulinga fruit may be linked to reduced membrane permeability (Liang *et al*., 1996) and increased membrane stability and integrity (Agarie *et al*., 1998) due to endogenous silicon.

Electrolyte leakage is one of the most important indicators of chilling injury in fruit and vegetables. Increased electrolyte leakage has been associated with appearance of chilling injury symptoms in mango (*Mangifera indica* L) (González-Aguilar *et al*., 2000), pepper (*Capsicum annuum*) (Vicente *et al*., 2005) and mamey

sapote fruit (*Pouteria sapota*) (Perez-Tello *et al*., 2009). Fruit source had an effect on electrolyte leakage with Ukulinga fruit showing higher electrolyte leakage compared with Ithala Farm. However, electrolyte leakage did not prove to be a reliable indicator of chilling injury in this study. Despite having high electrolyte leakage, Ukulinga fruit did not exhibit any symptoms of chilling injury. Theoretically, high electrolyte leakage means high membrane damage and subsequently high chilling injury (Wilson, 1976). Results of the electrolyte leakage assay are highly dependent on the thickness of lemon peel and solute concentration in the tissue (Wills *et al*., 1998). Ukulinga fruit had a thicker peel and, perhaps, higher solute concentration than Ithala fruit, hence higher electrolyte leakage.

The growing conditions of the fruit also affect the phenolic and flavonoid content of fruit. In this study, Ukulinga fruit had higher phenolic and flavonoid levels compared with Ithala fruit. These results concur with reports by Reddivari *et al*. (2007) who found phenolic content in potatoes (*Solanum tuberosum*) to be significantly different between locations. The difference in the flavonoid and phenolic content within location may also be possibly connected to differences in endogenous silicon concentration.

Storage temperature is the principal factor determining fruit susceptibility to chilling injury. Perez-Tello *et al*. (2009) found storage temperature to be inversely proportional to chilling injury. However, in the present study -0.5ºC persistently showed reduced chilling injury compared with high storage temperature (2ºC). Similar findings were reported for Clementine mandarins stored at -0.5ºC. They were found

to be less prone to rind breakdown or chilling injury when stored at -0.5ºC compared with 7.5ºC (Cronje *et al*., 2011).

In addition, different storage temperatures are known to affect weight loss and electrolyte leakage. However, storage temperatures showed no difference in both weight loss and electrolyte leakage at -0.5 and 2ºC. This was contrary to reports by Concellon *et al*. (2005) who found that electrolyte leakage of eggplant (*Solanum melongena)* was higher at 0ºC compared with 10ºC. These results suggest that, in lemon fruit, electrolyte leakage may not serve as an indicator for the development of chilling injury. Also, results of weight loss are in contrast to reports by Gonzalez-Aguilar *et al*. (1997) who found that mandarins stored at 2ºC had lower weight loss than those stored at 10ºC; our results showed that fruit stored at -0.5ºC had higher weight loss compared with fruit stored at 2ºC. Different storage temperatures had no effect on the levels of flavonoids and phenolics. Contrary to Ayala-Zavala *et al*. (2004) who reported that strawberry fruit (*Fragaria ananassa*) stored at 5 or 10ºC had higher flavonoids and phenolics than those stored at 0ºC, we found no such differences between storage temperatures. However, post-harvest shelf life, based on rind quality, was prolonged by storage at -0.5ºC than 2ºC.

Silicon has been proven to induce stress resistance in plants (Liang *et al*., 2008). In this study, 50 mg ℓ^{-1} K_2SiO_3 reduced the occurrence of chilling injury symptoms; these results are in agreement with several reports found in the literature Agarie *et al*. (1998); Bekker *et al*. (2007); Liang *et al*. (2008); Cai *et al*. (2009); Epstein

(2009) who found that Si played an important role in inducing stress resistance for different agricultural crops. However, high Si concentrations had a negative effect on rind quality. Chilling injury was exacerbated by 150 and 250 mg ℓ^{-1} K_2SiO_3 (Figure 3.1). This was probably caused by the glassy characteristic of Si that can potentially damage the cell hence increasing fruit water loss.

The reduced fruit weight loss and electrolyte leakage following treatment with Si also showed the potential of Si to retard stress as discussed by other researchers. The reduction in fruit weight at 50 mg ℓ^{-1} K_2SiO_3 was probably due to the modification of cell membranes after Si application that led to reduction of water loss and subsequently reduced fruit weight loss (Epstein, 2009). Silicon application also reduced electrolyte leakage and this was in agreement with Agarie *et al*. (1998) who reported the potential of Si to reduce electrolyte leakage in rice leaves. This may possibly be attributed to the improved strength and rigidity of tissue following Si application (Liang *et al*., 2007b). The high fruit weight loss and electrolyte leakage at high Si concentration was possibly caused by water channels caused by concentrated Si.

The application of Si has been proved to enhance flavonoid and phenolic content in plants (Maksimovic *et al*., 2007). In this study, 250 mg ℓ^{-1} K_2SiO_3 increased the free flavonoid and phenolic content (Table 3.2 and 3.3). However, conjugated flavonoids and phenolics were not affected by Si. These results are in line with an earlier study by (Bekker *et al.*, 2007) that showed enhancement of free phenolics on avocado trees following Si application.

Generally, fruit from Ukulinga had no chilling in contrast to Ithala fruit that had high incidence of chilling injury. Furthermore, Ukulinga fruit had high flavonoid and phenolic content that played a role in mitigating chilling injury.

Our findings indicated that lower concentration post-harvest Si dips reduced the incidence of chilling injury while higher concentration Si dips exacerbated chilling injury. Although high concentrations of Si improved the levels of flavonoids and phenolics, they had an adverse effect on fruit by increasing the occurrence of chilling injury. However, increased levels of flavonoids and phenolics in response to high concentrations of Si suggest that Si may be involved in modulating enzymes.

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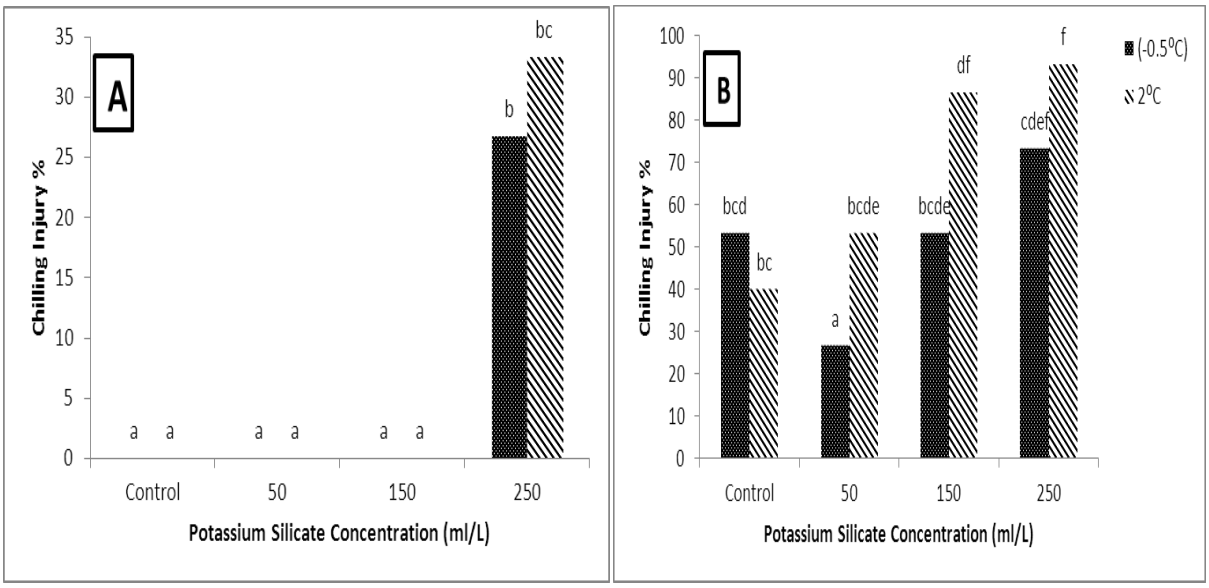

Figure 3.1: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 21 days (A) and 28 days (B) storage time.

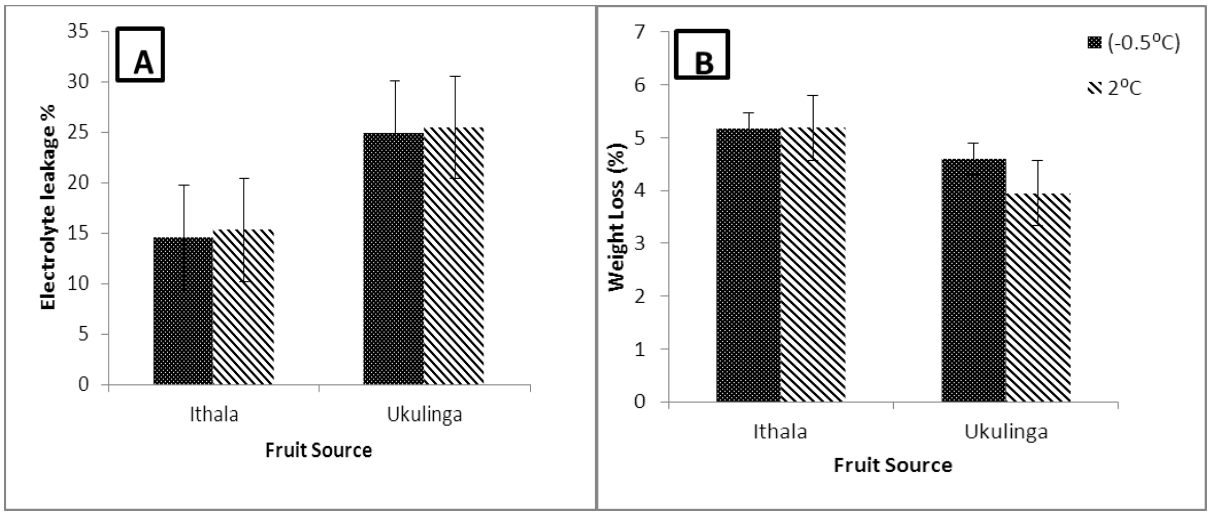

Figure 3.2: Effect of post-harvest K_2SiO_3 dips on electrolyte leakage (A) and weight loss (B) of lemon fruit sourced from Ithala and Ukulinga Farm after 28 days cold storage, plus 5 days at room temperature.

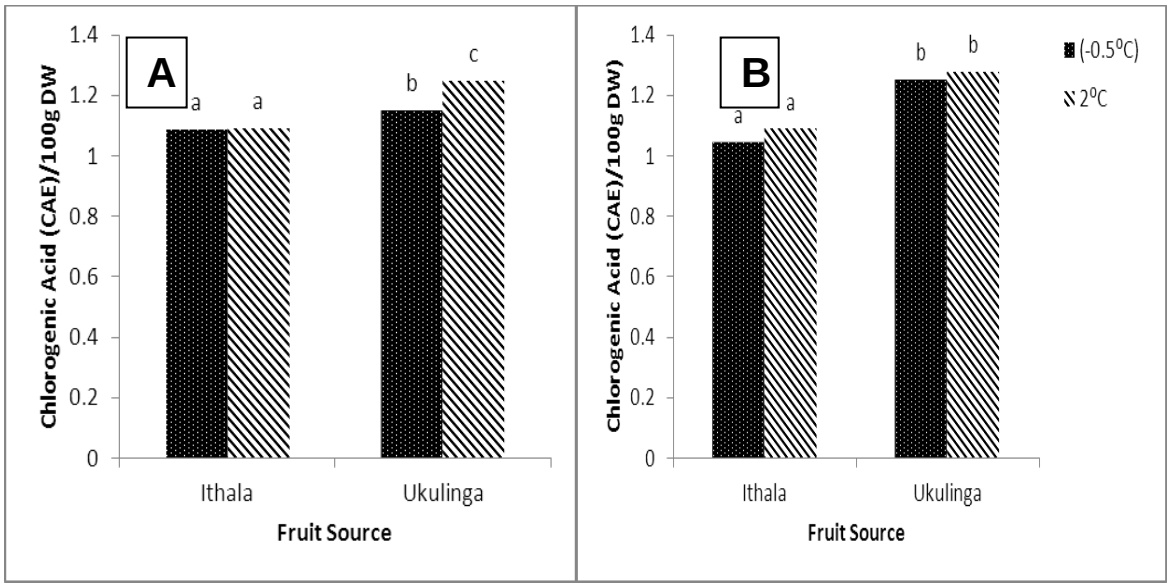

Figure 3.3: The effect of K_2SiO_3 concentration and storage temperature production of free phenolics (A) and conjugated phenolics (B) of fruit sourced from Ithala and Ukulinga Farm stored at -0.5^{0}C and 2^{0}C during 28 days of cold storage.

