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The potential of postharvest silicon dips to regulate phenolics in citrus peel as a method to mitigate chilling injury in lemons

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This study investigated the ability of silicon dips to enhance the phenolic content in order to reduce the incidence of chilling injury in lemon fruit. Fruits were obtained from two farms and dipped in 0, 50, 150 and 250 mg L^{-1} solutions of K_2SiO_3 for 30 min and afterward, fruit were air dried and waxed. Thereafter, fruits were stored at -0.5°C and sampled after 28 days for evaluation of phenolic content and chilling injury symptoms. Chilling susceptible fruit sourced from Ithala farm had significantly lower phenolics and flavonoids concentration when compared with chilling resistant lemons from Ukulinga farm. Phenolic and flavonoids content was improved by dipping fruit in silicon for almost all the concentrations. 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.

Key words: Silicon, lemon, antioxidants, phenolics, chilling injury.

INTRODUCTION

Citrus fruit production is very important 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 always a challenge to meet these quality requirements; this is due to convoluted marketing systems before the fruit reach consumers.

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 -0.5°C for 22 days in order to meet quarantine requirements (CRI, 2008). However, tropical and subtropical fruit are susceptible to chilling injury at temperatures below 12°C (Lafuente et al., 2005).

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 (Sapitniskaya et al., 2006; Mathaba et al., 2008), waxing (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 al., 2006) and methyl jasmonate (Gonzalez-Aguilar et al., 2000). These chemicals are applied to reduce

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chilling injury, possibly by reinforcing bioactive compounds that detoxify reactive oxygen species (ROS) (Sato et al., 2001). One of the effective bioactive compounds in protecting the fruit from oxidative damage is phenolics. Phenolics are often available in free and conjugated forms (Zuo et al., 2002; Robbins, 2003). Flavonoids are a subgroup of phenolics in plants, also with antioxidants capacity to deactivate ROS thereby reducing lipid peroxidation and cellular damage (Medina, 2006). Many chemicals and methods have proven to be 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 shown 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 (Liang et al., 2003; Hammerschmidt, 2005; Crusciol et al., 2009; Epstein, 2009; Keeping and Reynolds, 2009). Moreover, recent postharvest studies on avocado have proved Si to be a safe and effective antioxidant source (Tesfay et al., 2011). The aim of this work was to investigate whether silicon could be used to reduce chilling injury in lemon fruit. Mechanisms used by silicon to improve antioxidant capacity, in particular, phenolics where also investigated.

MATERIALS AND METHODS

Fruit were obtained from ten mature 'Eureka' lemon (*Citrus limon*) trees located in the Ukulinga Research Farm (29°40'00''S, 30°24'00''E) and Ithala Farm (29°52'00''S, 30°16'00''E), both located in the midlands of KwaZulu-Natal, South Africa. Agroclimatic conditions of both farms were found to be identical during the study. Fruit were collected in June when they reached their commercial maturity. 80 fruits (per location) were selected based on uniform appearance and size, washed with distilled water and Sporekill® solution, and left to air-dry prior to experimentation. Fruit were then soaked in different concentrations (0, 50, 150 and 250 mg L^{-1}) of potassium silicate (K_2SiO_3) dips for 30 min. For each location, fruit were sub-divided into four groups with 20 fruit per KSil treatment. The treatments were replicated four times, one replication contained five fruits. A randomised complete design (RCD) was assigned for statistical analysis. Fruits were later waxed with Avoshine® (Citrashine (Pty) Ltd), weighed and subsequently stored at -0.5°C at 85 to 90% relative humidity (RH) for 28 days to imitate commercial shipping and shelf life conditions. 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 -21°C for further physiological analyses.

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 from each fruit sample peel of the five fruits per replication 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 h, 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 h and allowed to cool to room temperature. The percentage of electrolyte leakage was calculated as:

Total electrolyte leakage (%) = (initial reading (EC1)/final reading (EC2) x100)

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(%) = (mass $_{\text{(before storage)}}$ - mass $_{\text{(after storage+5days at room temperature)}}$/mass $_{\text{(before storage)}}$ x100

Extraction of phenolics

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) from each replicate 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 min and centrifuged at 10 000 rpm for 10 min to remove the solid fraction. The resultant supernatant was used for determination of free phenolics and flavonoids.