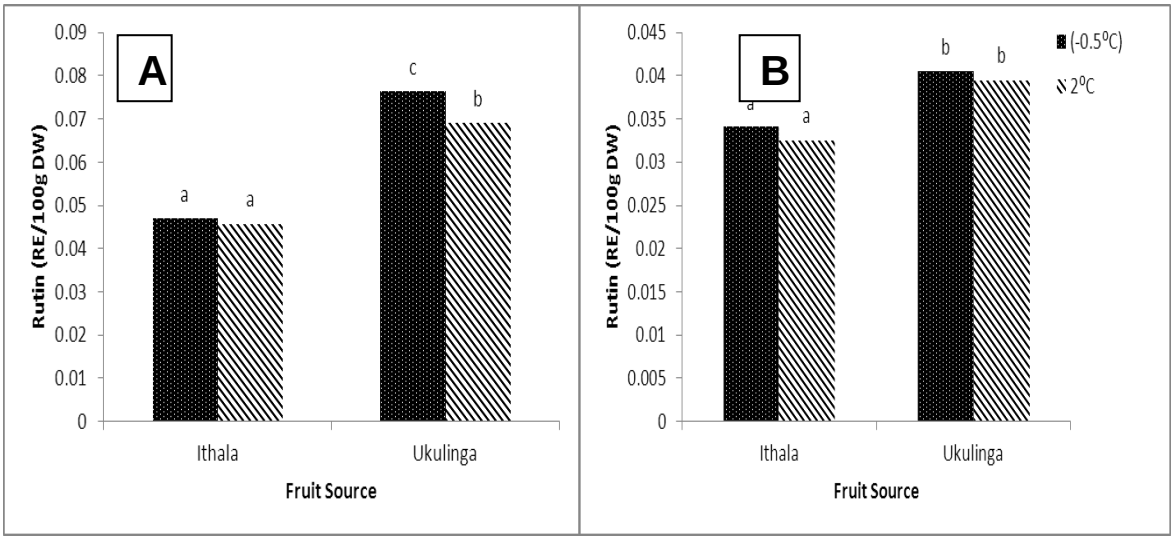

Figure 3.4: The effect of K_2SiO_3 concentration and storage temperature production of free flavonoids (A) and conjugated flavonoids (B) of fruit sourced from Ithala and Ukulinga Farm stored at -0.5^{0}C and 2^{0}C during 28 days of cold storage.

Table 3.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on lemon fruit weight loss, electrolyte leakage and chilling injury percentage under -0.5ºC and 2ºC storage temperatures.

			Ithala Farm 2011			Ukulinga Farm 2010		
Storage temperature	Cold storage time (days)	Treatments K_2SiO_3 (mg g^{-1})	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)	Weight loss after storage (%)	Weight loss 5 days shelf-life (%)	Electrolyte leakage (%)
-0.5°C		0	0.00±0.00	0.00±0.00	15.30±1.12	0.00±0.00	0.00±0.00	24.06±2.09
	0	50	0.00±0.00	0.00±0.00	16.55±3.78	0.00±0.00	0.00±0.00	18.70±1.12
		150	0.00±0.00	0.00±0.00	11.71±1.92	0.00±0.00	0.00±0.00	20.43±2.56
		250	0.00±0.00	0.00±0.00	16.04±1.39	0.00±0.00	0.00±0.00	22.5 ±0.98
	Mean		**0.00±0.00**	**0.00±0.00**	**14.90cde**	**0.00±0.00**	**0.00±0.00**	**21.43b**
		0	1.26±0.04	5.68±0.08	12.2±0.34	0.89±0.09	4.02±0.20	39.34±3.60
		50	1.29±0.01	5.39±0.23	8.28±0.56	0.95±0.06	4.13±0.30	27.59±3.18
	7	150	1.72±0.03	7.32±0.26	10.95±0.33	1.02±0.02	4.14±0.11	27.62±1.99
		250	2.41±0.15	8.44±0.41	11.03±2.46	1.37±0.03	5.38±0.30	26.09±2.16
	Mean		**1.43a**	**6.14ab**	**10.61a**	**1.06a**	**4.42a**	**30.16c**
		0	1.73±0.07	6.08±0.24	20.65±1.50	2.75±0.13	5.54±0.23	19.23±1.38
		50	1.87±0.09	5.92±0.34	17.74±3.60	2.93±0.12	5.69±0.29	16.42±2.21
	14	150	2.71±0.08	8.77±0.36	18.11±4.53	3.14±0.05	6.39±0.08	17.40±0.90
		250	3.48±0.21	9.65±0.66	17.50±2.33	4.44±0.20	8.50±0.55	18.00±1.24
	Mean		**2.11bc**	**6.93bc**	**18.50e**	**3.32c**	**6.53b**	**17.76a**
		0	2.12±0.11	5.28±0.28	12.77±3.03	1.58±0.05	5.30±0.11	30.09±0.39
		50	2.87±0.23	6.17±0.48	10.23±2.09	2.19±0.12	5.51±0.42	32.81±1.57
	21	150	3.94±0.19	9.40±0.34	10.41±1.64	3.04±0.13	7.22±0.44	32.39±3.06
		250	4.96±0.13	10.3±0.08	18.47±2.49	2.95±0.12	6.99±0.31	34.38±3.52
	Mean		**2.99de**	**6.95c**	**12.97de**	**2.45b**	**6.26b**	**32.42c**
		0	2.77±0.02	6.20±0.05	14.04±2.80	3.19±0.11	6.64±0.20	18.88±1.39
		50	2.89±0.07	5.95±0.16	10.00±1.41	3.79±0.09	7.48±0.15	21.29±2.62
	28	150	4.33±0.22	8.55±0.49	15.17±1.61	4.27±0.48	8.76±0.42	26.02±2.71
		250	5.75±0.34	10.5±0.53	23.05±6.45	5.76±0.14	10.8±0.03	24.69±1.11
	Mean		**2.89e**	**6.37c**	**15.57**	**4.26d**	**8.44c**	**22.72b**
2°C		0	0.00±0.00	0.00±0.00	15.30±1.12	0.00±0.00	0.00±0.00	24.06±0.76
		50	0.00±0.00	0.00±0.00	16.55±3.78	0.00±0.00	0.00±0.00	18.70±0.32
	0	150	0.00±0.00	0.00±0.00	11.71±1.92	0.00±0.00	0.00±0.00	20.43±1.28
		250	0.00±0.00	0.00±0.00	16.04±1.39	0.00±0.00	0.00±0.00	22.50±0.68
	Mean		**0.00±0.00**	**0.00±0.00**	**14.90bcde**	**0.00±0.00**	**0.00±0.00**	**21.43ab**
		0	1.60±0.02	5.23±0.05	9.59±2.19	1.02±0.03	3.57±0.06	29.63±5.11
		50	1.98±0.06	5.86±0.18	8.93±0.48	1.23±0.02	4.68±0.15	28.74±1.31
	7	150	2.51±0.27	7.69±1.01	16.98±3.68	1.63±0.03	5.60±0.18	30.13±2.03
		250	2.42±0.21	7.12±0.44	8.66±0.20	1.51±0.01	5.65±0.15	32.41±3.42
	Mean		**2.03ab**	**6.26a**	**11.04ab**	**1.35a**	**4.88a**	**30.23c**
		0	1.84±0.04	5.52±0.05	28.7±1.78	1.16±0.05	3.70±0.12	19.56±0.75
		50	2.04±0.06	5.68±0.13	19.5±1.48	1.63±0.06	4.43±0.19	19.18±0.57
	14	150	2.96±0.29	9.11±0.87	20.6±2.20	1.98±0.07	5.61±0.12	23.30±0.67
		250	5.83±2.64	12.3±2.27	20.7±3.10	1.93±0.04	5.08±0.20	22.21±2.25
	Mean		**2.29cd0**	**6.77c**	**22.41f**	**1.68ab**	**4.71a**	**21.06b**
		0	2.22±0.07	5.63±0.53	10.81±1.07	1.53±0.07	5.30±0.11	29.65±2.62
	21	50	2.53±0.06	5.72±0.05	8.44±0.38	2.19±0.12	5.51±0.42	34.50±3.48
		150	3.04±0.45	8.87±0.28	12.9±1.01	3.04±0.13	7.22±0.44	31.29±2.27
		250	4.15±0.33	10.2±0.52	14.28±4.15	2.95±0.12	6.99±0.31	31.91±1.74
	Mean		**2.59cd**	**6.74bc**	**11.62abc**	**2.43b**	**6.26b**	**31.84c**
		0	2.75±0.04	5.31±0.09	17.83±3.11	1.92±0.08	4.71±0.23	23.06±2.33
	28	50	3.00±0.09	5.44±0.23	17.02±2.31	2.71±0.06	6.53±0.03	18.42±1.71
		150	4.42±0.33	9.29±1.75	15.58±2.74	6.44±2.94	6.67±1.57	24.38±0.75
		250	4.71±0.22	8.90±0.45	15.49±0.89	3.74±0.09	8.37±0.37	27.79±1.12
	Mean		**3.39de**	**6.68abc**	**16.48de**	**3.71cd**	**6.57b**	**23.41b**
LSD P=0.05			**P<0.05 1.432**	**P<0.05 1.788**	**Ns 7.039**	**P<0.05 1.506**	**P<0.05 1.078**	**Ns 6.104**

Table 3.2: Table 1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on production of phenolic compounds on lemon fruit susceptible to chilling injury stored at-0.5ºC and 2ºC storage temperatures.

			Ithala 2011		Ukulinga 2010	
Storage temperature	Cold storage time (days)	Treatments K_2SiO_3 (mg g^{-1})	Phenolics(CAE/100g DW		Phenolics(CAE/100g DW	
			Free	Conjugated	Free	Conjugated
-0.5°C		0	1.03±0.07	0.81±0.01	1.25±0.10	1.37±0.03
	0	50	1.03±0.01	0.81±0.01	1.07±0.03	1.10±0.22
		150	1.06±0.05	0.81±0.01	0.93±0.02	0.98±0.19
		250	0.98±0.01	0.81±0.01	1.04±0.09	1.31±0.06
	Mean		**1.024a**	**0.810a**	**1.075ab**	**1.192ab**
		0	1.06±0.02	1.03±0.03	1.33±0.03	1.46±0.02
		50	1.08±0.05	0.99±0.02	1.13±0.08	1.13±0.08
	7	150	1.09±0.03	1.03±0.01	1.18±0.03	1.28±0.08
		250	1.06±0.03	1.00±0.01	1.25±0.03	1.31±0.09
	Mean		**1.071ab**	**1.017b**	**1.224cd**	**1.299bc**
		0	1.10±0.03	1.10±0.02	1.12±0.05	1.08±0.09
		50	1.11±0.01	1.23±0.03	1.19±0.02	1.29±0.08
	14	150	1.14±0.02	1.31±0.08	1.27±0.03	1.28±0.03
		250	1.19±0.05	1.22±0.05	1.21±0.03	1.26±0.06
	Mean		**1.137c**	**1.214d**	**1.199c**	**1.227ab**
		0	1.19±0.07	1.11±0.01	1.20±0.02	1.30±0.01
		50	1.12±0.03	1.06±0.02	1.21±0.03	1.32±0.04
	21	150	1.16±0.03	1.05±0.01	1.14±0.04	1.32±0.02
		250	1.13±0.02	1.02±0.01	1.20±0.01	1.17±0.01
	Mean		**1.154c**	**1.061b**	**1.187c**	**1.282bc**
		0	1.06±0.02	1.24±0.11	1.14±0.01	1.19±0.14
		50	1.04±0.02	1.10±0.01	1.03±0.05	1.23±0.07
	28	150	1.04±0.01	1.08±0.01	1.11±0.01	1.31±0.06
		250	1.06±0.01	1.09±0.02	1.01±0.06	1.31±0.02
	Mean		**1.058a**	**1.130c**	**1.074a**	**1.260abc**
2°C		0	1.03±0.07	0.81±0.01	1.25±0.10	1.36±0.02
		50	1.03±0.01	0.81±0.01	1.07±0.03	1.10±0.03
	0	150	1.06±0.05	0.81±0.01	0.93±0.02	0.99±0.22
		250	0.98±0.01	0.81±0.03	1.04±0.09	1.31±0.19
	Mean		**1.024a**	**0.810a**	**1.075ab**	**1.192ab**
		0	1.11±0.03	1.06±0.01	1.46±0.13	1.27±0.07
		50	1.03±0.01	1.01±0.01	1.36±0.03	1.39±0.17
	7	150	1.06±0.02	1.04±0.02	1.47±0.05	1.48±0.02
		250	1.03±0.01	1.06±0.02	1.25±0.02	1.44±0.04
	Mean		**1.058a**	**1.046b**	**1.384f**	**1.395c**
		0	1.12±0.02	1.14±0.05	1.36±0.01	1.39±0.02
		50	1.14±0.01	1.23±0.04	1.22±0.13	1.31±0.04
	14	150	1.09±0.02	1.21±0.02	1.40±0.02	1.40±0.09
		250	1.14±0.02	1.20±0.02	1.21±0.08	1.42±0.02
	Mean		**1.123c**	**1.197d**	**1.297de**	**1.379c**
		0	1.19±0.07	1.09±0.02	1.34±0.03	1.17±0.03
	21	50	1.16±0.04	1.20±0.02	1.30±0.01	1.30±0.09
		150	1.11±0.03	1.06±0.03	1.35±0.01	1.33±0.02
		250	1.04±0.03	1.07±0.02	1.34±0.03	1.35±0.02
	Mean		**1.123c**	**1.082bc**	**1.332ef**	**1.288bc**
		0	1.02±0.05	1.19±0.03	1.14±0.05	1.07±0.01
	28	50	1.09±0.02	1.27±0.03	1.12±0.04	1.17±0.11
		150	1.22±0.02	1.27±0.20	1.20±0.08	0.98±0.06
		250	1.18±0.08	1.53±0.03	1.17±0.03	1.30±0.20
	Mean		**1.127c**	**1.314e**	**1.155ac**	**1.129a**
LSD P=0.05			**P<0.05 0.156**	**P<0.05 0.260**	**P<0.05 0.135**	**P<0.05 0.206**

Note: Values are means± SE; DW= Dry weight; values followed by different letter are significant different

Table 3.3: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3) on production of flavonoids on lemon fruit susceptible to chilling injury stored at-0.5ºC and 2ºC storage temperatures (RE is Rutin equivalent)

			Ithala 2011		Ukulinga 2010	
Storage temperature	Cold storage time (days)	Treatments K_2SiO_3 (mg/ℓ)	Flavonoids(RE/100g DW)		Flavonoids(RE/100g DW)	
			Free	Conjugated	Free	Conjugated
-0.5°C		0	0.056±0.005	0.045±0.003	0.078±0.001	0.041±0.003
	0	50	0.056±0.003	0.043±0.006	0.080±0.001	0.041±0.001
		150	0.058±0.002	0.047±0.006	0.067±0.008	0.038±0.003
		250	0.048±0.004	0.041±0.006	0.078±0.006	0.036±0.006
	Mean		**0.050cd**	**0.044d**	**0.076bcd**	**0.039bc**
		0	0.043±0.005	0.027±0.008	0.091±0.001	0.055±0.007
		50	0.037±0.006	0.033±0.003	0.072±0.002	0.032±0.004
	7	150	0.034±0.004	0.038±0.009	0.078±0.004	0.044±0.010
		250	0.035±0.003	0.036±0.007	0.082±0.001	0.042±0.009
	Mean		**0.032a**	**0.034bc**	**0.080d**	**0.043bc**
		0	0.047±0.004	0.023±0.004	0.072±0.002	0.037±0.007
		50	0.059±0.007	0.036±0.012	0.063±0.001	0.032±0.004
	14	150	0.061±0.007	0.033±0.006	0.081±0.001	0.031±0.005
		250	0.058±0.011	0.021±0.003	0.080±0.002	0.047±0.004
	Mean		**0.056d**	**0.028ab**	**0.074bcd**	**0.037b**
		0	0.046±0.006	0.024±0.004	0.077±0.004	0.050±0.002
		50	0.049±0.001	0.028±0.002	0.082±0.003	0.045±0.005
	21	150	0.046±0.005	0.029±0.002	0.079±0.003	0.045±0.005
		250	0.039±0.003	0.025±0.005	0.080±0.001	0.039±0.010
	Mean		**0.044abc**	**0.027ab**	**0.079cd**	**0.045bc**
		0	0.045±0.001	0.046±0.004	0.069±0.002	0.026±0.003
		50	0.043±0.005	0.038±0.005	0.062±0.003	0.034±0.001
	28	150	0.033±0.002	0.036±0.004	0.079±0.002	0.049±0.002
		250	0.045±0.004	0.032±0.001	0.075±0.005	0.043±0.006
	Mean		**0.042ab**	**0.038cd**	**0.072b**	**0.038bc**
2°C		0	0.056±0.005	0.045±0.003	0.078±0.001	0.041±0.003
		50	0.057±0.003	0.043±0.006	0.080±0.008	0.041±0.001
	0	150	0.058±0.003	0.047±0.005	0.068±0.006	0.038±0.003
		250	0.048±0.004	0.041±0.006	0.078±0.002	0.036±0.006
	Mean		**0.056d**	**0.044d**	**0.076bcd**	**0.039bc**
		0	0.043±0.005	0.027±0.008	0.076±0.004	0.042±0.007
		50	0.037±0.003	0.028±0.003	0.068±0.005	0.046±0.007
	7	150	0.034±0.005	0.034±0.002	0.077±0.004	0.045±0.007
		250	0.035±0.006	0.036±0.002	0.065±0.001	0.049±0.004
	Mean		**0.037a**	**0.031abc**	**0.071b**	**0.045c**
		0	0.036±0.005	0.027±0.003	0.057±0.013	0.061±0.009
		50	0.050±0.002	0.028±0.005	0.067±0.004	0.029±0.006
	14	150	0.043±0.005	0.029±0.002	0.065±0.008	0.052±0.002
		250	0.040±0.002	0.025±0.004	0.064±0.004	0.040±0.003
	Mean		**0.043ab**	**0.027ab**	**0.064a**	**0.046c**
		0	0.044±0.001	0.038±0.002	0.076±0.002	0.029±0.001
	21	50	0.042±0.002	0.031±0.002	0.078±0.002	0.031±0.001
		150	0.044±0.002	0.033±0.001	0.068±0.005	0.047±0.002
		250	0.047±0.003	0.033±0.001	0.067±0.008	0.045±0.005
	Mean		**0.044abc**	**0.034bc**	**0.073bc**	**0.038bc**
		0	0.043±0.001	0.033±0.006	0.059±0.007	0.020±0.001
	28	50	0.047±0.007	0.021±0.004	0.055±0.006	0.031±0.003
		150	0.052±0.008	0.024±0.003	0.062±0.008	0.024±0.004
		250	0.059±0.002	0.024±0.004	0.068±0.008	0.041±0.004
	Mean		**0.055cd**	**0.026a**	**0.061a**	**0.029a**
LSD P=0.05			**P<0.05 0.014**	**P<0.05 0.015**	**P<0.05 0.013**	**P<0.05 0.015**

CHAPTER 4

AN INVESTIGATION INTO THE EFFICIENCY OF THE TOTAL ANTIOXIDANTS ASSAYS IN SILICON TREATED LEMON

ABSTRACT

The effect of postharvest silicon dips on the concentration of total antioxidants, total phenolics and malondialdehyde was investigated as a measure to reduce chilling injury of citrus. Previous studies in plants have shown a strong relationship between silicon and antioxidants capacity. The aim of this study was therefore to evaluate the effect of postharvest silicon dips to increase antioxidant level as to counteract chilling injury. Briefly, fruit from two sources were dipped into different silicon concentrations (0, 50, 150, and 250 mg ℓ^{-1}) for thirty minutes, and stored at -0.5ºC or 2ºC for 28 days with weekly evaluations for chilling injury incidence. The amount of antioxidants present in the fruit were determined using the FRAP, ABTS, and DPPH assays (spectrophotometically). Postharvest silicon applications had no effect on the flavedo's total phenolic content but increased the total antioxidants and reduced lipid peroxidation/membrane damage. However, high silicon concentrations (i.e. 150 and 250 mg ℓ^{-1}) were found to reduce the visual fruit quality. Chilling injury symptoms were reduced using 50 mg ℓ^{-1} K_2SiO_3 while antioxidants and total phenolics were significantly reduced with an application of 150 and 250 mg ℓ^{-1} K_2SiO_3. Fruit, which was seemingly chilling resistant, had a higher concentration of total antioxidants, total

phenolics and low malondialdehyde, as well as higher endogenous silicon concentration than fruit from the chilling susceptible location. Therefore, silicon (either applied at preharvest or postharvest) has the potential to reduce postharvest chilling injury. Preharvest silicon application should therefore be considered as an option to improve the rind's antioxidants concentration prior to cold storage. The tested antioxidant assays (FRAP, ABTS and DPPH) gave comparable results for antioxidants present in the lemon flavedo with ABTS and DPPH detecting the high correlation. Therefore, ABTS and DPPH would be the preferred assays to measure total antioxidants in lemon flavedo. The major antioxidants in lemon flavedo were found to be Vitamin C, phenolics and flavonoids.

4.1 INTRODUCTION

The citrus industry is an important component of the South African (Medina, 2006) economy. Internationally, South Africa is the second largest exporter of citrus, following Spain (Solomon, 2010). Approximately 61% is of the SA citrus production is exported comprising of Valencia (45.7%), navels (22.8%), grapefruit (13.6%), lemons (10.1%) and soft citrus (7.8%) (Solomon, 2010). The presence of the Mediterranean fruit fly (*Ceratitis capitata*) and false codling moth (*Thaumatotibia leucotreta*) requires cold sterilisation as a quarantine treatment to export citrus fruit (Serry, 2010). However, such cold treatment can result in chilling injury a condition characterized by dark brown spots on the flavedo, pitting, and eventually decay once fruit is moved to room temperatures (Perez-Tello *et al*., 2009).