Conjugated phenolics

5 ml 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 h, with vortexing every 30 min. Thereafter, samples were allowed to cool down to room temperature, they were diluted to 10 ml with methanol and centrifuged at 10 000 rpm for 10 min to remove the solid fraction. The supernatant was used for determination of conjugated phenolics and flavonoids.

Determination of phenolics content

Free and conjugated phenolics of flavedo extract were measured using a modified colorimetric Folin-Ciocalteu method (Abeysinghe et al., 2007). 4 ml 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 of saturated sodium carbonate. Absorbance was read at 760 nm after 3 h 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.

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) from each replicate 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 min of incubation at 40°C using a spectrophotometer (Beckman Coulter

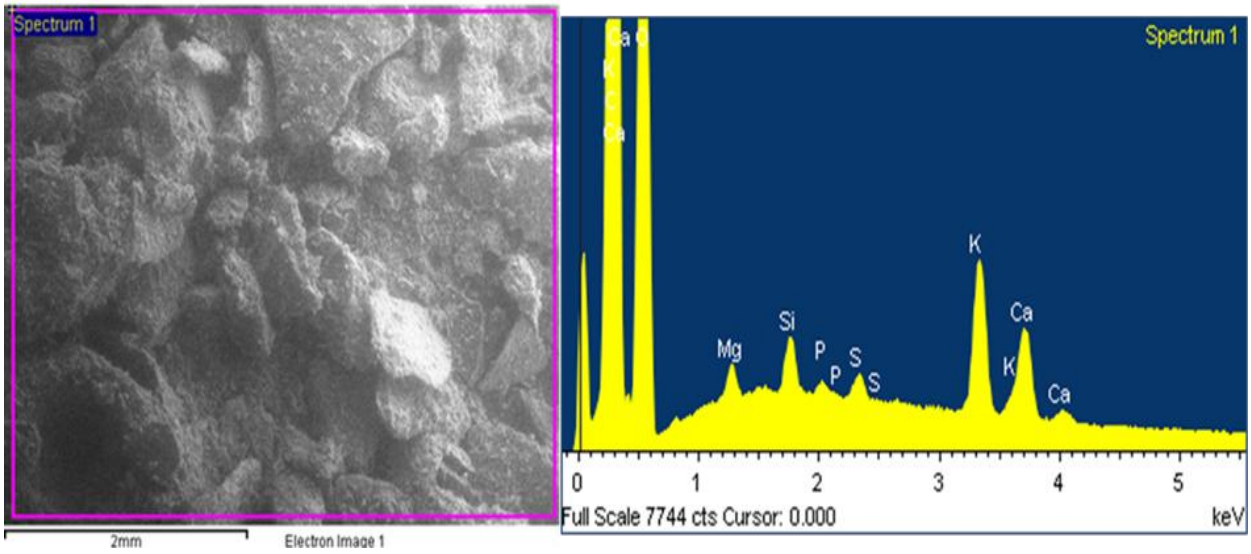

Figure 1a. Mineral analysis as determined by energy dispersive X-ray spectroscopy (EDX).

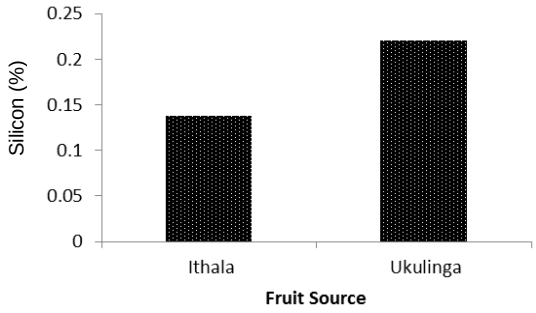

Figure 1b. Silicon content of lemon peel from Ithala and Ukulinga farm.

DU-800, USA). Rutin was used as a standard and total flavonoid content was expressed as mg rutin equivalent (RE/100 g DW).

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 1a).

Statistical analysis

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

RESULTS

Mineral analysis

Significant differences were found between Ukulinga and Ithala fruit in relation to potassium silicate concentration. The fruit sourced from Ukulinga farm had significantly high silicon concentration as compared to Ithala farm (Figure 1b).