Several techniques have been used to reduce chilling injury in order to extend fruit shelf life. These techniques have proved to be successful in many horticultural crops, such as hot water dips in lemons (Mclauchlan *et al.*, 1997b; Sapitniskaya *et al.*, 2006; Mathaba *et al.*, 2008), waxing of grapefruit and orange fruit (Wild, 1993; Petracek *et al.*, 1998; Perez Gago *et al.*, 2002), application of molybdenum-containing compounds in wheat and lemons (Xue-Cheng *et al*., 2006; Mathaba *et al*., 2008), ethylene in nectarines (Wang, 1990; Zhou *et al*., 2001) and methyl jasmonate in mango and loquat fruit (Gonzalez-Aguilar *et al*., 2000; Cao *et al*., 2009). However, health concerns regarding certain chemicals have been raised hence there has been a move towards less hazardous chemicals such silicon; a compound that is reported to be able to reduce postharvest stress (Liang *et al*., 2007b; Tesfay *et al*., 2011). The element has been shown to be involved in the metabolism and utilization of phenolic compounds in cucumber (*Cucumis sativus*) when grown in excess manganese (Maksimovic *et al*., 2007). The accumulation of phenolics in avocado(*Persea americana*) trees following silicon application has been reported (Bekker *et al.*, 2007), as well as improvement in postharvest quality due to increased total antioxidant capacity (Tesfay *et al*., 2011).

Chilling injury is an oxidative stress caused by the increased production of free radicals such as superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH·) (Mathaba *et al*., 2008; Lafuente *et al*., 2005). Such free radicals contribute to membrane breakdown (Liang *et al*., 2008). Chilling injury is preceded by lipid breakdown (Wongsheree *et al.*, 2009). Antioxidants alleviate the effect of free radicals which contribute to the development of chilling injury. Citrus flavedo has an

array of antioxidant compounds that are responsible for scavenging reactive oxygen species (ROS) and thereby alleviating stress (Abeysinghe *et al*., 2007; Mathaba *et al*., 2008; Cronje *et al*., 2011). Antioxidants have different scavenging mechanisms; it is therefore imperative to perform several antioxidant measurements to gain a holistic view of mechanisms counteracting ROS effects (Wong *et al*., 2006).

Several assays can be followed to measure the concentration of antioxidant present in the tissues. Previous studies have revealed that these assays either measure hydrophilic (ascorbic acid and phenolic groups) or lipophilic antioxidants (α-tocopherol, β-carotene and lycopene) (Wong *et al*., 2006; Pérez-Jiménez *et al*., 2008). The ferric reducing antioxidant power (FRAP) assay which is based on the ability of the antioxidant to reduce ferric-tripyridyltriazine to ferrous form (Benzie and Strain, 1996), while the oxygen radical absorbance capacity (ORAC) assay is based on hydrogen atom transfer from the antioxidant (Ou *et al*., 2002); the 2,2'-azinobis-(2-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay is based on hydrogen donating antioxidants and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay is based on the capacity of antioxidant to scavenge the 1,1-diphenyl-2-picrylhydrazyl radical (Wong *et al*., 2006). It is therefore imperative to assess whether different methods can equivalent antioxidant values for the sample.

With silicon (Si) having been shown to increase antioxidant concentration and subsequently reduce stress (Gunes *et al.*, 2007a; Gunes *et al.*, 2007b; Tesfay *et al.*, 2011), the aim of this study was to determine the potential of Si to mitigate chilling injury through increasing the rind antioxidant concentration, and to further investigate

which antioxidant assay is efficient in measuring holistic antioxidant action. Principal component analysis (PCA) is used to identify the total variation in the antioxidant activities of the fruit by the methods used.

4.2 MATERIALS AND METHODS

Lemon fruit were harvested in July 2010 from the University of KwaZulu-Natal Research Farm, Ukulinga (29º40'00''S, 30º24'00''E) and as well as Ithala Farm (29º52'00''S, 30º16'00''E) located in the KwaZulu-Natal Midlands. Fruit was transported to laboratory, where it was selected according to good appearance and absence of blemishes. Prior to treatments fruit was dipped in Sporekill® solution. Fruit was treated with various concentrations (0, 50, 150, 250 mg ℓ^{-1}) of potassium silicate (K_2SiO_3) dips for 30 minutes. The fruit was waxed with Avoshine® (Citrashine (Pty) Ltd) and weighed and subsequently stored at -0.5 or 2°C under 85-90% relative humidity (RH) for up to 7, 14, 21 or 28 days. After storage the fruit was evaluated for weight loss and kept at room temperature for five days before weight loss was recorded again and chilling injury evaluated. The percentage of chilling injury was also evaluated. Thereafter the flavedo of fruit was peeled, freeze-dried, milled and stored at -21ºC for further analysis.

4.2.1 Chemicals

The chemicals were obtained from Sigma Chemical Co. (St Louis, MO, USA) and Merck (Darmstadt, Germany): Ferric chloride ($FeCl_3$), tripyridyltriazine (TPTZ), sodium

acetate, thiobarturic acid (TBA), trichloroacetic acid (TCA). Deionized water was used for making solutions, hydrochloric acid (HCl), dimethyl sulfoxide (DMSO), Folic-Ciocalteu reagent, sodium carbonate, and chlorogenic acid.

4.2.2 Chilling injury evaluation

After 7, 14, 21 and 28 days cold storage at -0.5 or 2^0C plus 5 days shelf-life fruit were evaluated for appearance of chilling symptoms and chilling injury was expressed as percentage.

Chilling injury (%) = (Number of fruit with chilling symptoms/total number of fruit evaluated)*100

4.2.3 Total antioxidants capacity (TAC) Assays

Comparison of assays used to determine total antioxidant concentration as to gain a well-round view of mechanisms counteracting ROS effects. The total antioxidant concentrations were all standardized to µmol Trolox equivalents per g DW as to compare the assays.

4.2.3.1 Ferric reducing antioxidant power (FRAP) assay

Total antioxidant capacity (TAC) was determined using the FRAP assay according to the method of Abeysinghe *et al*. (2007), with slight modifications. The FRAP assay measures the ability of the antioxidants in the sample to reduce ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to the blue coloured ferrous form (Fe^{2+}) which absorbs light at 593 nm (Khanizadeh *et al*., 2008). The FRAP reagent was prepared freshly by mixing 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution, 20 mM ferric chloride solution in the ratio of 10:1:1 (v/v/v). Total antioxidants were extracted from 0.1 g milled lemon peel. The absorbance at 593 nm was measured 8 to 10 min after mixing 20 µl fruit extract with 1ml FRAP reagent, using a spectrophotometer (Beckman Coulter DU-800, USA). The TAC was expressed as µmol Trolox equivalents per g DW.

4.2.3.2 Total antioxidant activity (TAOA)

The ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay was performed according to Re *et al*. (1999) with some modifications. To determine the hydrophilic and lipophilic antioxidant fractions of the sample ABTS was dissolved in water to a 7 mM concentration. The ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting the ABTS solution with 2.45 mM ammonium persulfate, allowing the mixture to stand in the dark at room temperature for 3 to 6 hours. After addition of 1.0 mL activated ABTS solution (A_{734}= 0.700 ± 0.02) to 10 µL flavedo extract in 0.5M acetate buffer

(pH 4.0) was recorded after 6 min. The antioxidant capacity of the extract was expressed as µmol Trolox equivalents per g DW.

4.2.3.3 DPPH assay

The procedure of Wong *et al*. (2006) was followed with some modifications. Briefly, a 0.1 mM solution of DPPH in methanol was prepared. The initial absorbance of DPPH in methanol was measured at 515 nm and was kept constant throughout the period of assay. An aliquot (40 µl) of the extract was added to 3 ml methanolic DPPH solution. The change in absorbance at 515 nm was measured after 30 min. The antioxidant capacity, based on the DPPH free radical scavenging ability of the extract, was expressed as µmol Trolox equivalents per g DW.

4.2.4 Total phenolics

The free phenolic content was determined according to Abeysinghe *et al*. (2007), with some modification. Pulverized lemon peel (0.5 g) was accurately weighed in a screw-capped test tube. The samples were extracted with 5 ml of 50% DMSO: 50% of 1.2 M HCl in 80% methanol/water, vortexed for 1 minute and centrifuged at 10 000 rpm for 10 min. The supernatant was used for determination of free phenolics. Total phenolics of the flavedo extract were measured using a modified colometric Folic-Ciocalteu method (Abeysinghe *et al*., 2007). Four milliliters of distilled water and 0.5 ml of properly diluted flavedo extract were placed in a glass test tube. Folin-Ciocalteu

reagent (0.5 ml) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 1 ml saturated sodium carbonate. Absorbance at 760 nm was read after 3 hours, using a spectrophotometer. Chlorogenic acid was used as a standard and data were expressed as mg chlorogenic acid equivalents (CAE)/100 g DW.

4.2.5 Lipid peroxidation determination

Lipid peroxidation is measured by the accumulation of malondialdehyde (MDA) with high MDA accumulation signifying high lipid peroxidation. The malondialdehyde (MDA) concentration of rind tissue was determined by thiobarturic acid (TBA) reaction according to Abeysinghe *et al*., (2007). Freeze-dried flavedo tissue (0.5g) was homogenized in a chilled mortle and pestle with 10 ml of ice cold 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 20 000 x g for 10 minutes at 4°C. A 1ml aliquot of the supernatant was thoroughly mixed with 4 ml 0f 20% TCA containing 0.5% TBA. The mixture was incubated at 95°C for 30 minutes and thereafter quickly cooled in an ice bath. After centrifugation at 20 000 x g for 10 minutes at 4°C, the absorbance of the supernatant was recorded at 532 nm and corrected for nonspecific absorbance at 600 nm using spectrophotometer. The MDA concentration was calculated according to the following formula

Total MDA (nmol/g DW) = (Amount of extraction buffer (ml) x amount of supernatant (ml) x [(Abs 532nm-Abs 600nm/155mMcm^{-1}] x10^{3})* Amount of sample (g)$^{-1}$

4.2.6 Mineral analysis

To determine the Si concentration in the sample, all samples were observed under a Scanning Electron Microscope equipped with EDX detector (Zeiss EVO LS15, Oxford XMax detector, and INCA Energy EDX software). Solid particles were dispersed on a graphite adhesive tab placed on an aluminum stub.

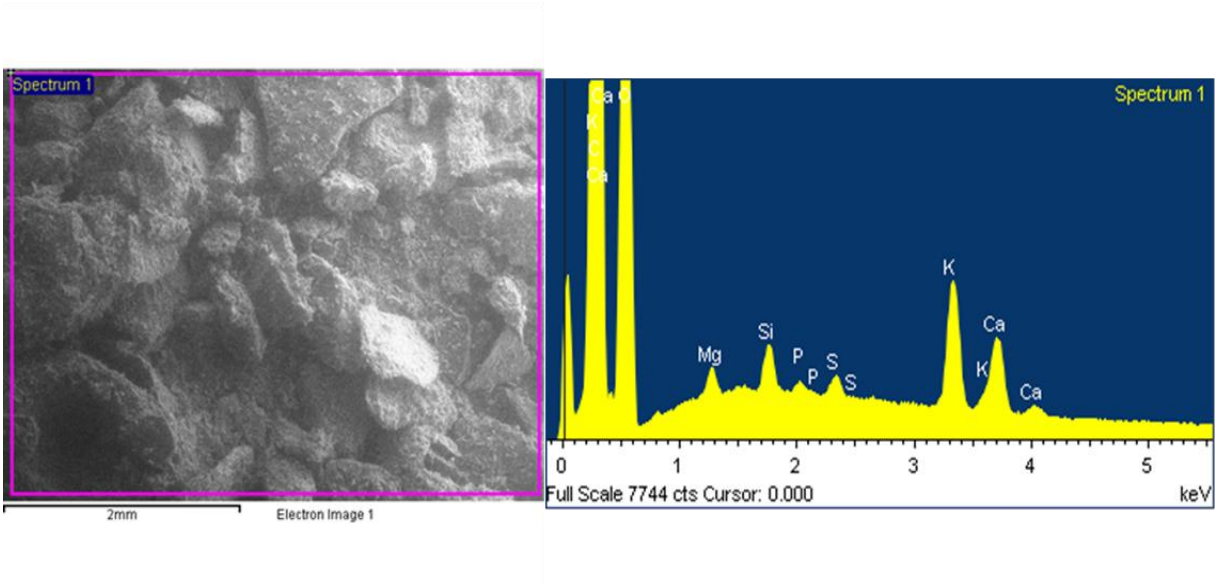

Figure 4.1: Mineral analysis as determined by energy dispersive X-ray spectroscopy (EDX)

4.2.7 Statistical analysis

Data were subjected to analysis of variance (ANOVA) using GenStat, 12th edition. Means were separated using Duncan's test at P≤0.05 levels. Furthermore, data was subjected to principle component analysis (PCA) using Unscrambler (version 9.8).

4.3 RESULTS

4.3.0 Antioxidant strength of antioxidant compounds occurring in citrus

The total antioxidant assay plays an important role in determining the contribution of individual antioxidants to total antioxidants capacity. In this study, Vitamin C was the main contributor to the total antioxidant capacity with FRAP, ABTS and DPPH assay (Figure 4.3). The contribution of individual antioxidants according to FRAP assay as in order of decreasing effectiveness was Vitamin C (ASE) > Phenolics (CAE) > Flavonoids (RUE) > Naringin (NE) > Hesperidin (HE) (Figure 4.3A). The contribution of individual antioxidants according to ABTS assay was Vitamin C > Phenolics > Flavonoids > Hesperidin > Naringin (Figure 4.3B). Lastly, according to DPPH the contribution of individual antioxidants was Vitamin C = Flavonoids ≥ Phenolics > Hesperidin > Naringin (Figure 4.3C).

4.3.1 Effect of silicon on chilling injury symptoms during cold storage

Fruit from Ukulinga Farm did not show chilling injury symptoms during cold storage and after the 5 days shelf-life. However, fruit from Ithala Farm developed chilling injury symptoms after 21 and 28 days of cold storage (Figure 4.1 A&B). Overall treatments, storage temperature had an impact on chilling injury percentage, with -0.5^{0}C showing a tendency to less chilling injury than 2^{0}C. Treatments with 50 mg ℓ^{-1} K_2SiO_3 significantly reduced chilling injury symptoms compared with the control and high silicon concentration after 28 days cold storage at -0.5ºC (Figure 4.1 A&B).

However, 250 mg ℓ^{-1} K_2SiO_3 displayed chilling injury at 21 days for both storage temperatures. In addition, control, 150 and 250 mg ℓ^{-1} K_2SiO_3 treatments showed significantly higher chilling injury at 28 days cold storage at -0.5ºC (Figure 4.1 A&B). Chilling symptoms tended to increase with K_2SiO_3 concentration (Figure 4.1). Furthermore, fruit treated with 150 and 250 mg ℓ^{-1} K_2SiO_3 displayed whitish patches of Si disposition.

4.3.2 Effect of fruit source on total antioxidant capacity and lipid peroxidation in lemon rind

Chilling susceptible control fruit from Ithala Farm showed significantly lower total antioxidant capacity than the chilling resistant fruit from Ukulinga Farm (Figure 4.2A, B and C); antioxidant assays giving similar trends for both Ithala and Ukulinga fruit (Figure 4.2A, B and C). However, chilling resistant fruit from Ukulinga contained significantly less total phenolics compared with the chilling susceptible Ithala fruit (Figure 4.2F).

The lipid peroxidation expressed as malondialdehyde (MDA) concentration was significantly higher in Ithala fruit compared with Ukulinga fruit (Figure 4.2E). Ukulinga fruit contained a significantly less MDA indicating resistance to chilling injury (Figure 4.2E). Furthermore, such fruit had a significantly higher endogenous Si concentration (control) compared with those from Ithala Farm (Figure 4.2D).

4.3.3 Effect of storage temperature on total antioxidants, total phenolics and lipid peroxidation of lemon flavedo during

The FRAP and DPPH assay detected a significant higher antioxidant capacity in the rind of fruit at -0.5ºC compared with 2ºC; however, the ABTS assay showed a significantly lower antioxidant capacity for Ukulinga fruit at -0.5ºC (Figure 4.2A, B & C). The rind of Ithala fruit had a higher antioxidant capacity when stored at 2ºC (FRAP) (Figure 4.2A) than the antioxidants determined using the DPPH assay which detected a higher antioxidant capacity at -0.5ºC (Figure 4.2B). The ABTS assay showed no significant differences in antioxidants between storage temperatures (Figure 4.2C).

The total phenolics concentration of the rind of Ukulinga fruit was not affected by storage temperature; however, in Ithala fruit 2ºC storage increased the rind total phenolics (Figure 4.2F). Lipid peroxidation, expressed as MDA, was significantly higher in the rind of the fruit for Ithala fruit stored at 2ºC compared with -0.5ºC storage (Figure 4.1 and 4.2E), while, storage temperature did not influence lipid peroxidation of the rind of Ukulinga fruit (Figure 4.2E).

4.3.4 Effect of cold storage period on total antioxidants, total phenolics and lipid peroxidation of lemon flavedo.

Cold storage time did not influence the total antioxidant capacity in the rind of Ithala fruit when determined by the FRAP assay; however, after 28 days in the cold storage

the highest antioxidant capacity was detected (Table 4.1 and 4.2). Ukulinga fruit followed the same trend, (28 days showing high antioxidant capacity even though it was not significantly different). The DPPH assay detected high total antioxidant capacity at 7 and 28 days for both Ithala and Ukulinga fruit. The ABTS assay detected high total antioxidant capacity at 0 days and low antioxidant at 28 days for Ithala fruit, in contrary high total antioxidant at 28 days was detected under ABTS (Figure 4.1 and 4.2).

Total phenolics were significantly higher after 28 days of cold storage in the rind of Ukulinga fruit; however, Ithala fruit showed an expected decrease in total phenolics as the cold storage time increased (Table 4.1 and 4.2). Cold storage time did not influence the lipid peroxidation of the rind of Ukulinga fruit (Table 4.1 and 4.2). Ithala fruit showed Significantly ($P<0.05$) low lipid peroxidation was shown in the rind of Ithala fruit after 7 days of cold storage, however, high lipid peroxidation after 0 days of cold storage was unexpectedly shown (Table 4.1 and 4.2).

4.3.5 Effect of silicon dips on total antioxidants, total phenolics and lipid peroxidation on lemon flavedo

Postharvest dips of 250 mg ℓ^{-1} K_2SiO_3 significantly ($P<0.05$) increased total antioxidant (FRAP) compared with other treatment (Table 4.1 and 4.2). Postharvest dips of 250 mg ℓ^{-1} K_2SiO_3 significantly increased the antioxidant capacity for Ithala fruit (250>150>50>0 mg ℓ^{-1}) under FRAP assay. However, silicon dips did not significantly impact on the rind of Ukulinga fruit. Silicon dips at 250 mg ℓ^{-1} K_2SiO_3 maintained a high

antioxidant capacity from 7 to 28 days for Ithala fruit, however, 150 mg ℓ^{-1} K_2SiO_3 decreased antioxidant capacity after 7 days whilst antioxidant capacity was improved after 28 days of cold storage (Table 4.1 and 4.2). The rind of Ukulinga fruit had a higher antioxidant capacity compared with that of Ithala fruit (Figure 4.2). The ABTS assay also detected a high antioxidant capacity at 150 mg ℓ^{-1} K_2SiO_3. Postharvest dips did not improve the antioxidant capacity of Ithala fruit; however, 250 mg ℓ^{-1} K_2SiO_3 increased the antioxidant capacity for Ukulinga fruit (Table 4.1 and 4.2).

Silicon dips did not improve the antioxidant capacity when measured under the DPPH assay. Similarly, postharvest silicon dips did not affect total phenolics in the rind of both, Ithala and Ukulinga fruit. However, Ukulinga fruit had higher total phenolics compared with Ithala the chilling susceptible Ithala fruit (Table 4.1 and 4.2).

Fruit sourced from Ithala Farm had significantly ($P<0.05$) lower MDA at 150 and 250 mg ℓ^{-1} K_2SiO_3; however, no significant difference was found on Ukulinga fruit even though low MDA tendencies were observed (Table 4.1 and 4.2). Low MDA was observed for Ithala fruit following 7 to 28 days after 250 mg ℓ^{-1} K_2SiO_3 dips.

4.3.6 Principal component analysis

Subjecting data to principal component analysis (PCA) led to variation of 90% with principal component 1 (PC1) explaining majority of variation (80%) and principal component 2 (PC2) explaining 11% of total variation (Figure 4.4). Variation explained by PC1 relates with DPPH and PC2 relates with ABTS (Figure 4A). When grouping

the data, Ithala which was chilling susceptible fruit showed different grouping compared with non-chilled fruit sourced from Ukulinga (Fig 4.4B).

4.4 Discussion and Conclusion

Antioxidants play an important role in stress reduction. Antioxidants have the ability to delay, reduce or prevent the destructive action of free radicals produced during stress (Halliwell, 1990; Salah *et al*., 1995). There are many citrus fruit antioxidants that have been identified to play a role in reducing stress, including vitamin C, phenolics, flavonoids and others. Antioxidants in the rind/exocarp of the fruit are most important in mitigating postharvest stresses such as chilling injury.

Silicon as it has been proven to induce stress resistance and enhancing antioxidant capacity in plants (Liang *et al*., 2008). Similarly, Si applied at 50 ml ℓ^{-1} K_2SiO_3 was involved in the reduction of chilling injury in this study (Figure 4.2B). The difference in silicon concentration between Ithala and Ukulinga fruit seemed to be an important factor for the susceptibility of Ithala Farm fruit and resistant of Ukulinga Farm fruit to chilling injury (Figure 4.1 and 4.2D). Postharvest treatment with 50 mg ℓ^{-1} K_2SiO_3 reduced chilling injury symptoms; a finding in agreement with Agarie *et al*. (1998), Bekker *et al*. (2007), Liang *et al*. (2008), Cai *et al*. (2009) and Epstein (2009). These authors found Si to play an important role in inducing stress resistance. However, high Si concentrations proved to be disadvantageous to rind quality as chilling injury was worsened by 150 and 250 mg ℓ^{-1} K_2SiO_3 compared to the control

treatment (Figure 4.1). This could be caused by the glassy characteristic of Si that damages the cell and subsequently increases fruit water loss.

Source of plant material can have an impact on total antioxidant capacity, as previously discovered on moringa (*Moringa oleifera*) leaves where production location has demonstrated a profound effect on total antioxidants (Iqbal and Bhanger, 2006). The difference in silicon concentration between the fruit source may explain the difference in total antioxidants as silicon increases the antioxidant capacity (Maksimovic *et al.*, 2007; Liang *et al.*, 2007b; Cai *et al.*, 2009). Furthermore, the reduced lipid peroxidation in the fruit containing high endogenous silicon concentrations fruit source further proves relationship between region of harvest and chilling injury.

Antioxidant capacity was not largely influenced by storage temperature (Figure 4.1A, B, C). Previous studies on apple (*Malus X domestica*) fruit have also revealed that refrigerator temperature does not influence antioxidant concentration nor activity (Van Der Sluis *et al.*, 2001). Fruit were probably pre-conditioned on the field hence the storage temperature did not affect antioxidant concentration.

The fruit storage time has an effect on total fruit antioxidants. Factors that impact on antioxidant capacity of fruit include postharvest storage conditions, such as which storage time (Connor *et al*., 2002). In this study, total antioxidants and total phenolics were not largely affected by storage time as determined by the FRAP and DPPH assays (Table 4.1 and 4.2). Connor *et al*. (2002) found that the antioxidant activity of blueberry (*Vaccinium* L. sp) increased when stored at 5ºC for 7 weeks. With

increased storage time, lipid peroxidation also increased a result in line with previous findings by Parkin and Kuo (1989) in cold-stored cucumbers (4ºC).