Chilling injury

Fruit from Ukulinga farm did not show chilling injury symptoms during cold storage and shelf-life (data not shown). However, fruit from Ithala farm showed chilling injury symptoms after 21 and 28 days of cold storage (Figure 2). There were significant differences ($P<0.05$) between treatments with 50 mg L^{-1} K_2SiO_3 significantly ($P<0.05$) reducing chilling injury at 28 days cold storage (Figure 2B). Only 27% of the fruit stored at -0.5°C and treated with 50 mg L^{-1} K_2SiO_3 had chilling injury. However, control, 150 and 250 mg L^{-1} K_2SiO_3 treatments had 40 to 97% chilling injury after 28 days of cold storage. Chilling symptoms increased with K_2SiO_3 concentration (Figure 2A and B).

Weight loss

Fruit sourced from Ithala farm showed significantly ($P<0.05$) higher fruit weight loss when compared with Ukulinga fruit (Figure 3). Fruit weight loss increased with increasing K_2SiO_3 concentration; however, the 50 mg L^{-1} K_2SiO_3 significantly reduced weight loss for both Ithala and Ukulinga Farm (Figure 3A). The high concentrations of potassium silicate increased fruit weight loss for both localities.

Electrolyte leakage (EL)

Electrolyte leakage also differed ($P<0.05$) between fruit

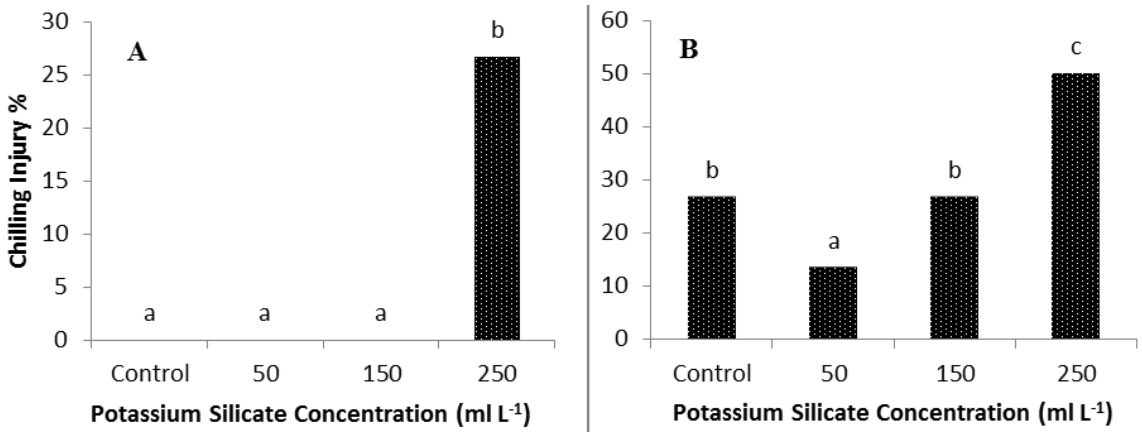

Figure 2. Effect of potassium silicate concentration on chilling injury percentage after 21 (A) and 28 days (B) cold storage in fruit sourced from Ithala farm.