The success of silicon to maintain or increase the antioxidant capacity, and subsequently reduce the risk of abiotic stress, has been reported in previous studies (Agarie *et al.*, 1998; Cai *et al.*, 2009; Tesfay *et al.*, 2011). In this study, silicon enhanced the antioxidant capacity (FRAP) increasing with silicon concentration used. However, when using the ABTS assay total antioxidant capacity was reduced at 250 mg ℓ^{-1} K_2SiO_3. Total phenolics were not influenced by postharvest silicon dips, a finding in contrast with the increased phenolics in the avocado exocarp stored at 5.5ºC (Tesfay *et al*., 2011). The reduced lipid peroxidation, as determined by malondialdehyde accumulation (MDA) following silicon treatment is in agreement with the reduction in MDA in salt-treated barley leaves (Liang, 1999).

Our data has shown that the endogenous silicon concentration is correlated to the antioxidant capacity as measured by DPPH and ABTS assay thereby reducing lipid peroxidation and ultimately reducing chilling injury (Figure 4.2). The rind of Ukulinga fruit had a higher silicon concentration than Ithala fruit, probably responsible for chilling resistance of Ukulinga Farm fruit. The silicon effect of increasing antioxidant activity and reduction of MDA has been reported by Liang (1999), Liang *et al*. (2007), Cai *et al*. (2009) and Tesfay *et al*. (2011). Furthermore, postharvest silicon dips were effective in reducing chilling injury. Moreover, in as much as postharvest silicon dips have shown to improve total antioxidants, and reduce MDA, high silicon

concentration (150 and 250 mg ℓ^{-1} K_2SiO_3) have shown to impair fruit visual quality as whitish deposits are often observed on the fruit surface.

Total antioxidant assays have been proved to differ in determining the antioxidants due to the complexity of antioxidants. It is therefore important to use different antioxidants assay to get a holistic view of antioxidants involved in chilling stress mitigation in citrus. However, different antioxidant assays have never been correlated in relation to chilling injury of citrus. Wong *et al*. (2006) using principal component analysis found a strong correlation between total antioxidant capacity values of sweet potato (*Ipomea babatas*, C.) obtained for DPPH and FRAP assay. In this study, principal component analysis (Figure 4.4) showed a correlation between ABTS and DPPH, with DPPH probably estimating both, lipophilic and hydrophilic antioxidants (Wong *et al*., 2006). Moreover, Awika *et al*. (2003) found a correlation between ABTS and DPPH in sorghum. All three assays rank the strength of antioxidants in order of: Vitamin C > Phenolics > Flavonoids > Hesperidin > Naringin (Figure 4.3). The ABTS and DPPH assay had similar antioxidants except that DPPH detected a higher value for flavonoids than ABTS. Furthermore, rind antioxidants as determined by DPPH assay had slightly higher values than determined with other antioxidant assay. The use of FRAP, DPPH and ABTS provided a holistic picture of antioxidants involved in stress mechanism since the use of one dimensional method may be misleading due to complexity of some antioxidants (Nilsson *et al*., 2005; Awika *et al*., 2003).

In conclusion, fruit source had a great impact on the antioxidants present in the flavedo, lipid peroxidation and chilling injury. The use of different total antioxidant assays (FRAP, ABTS, DPPH) seems important to obtain a holistic estimate of the total antioxidant activity on flavedo. Moreover, the silicon concentration of a certain fruit source can be used to determine chilling susceptibility of a fruit, with high silicon concentrations resulting in less chilling susceptibility. Postharvest silicon dips increase the total antioxidant capacity, on fruit source and storage time; however, the visual fruit quality is partly impaired by postharvest silicon dips, preharvest silicon applications should be considered as a method to reduce postharvest chilling injury. The concentration applied is critical so the negative effect of the low visual fruit quality is not an issue. The FRAP, ABTS and DPPH assays gave comparable results for the antioxidant strength of lemon flavedo with ABTS and DPPH showing high correlation. Therefore, these assays constitute the best techniques for measuring the total antioxidants of lemon flavedo with major contributors to the total antioxidant capacity of lemon flavedo being Vitamin C, phenolics and flavonoids.

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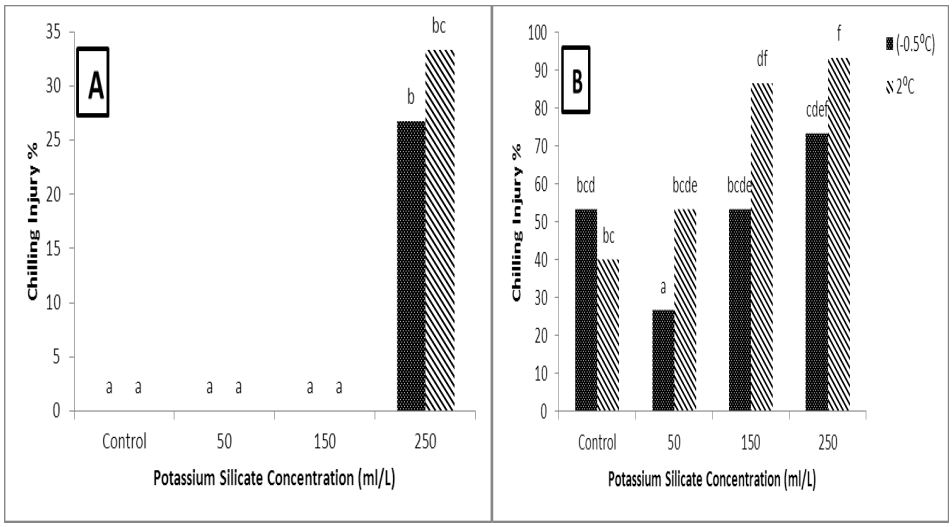

Figure 4.2: The effect of K_2SiO_3 concentration and storage temperature on chilling injury percentage of lemon fruit sourced from Ithala Farm at 21 days (A) and 28 days (B) storage time.

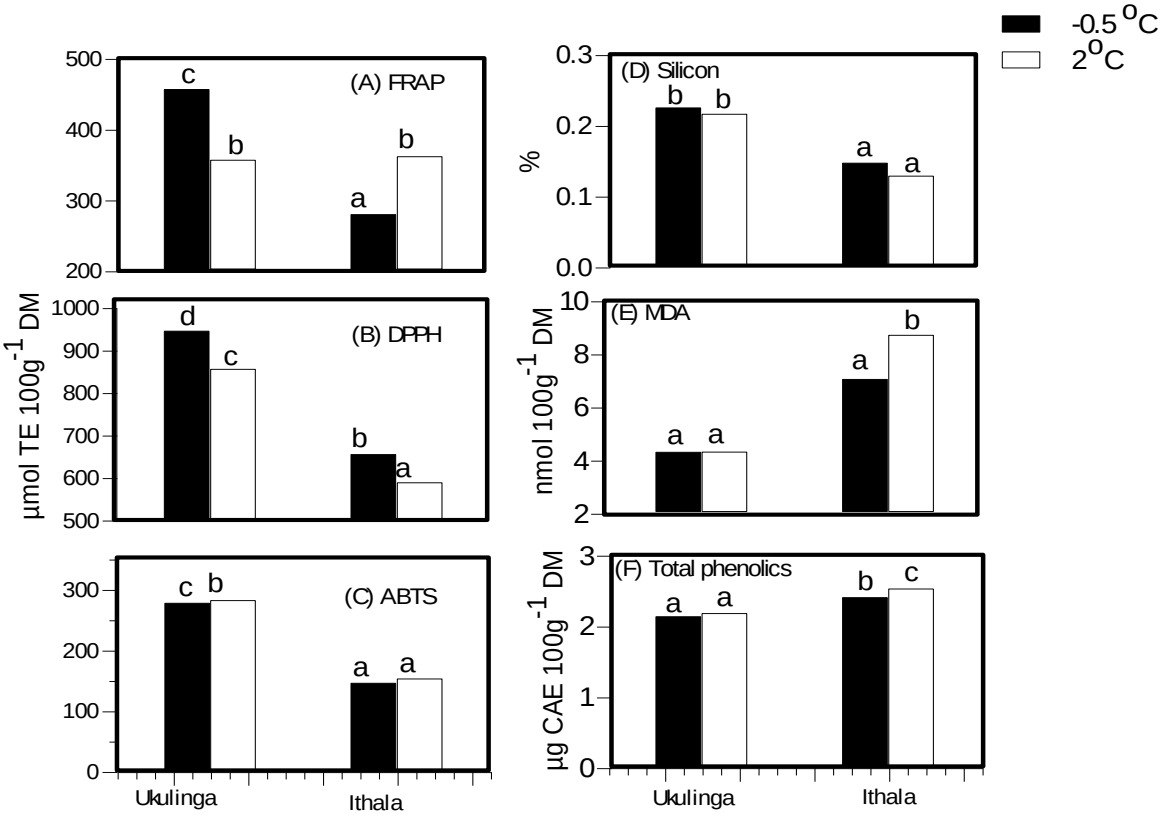

Figure 4.3: Effect of fruit source on total antioxidants, FRAP (A), DPPH (B), ABTS (C), and silicon (D),lipid peroxidation (MDA) (E) and total phenolics (F).

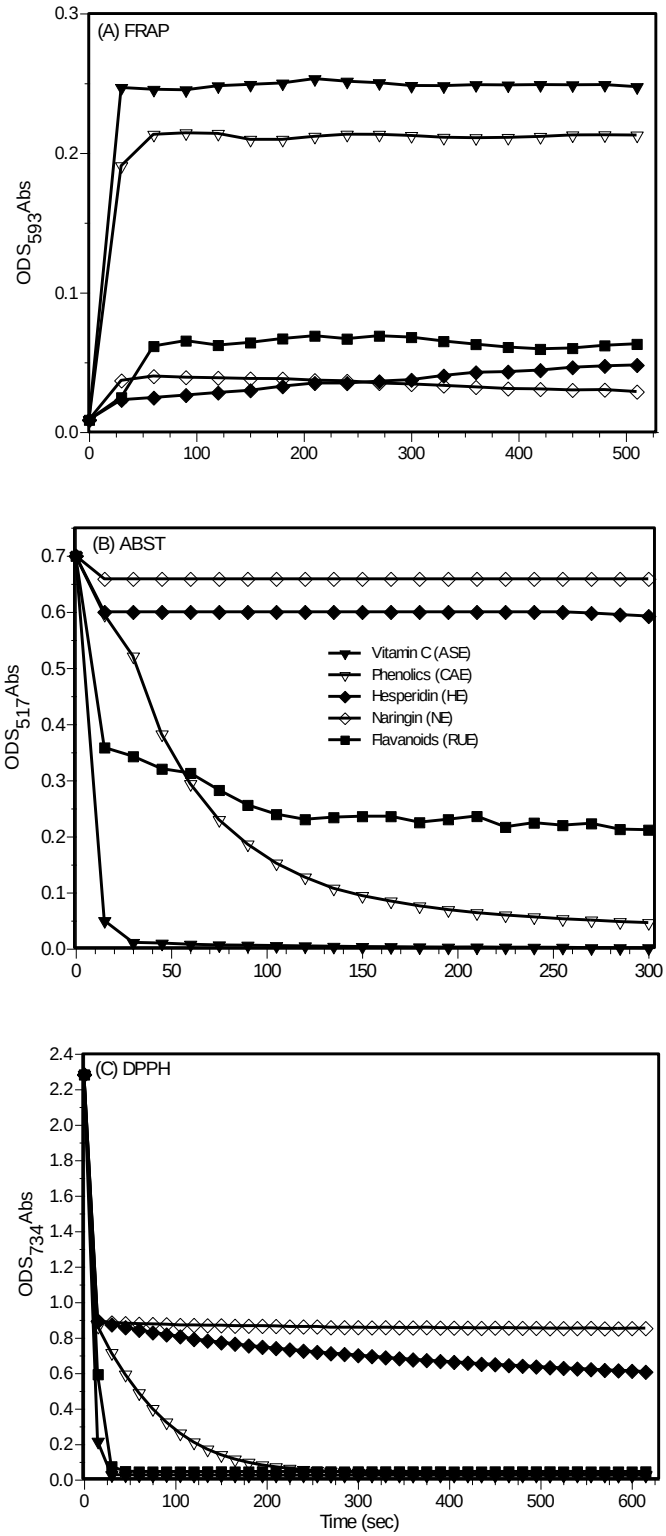

Figure 4.4: Evaluation of antioxidant capacity over different trolox equivalent using FRAP (A), ABTS (B) and DPPH (C) assays.

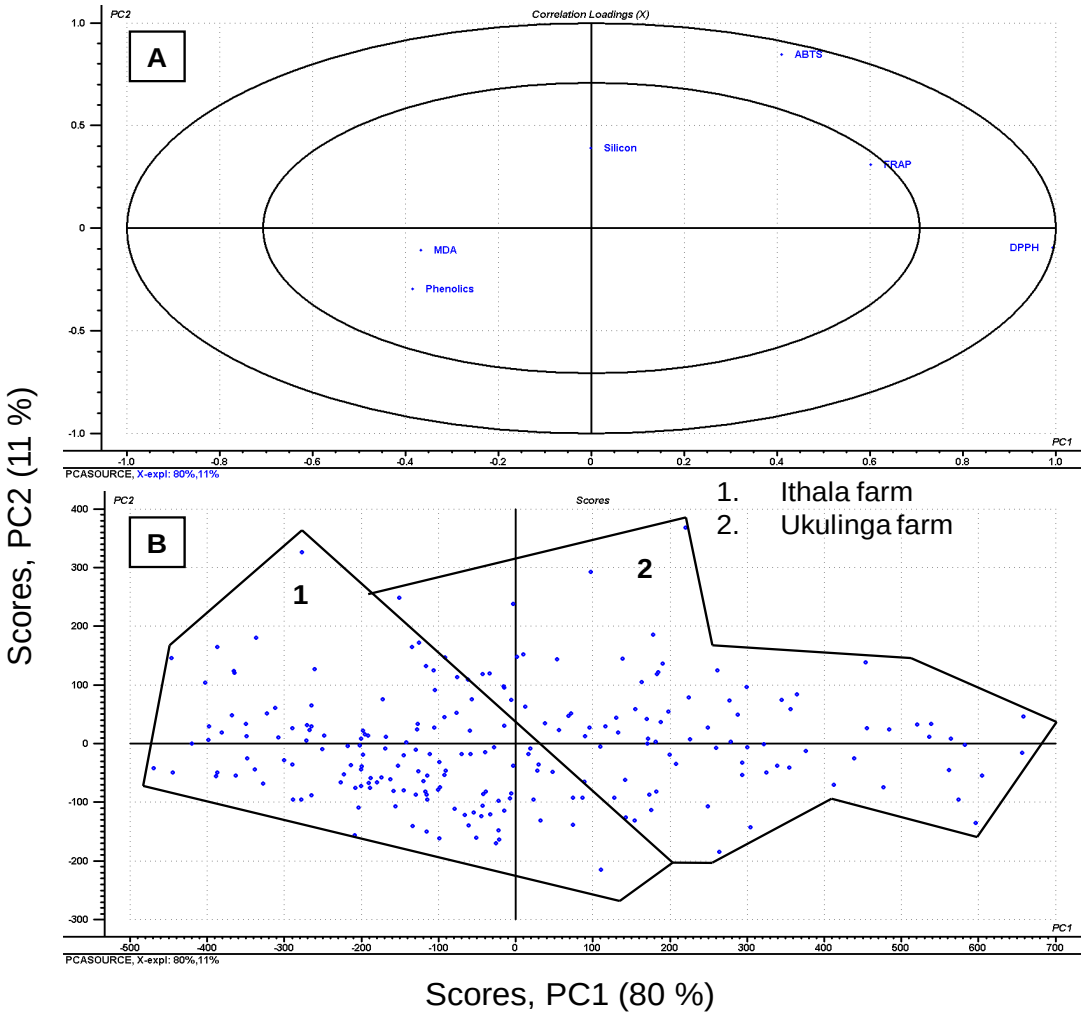

Figure 4.5: Principal component analysis (PCA) showing correlation loadings (A). Score plot lemon total antioxidants (FRAP, DPPH, ABTS), MDA, Silicon, and total phenolics (B). Principal component analysis (PCA) led to variation of 90% with principal component 1 (PC1) explaining majority of variation (80%) and principal component 2 (PC2) explaining 11% of total variation.

Table 4.1: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3), cold storage time, fruit source on total antioxidants (FRAP, ABTS, DPPH), total phenolics, and lipid peroxidation (MDA) of lemons stored at -0.5^{0}C.

		Ukulinga University Research Farm					Ithala Farm				
Cold storage time (days)	Treatments K_2SiO_3 (mg/g)	Lipid Peroxidation (MDA) nmol/g	FRAP (TE/100g)	DPPH (TE/100g)	ABTS (TE/100g)	Total Phenolics (mg/100g)	Lipid Peroxidation (MDA) (nmol/g)	FRAP (TE/100g)	DPPH (TE/100g)	ABTS (TE/100g)	Total Phenolics (mg/100g)
0	Control	300.1±0.48	537.7±10.76	999.5±54.61	256.9±56.21	1.84±0.07	256.9±0.31	264.5±28.20	747.4±36.78	300.1±36.22	2.62±0.09
	50	270.2±0.37	498.8±15.85	960.5±60.14	297.1±15.38	1.83±0.01	297.1±7.13	293.3±30.17	714.3±36.78	270.2±43.00	2.18±0.20
	150	276.3±1.88	342.5±15.85	701.6±80.23	216.9±15.62	1.86±0.06	216.9±2.38	294.4±23.14	645.7±24.44	276.3±39.35	1.91±0.22
	250	270.9±0.12	358.9±53.99	848.7±71.84	151.9±17.96	1.79±0.02	151.9±0.63	406.8±14.97	693.6±50.83	270.9±17.25	2.35±0.03
7	Control	241.8±0.17	457.5±35.35	918.0±85.35	154.7±46.15	2.09±0.03	154.7±0.04	275.7±55.63	666.2±59.42	241.8±04.95	2.79±0.02
	50	235.1±0.18	407.9±44.96	999.9±27.21	135.8±30.95	2.08±0.04	135.8±1.12	263.1±26.94	566.4±99.64	235.1±33.54	2.27±0.04
	150	238.6±0.28	545.8±19.89	971.9±23.61	130.6±11.09	2.12±0.01	130.6±0.60	228.2±18.53	730.1±26.11	238.6±06.35	2.46±0.10
	250	217.7±0.58	467.2±76.68	821.2±94.53	112.7±05.52	2.06±0.01	112.7±0.94	264.5±14.41	662.5±59.22	217.7±10.76	2.57±0.12
14	Control	318.4±0.61	367.6±19.41	959.7±60.08	100.2±36.25	2.21±0.05	100.2±0.35	279.0±06.04	685.4±49.58	318.4±08.38	2.19±0.13
	50	293.7±4.47	421.3±40.27	929.1±44.62	118.5±22.00	2.34±0.01	118.5±0.73	224.4±03.85	566.1±81.07	293.7±23.01	2.48±0.02
	150	372.3±0.53	438.2±15.51	899.1±03.21	148.4±28.92	2.45±0.09	148.4±0.08	267.5±32.42	446.3±65.48	372.3±08.24	2.56±0.06
	250	296.0±0.74	512.4±51.85	833.9±80.31	272.2±12.56	2.41±0.04	272.2±1.09	233.8±21.08	572.4±80.22	296.0±90.25	2.47±0.03
21	Control	241.3±0.43	347.5±50.51	729.2±09.38	110.0±12.28	2.30±0.07	110.0±0.22	231.4±13.84	630.4±48.44	241.3±13.46	2.50±0.05
	50	283.1±0.46	402.8±24.55	706.6±51.30	097.3±35.16	2.19±0.04	097.3±0.69	252.6±15.78	531.9±99.02	283.1±19.35	2.54±0.01
	150	301.5±0.73	468.6±68.08	653.2±53.31	079.6±12.63	2.21±0.02	079.6±0.95	277.7±20.24	406.9±58.85	301.5±09.89	2.46±0.04
	250	261.3±0.37	444.6±42.72	685.9±10.36	096.6±18.21	2.15±0.03	079.6±0.49	309.2±12.49	398.4±31.26	261.3±10.79	2.38±0.15
28	Control	304.9±0.58	532.1±17.66	1290.1±15.47	116.0±10.95	2.30±0.09	116.0±0.77	287.7±37.30	832.2±40.67	304.9±42.40	2.34±0.08
	50	259.8±0.06	576.5±71.60	1317.9±31.48	153.1±86.71	2.15±0.01	153.1±0.82	321.8±28.28	907.9±21.73	259.8±03.17	2.26±0.04
	150	265.1±0.65	477.5±40.86	1259.8±41.83	086.8±34.35	2.12±0.02	086.8±1.59	284.6±04.72	799.3±64.25	265.1±19.12	2.42±0.01
	250	301.7±0.50	521.0±23.42	1290.6±48.83	096.6±08.85	2.16±0.02	096.6±1.13	330.2±31.65	882.3±90.23	301.7±12.94	2.32±0.08
		P<0.05	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**	**P<0.05**
P=0.05	**LSD**	**4.065**	**155.6**	**208.8**	**94.52**	**0.240**	**4.065**	**155.6**	**208.8**	**94.52**	**0.240**

Note: Values are means± SE

TE= Trolox equivalent

Table 4.2: Effect of post-harvest dips of Potassium Silicate (K_2SiO_3), cold storage time, fruit source on total antioxidants (FRAP, ABTS, DPPH), total phenolics, and lipid peroxidation (MDA) of lemons stored at 2^0C.