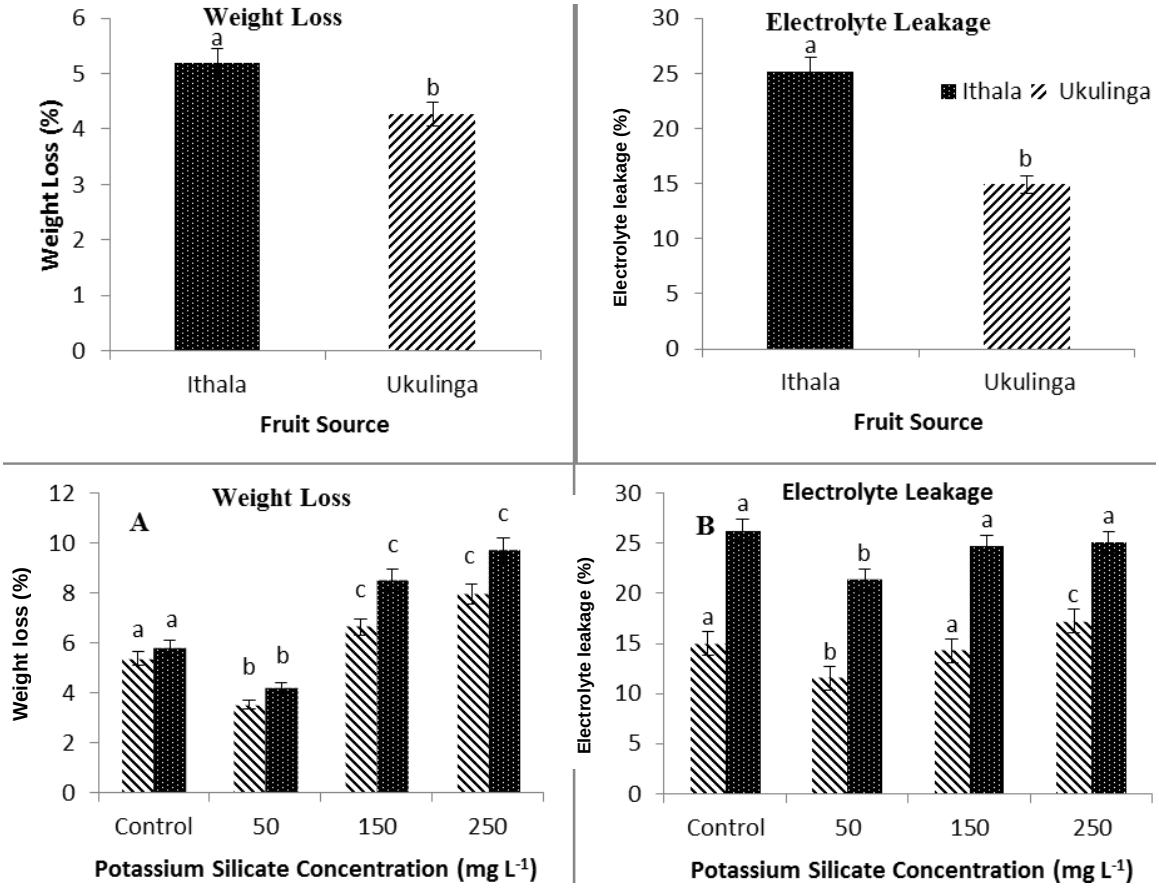

Figure 3. Effect of fruit source on fruit weight loss and electrolyte leakage, and effect of potassium silicate concentration on fruit weight loss (A) and electrolyte leakage (B) after 28 days cold storage.

sources; Ithala fruit showed higher EL when compared with fruit sourced from Ukulinga (Figure 3). There were significant differences ($P<0.05$) between treatments, with respect to EL (Figure 3B). Both Ithala and Ukulinga fruit had significantly lower EL at 50 mg L^{-1} K_2SiO_3 when compared with other treatments. The high concentrations of potassium silicate (150 and 250 mg L^{-1} K_2SiO_3) also increased the electrolyte leakage.

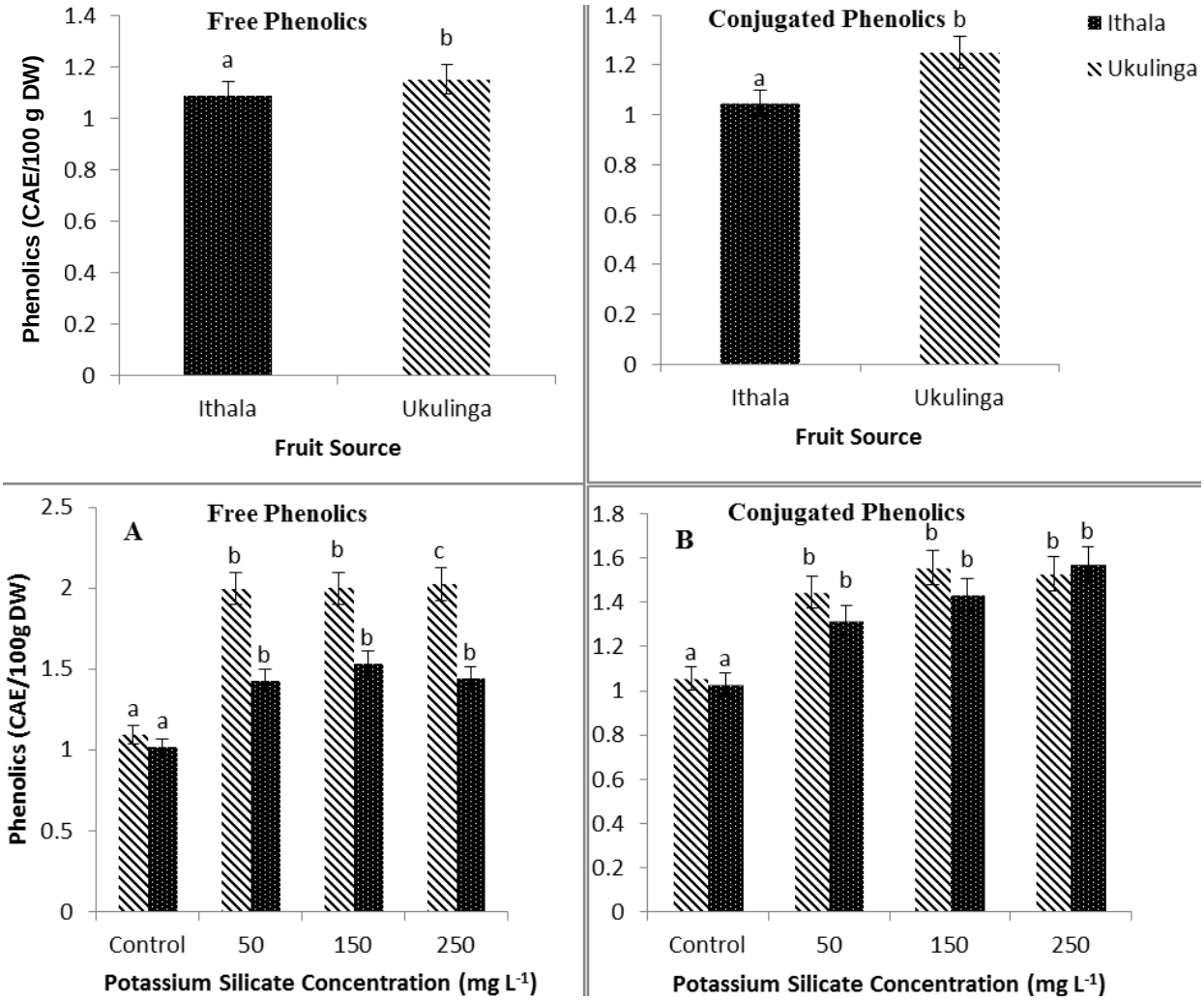

Figure 4. Effect of fruit source on free and conjugated phenolics, and change of free (A) and conjugated phenolics (B) after potassium silicate dips on fruit cold stored for 28 days.

Phenolics

Free phenolics

Fruit sourced from Ukulinga farm had significantly ($P < 0.05$) higher free phenolics when compared with Ithala fruit (Figure 4). There were significant differences in free phenolic content between treatments (Figure 4A) with all potassium silicate treatments increasing the phenolic content when compared with non-treated fruit.

Conjugated phenolics

Conjugated phenolic content was significantly ($P<0.05$) higher in fruit sourced from Ukulinga when compared with fruit from Ithala farm (Figure 4). There were significant differences ($P<0.05$) in conjugated phenolics between treatments (Figure 4B). In general, the amount of conjugated phenolics was shown to increase with increasing concentrations of K_2SiO_3 as in the case of free phenolics.

Flavonoids

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 when compared with Ithala fruit (Figure 5). Significant differences between treatments with respect to free flavonoids content were found in Ukulinga fruit (Figure 5A). Potassium silicate treatments exponentially increased the content of free Flavonoids with 150 and 250 mg L^{-1} K_2SiO_3 resulting in higher levels of free flavonoids content. However, potassium silicate treatments did not affect free flavonoids pool of Ithala fruit hence no signi-