		Ukulinga University Research Farm					Ithala Farm				
Cold storage time (days)	Treatments K_2SiO_3 (mg/g)	Lipid Peroxidation (MDA) nmol/g	FRAP (TE/100g)	DPPH (TE/100g)	ABTS (TE/100g)	Total Phenolics (mg/100g)	Lipid Peroxidation (MDA) nmol/g	FRAP (TE/100g)	DPPH (TE/100g)	ABTS (TE/100g)	Total Phenolics (mg/100g)
0	Control	300.1±0.48	537.7±10.76	999.9±54.61	256.9±56.21	1.84±0.07	256.9±0.31	264.5±28.70	590.2±46.10	300.1±36.22	2.62±0.09
	50	270.2±0.37	498.8±15.85	960.5±60.14	297.1±15.38	1.83±0.01	297.1±7.13	293.3±30.17	420.6±35.61	270.2±43.00	2.18±0.20
	150	276.3±1.88	342.5±15.85	701.6±82.31	216.9±15.62	1.86±0.06	216.9±2.38	294.4±23.14	372.6±31.46	276.3±39.35	1.91±0.22
	250	270.9±0.12	358.9±53.99	848.7±71.84	151.9±17.96	1.79±0.02	151.9±0.63	406.8±14.97	405.7±44.59	270.9±17.26	2.35±0.03
7	Control	253.5±0.62	408.8±55.64	999.1±82.76	154.6±22.99	2.17±0.03	154.6±0.67	275.6±47.36	798.0±19.09	253.5±20.89	2.73±0.22
	50	373.5±0.58	335.7±33.42	811.0±90.24	086.4±28.42	2.04±0.01	086.4±0.52	303.4±22.01	677.8±48.89	373.5±27.58	2.75±0.05
	150	356.3±1.09	294.3±36.14	875.1±74.98	172.8±56.47	2.11±0.02	172.8±0.34	233.3±37.39	640.9±27.72	356.3±09.71	2.95±0.03
	250	269.2±1.63	381.3±60.61	856.0±99.98	144.3±11.61	2.09±0.01	144.3±1.16	313.1±80.64	539.4±84.14	269.2±36.44	2.68±0.01
14	Control	231.6±0.14	521.5±38.72	909.9±98.23	134.5±19.00	2.26±0.03	134.5±0.55	317.6±32.29	581.5±07.59	231.6±23.62	2.75±0.05
	50	221.5±0.46	374.6±67.17	989.6±89.20	140.7±20.64	2.37±0.05	140.7±1.19	265.7±03.11	508.1±29.46	221.5±29.33	2.53±0.07
	150	202.8±0.58	290.3±21.88	897.8±71.06	176.6±26.48	2.30±0.05	176.6±0.59	272.7±14.27	540.4±27.65	202.8±27.45	2.80±0.03
	250	234.3±0.48	193.7±14.97	557.3±46.17	100.6±03.69	2.35±0.01	100.6±1.04	381.0±06.21	607.5±24.42	234.3±28.45	2.62±0.08
21	Control	234.9±0.03	274.4±12.19	996.9±55.48	114.5±31.83	2.28±0.08	114.5±0.92	289.6±31.11	662.8±76.94	234.9±26.54	2.51±0.09
	50	235.7±0.55	345.4±48.18	935.1±93.78	137.7±11.31	2.25±0.06	137.7±0.75	339.8±53.47	576.4±17.92	235.7±02.41	2.61±0.03
	150	207.3±0.24	403.1±29.34	769.9±59.44	164.4±14.46	2.18±0.02	164.4±0.48	304.4±12.59	641.3±24.09	207.3±18.58	2.68±0.02
	250	246.2±0.22	429.3±26.49	781.8±92.08	147.2±25.90	2.10±0.03	147.2±0.71	411.6±78.34	664.5±90.52	246.2±09.55	2.68±0.01
28	Control	327.2±0.56	328.0±37.97	772.1±77.76	096.4±54.24	2.22±0.05	096.4±0.96	386.5±10.98	665.7±40.51	327.2±03.81	2.21±0.14
	50	417.3±0.14	332.6±43.37	659.1±90.25	089.8±50.21	2.35±0.05	089.8±0.51	476.6±54.23	705.1±32.47	417.3±20.85	2.28±0.05
	150	459.7±0.71	389.2±22.81	794.2±42.08	143.4±41.94	2.48±0.04	143.4±0.89	635.3±56.23	567.1±15.00	549.7±07.56	2.18±0.27
	250	248.4±0.48	359.0±02.57	571.2±55.44	129.1±13.72	2.71±0.22	129.1±1.03	762.6±45.23	591.9±12.50	248.4±03.96	2.47±0.03
LSD P=0.05		**P<0.05 4.065**	**P<0.05 155.6**	**P<0.05 208.8**	**P<0.05 94.52**	**P<0.05 0.240**	**P<0.05 4.065**	**P<0.05 155.6**	**P<0.05 208.8**	**P<0.05 94.52**	**P<0.05 0.240**

Note: Values are means± SE

TE= Trolox equivalent

CHAPTER 5

5.1 General Discussion

Temperature is a critical factor in the maintenance of quality produce (Tsaneem, 2004). Generally, low temperatures lessen the rate of metabolic processes, and are an effective method of prolonging postharvest life of horticultural produce (Lyons, 1973). Unfortunately, although low temperature storage prolongs shelf-life and maintains high produce quality, there are negative effects. Exposing fruit tissue to chilling stress results in accumulation of reactive oxygen species which are damaging to plant tissue. It is important for fruit to have a pool of antioxidant systems so as to effectively mitigate any kind of stress, such as chilling injury. To minimize the incidence of chilling injury in lemons (*Citrus limon*), certain postharvest treatments are used. However, almost all current methods used to alleviate chilling injury have health concerns.

Various experiments have been conducted in pursuit of the most economic and consumer friendly ways of reducing postharvest chilling injury in citrus fruit, lemons in particular. Many chemicals and methods have been used as discussed in the previous chapters. However, the risk posed by use of chemicals on consumer health has become a major concern. The discovery of silicon as having the potential to alleviate abiotic and biotic stresses has made it possible to investigate the use of silicon as a safe alternative for alleviating chilling injury in fruit. Previous research

showed that preharvest application of silicon increased the antioxidant pool in avocado fruit stored at 5.5ºC (Tesfay *et al*., 2011). The overall objective of this study was to evaluate the potential of postharvest Si applications in decreasing chilling injury of lemon fruit.

The first study (Chapter 2) showed that silicon had potential to regulate the glucose-ascorbic acid relationship. Ascorbic acid (AA) is one of the antioxidants involved in postharvest fruit life and quality. Thus, it is important to ensure fruit has enough AA at postharvest. Under stress conditions, such as chilling injury when increased AA is required, the cells accumulate sugars which are conveyed for AA production (Ioannidi *et al*., 2009). Therefore, carbohydrates play a significant role in reducing the appearance of chilling symptoms specifically in citrus fruit (Holland *et al*., 2002). The role of silicon in increasing carbohydrate content was also found in this study. The conversion of glucose to ascorbic acid at lower concentration silicon dips significantly reduced appearance of chilling injury symptoms by inducing an enzymatic conversion of glucose to ascorbic acid, thereby increasing the antioxidant capacity of chilling susceptible fruit. This work reinstates the validity of recent work on glucose-ascorbic relationship in lemon stored at -0.5^{0}C for up to 28 day (Bower *et al*., In press). However, based on observations from Ukulinga fruit, this relationship seems to be found only for fruit with high glucose before storage.

The positive effects of ascorbic acid and glucose associated with silicon were evident after cold storage. Pre-storage silicon treatment resulted in reduced electrolyte leakage, lipid peroxidation, weight loss, increased bioactive compound expression and capacity as well as reduced chilling injury (Chapters 2 and 3).

Increased resistance to cell breakdown has been associated with such responses (Tesfay, 2009). The protection of cell membrane after silicon treatment has previously been achieved (Liang *et al.*, 2007). Rice leaves grown with silicon are often found to have low electrolyte leakage than those with no silicon (Agarie *et al*., 1998). In addition, the effect of silicon on lipid peroxidation of agricultural crops has been observed under drought stress (Liang, 1998; Lobato *et al.*, 2009). The reduced incidence of chilling injury following silicon application revealed the importance of increasing the pool of sugars and ascorbic acid prior to cold storage.

The potential of postharvest silicon dips to regulate phenolics and flavonoids in citrus peel as a method to mitigate chilling injury in lemons was also studied (Chapter 3). Phenolics and flavonoids are very efficient in scavenging reactive oxygen species which are the principal cause of chilling injury. This study revealed the potential of silicon to increase phenolics and flavonoids in citrus fruit. Similar results have previously been reported in avocado (Bekker *et al.*, 2007). Tesfay *et al*. (2011) also reported improved fruit quality in avocado following silicon application. They associated the improvement in quality with increased phenolic content in the fruit following treatment with silicon.

An investigation on the efficiency of the total antioxidants assays in silicon treated lemon was also studied (Chapter 4). The aim was not only to study the effects of Si but also the efficiency of total antioxidant assays. Total antioxidant assays have been correlated by other researchers (Awika *et al.*, 2003; Nilsson *et al.*, 2005; Wong *et al.*, 2006). However, different antioxidant assays have never been correlated in relation to chilling injury of citrus. The use of FRAP, DPPH and ABTS showed a good

performance of antioxidants since the use of one dimensional method may be misleading due to complexity of some antioxidants (Awika *et al.*, 2003; Nilsson *et al.*, 2005). The FRAP, ABTS and DPPH gave comparable results for the total antioxidant capacity of lemon flavedo. High correlation was found on ABTS and DPPH assays. Therefore, ABTS and DPPH would be the most suited techniques for measuring total antioxidants of lemon flavedo. Silicon enhanced antioxidant concentrations and reduced chilling injury. The difference in silicon concentration between Ithala and Ukulinga fruit rind, further provided a solid reason on the susceptibility of Ithala Farm fruit and resistant of Ukulinga Farm fruit to chilling injury (Figure 4.1 and 4.2D).

The differences observed in fruit susceptibility to chilling injury reinforced reports that the region of harvest determines fruit susceptibility to chilling injury (Mclauchlan *et al.*, 1997; Mathaba *et al.*, 2008). Apart from visual evidence of chilling injury, the question of why Ukulinga fruit was chilling resistant was addressed. Ukulinga fruit had high concentrations of bioactive compounds; phenolics, flavonoids, sugars, ascorbic acid and total antioxidants were higher compared to Ithala fruit which was chilling susceptible (Chapter 2, 3 and 4). Therefore, the high antioxidant power of Ukulinga fruit was responsible for chilling resistance observed. Physiologically, the amount of reactive oxygen species produced should be less than or equal to the available antioxidants in the fruit. The high antioxidants of Ukulinga fruit neutralized the reactive oxygen species and subsequently reduced chilling injury.

The effect of fruit source in determining chilling injury susceptibility may be closely related to pre-harvest practices and mineral nutrients in the soil. In the present study, high antioxidant power and chilling resistance of Ukulinga fruit coincided with

significantly high endogenous silicon. Moreover, chilling susceptible fruit were not only characterized by low antioxidant power, but also low endogenous silicon. The effect of silicon on phenolics and flavonoids as reported by (Bekker *et al.*, 2007) and on total antioxidants (Gunes *et al.*, 2007; Islam and Saha, 1969; Liang, 1999; Liang *et al.*, 2007) was evident on this study.

Lastly, the study evaluated quality differences due to storage temperature. Low chilling injury and good fruit quality was achieved at -0.5ºC. Fruit stored at 2ºC had a higher incidence of chilling injury. However, there were no differences observed, in terms of antioxidants, between storage temperatures. Zhang *et al*. (2010) found that peach fruit stored at 0ºC had low electrolyte leakage and consequently low chilling injury compared to fruit stored at 5ºC. Furthermore, enzyme activity was found to be very high in peach fruit stored at 0ºC. It can therefore be concluded that the expression and activity of bioactive compounds/antioxidants involved in metabolic pathways is not highly affected at -0.5ºC than at 2ºC. Moreover, the membrane possibly performed its normal functions at -0.5ºC than 2ºC hence percentage chilling injury was high.

5.2 Conclusions

The research reported in this thesis showed the effects of postharvest silicon dips of lemon and physiological responses of lemons after silicon dips treatments. The effects of silicon on lemon postharvest chilling injury have also been found. This was achieved, in part, by the regulation of AA, phenolics and antioxidants, all of which are involved in stress resistance mechanisms. The role of silicon in regulating ascorbic acid, sugars, phenolics and total antioxidants and subsequently reducing chilling stress was observed. 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 replacement for methods currently used in the citrus industry. However, the issue of appropriate concentrations to be used, particularly on lemons, requires further study. It was also shown that storing fruit at -0.5˚C resulted in reduced incidence of chilling injury compared to storing fruit at 2˚C. However, the reasons for this were unclear; there were no differences in levels of antioxidants, AA and sugars between the two storage temperatures. Lastly, fruit susceptibility to chilling injury may be related to pre-harvest conditions as shown by the effect of fruit source on chilling injury susceptibility.

5.3 Recommendations and Future research

- Generally, preharvest practices always determine the postharvest quality. Preharvest practices should increase antioxidants on fruit to ensure stress resistance at postharvest.
- It is widely postulated that antioxidants are related to postharvest quality due to their antagonistic power over reactive oxygen species.
- This study has clearly shown that silicon has a potential to reduce chilling injury, however, high silicon concentration has raised some concern in particularly on visual quality.
- Preharvest silicon application should therefore be considered on citrus trees. Furthermore, the results of this work have revealed that endogenous silicon has an ability to increase the antioxidant pool and subsequently reduce chilling injury.

The future research will have to look at the effects of preharvest silicon application on postharvest chilling injury.

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