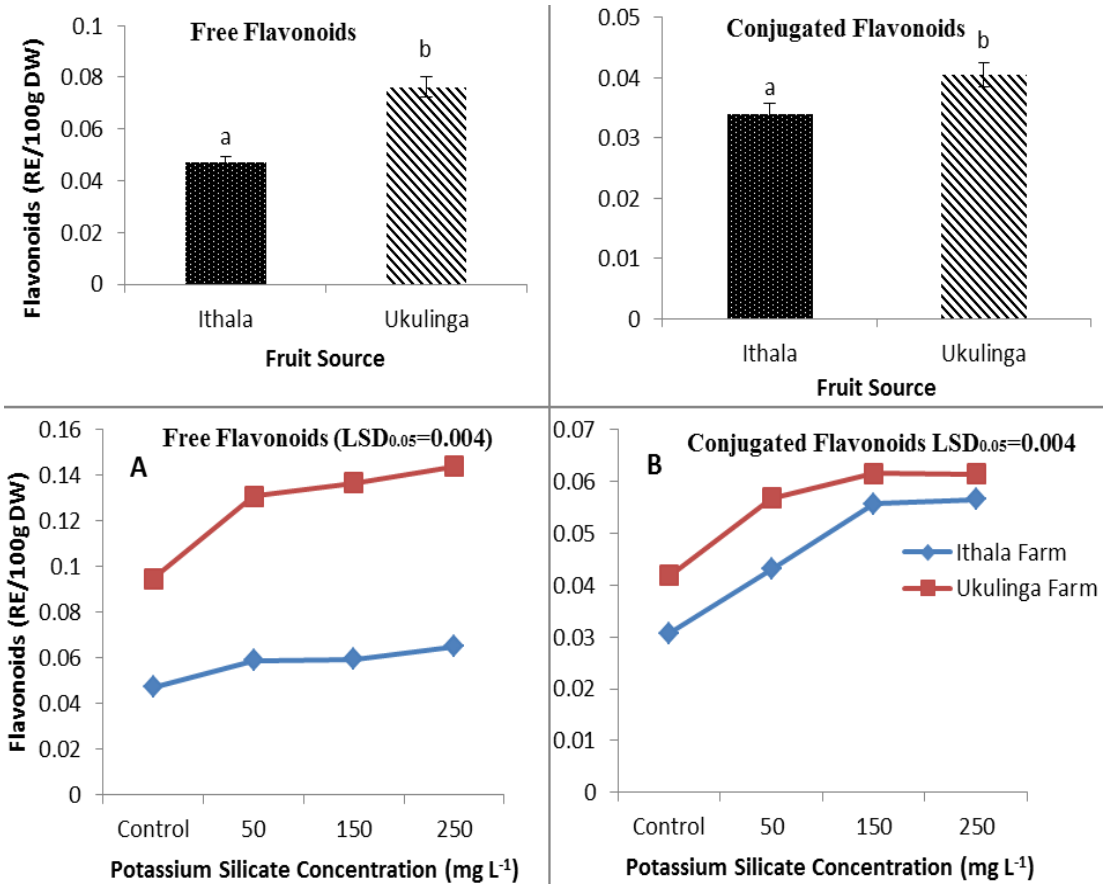

Figure 5. Effect of fruit source on free and conjugated flavonoids, and change of free (A) and conjugated flavonoids (B) after potassium silicate dips on fruit cold stored for 28 days.

ficant difference was found between the treatments with the fruit of this locality.

Conjugated flavonoids

The content of conjugated flavonoids was significantly ($P<0.05$) affected by fruit source. Ukulinga fruit had significantly higher conjugated flavonoids when compared with Ithala fruit (Figure 5). Significant differences in conjugated flavonoids were observed between treatments (Figure 5B). High conjugated flavonoid content at 50, 150 and 250 mg L^{-1} K_2SiO_3 relative to the control was observed for both Ukulinga and Ithala fruit.

DISCUSSION

The difference in chilling susceptibility of Ithala fruit when compared with Ukulinga fruit shows that environmental or growing conditions may determine fruit susceptibility to chilling injury (Mclauchlan et al., 1997; Mathaba et al., 2008). High chilling injury susceptibility of fruit from Ithala farm compared to fruit from Ukulinga farm might be attributed to difference in silicon content of the two locations. The high endogenous Si of Ukulinga lemons might be cited for 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 and EL. The increased fruit weight loss and EL for Ithala farm may be connected to loss of membrane integrity, cellular breakdown and perhaps the removal of epicuticular waxes known for reducing water loss through the rind (González-Aguilar et al., 2000). In addition, low fruit weight loss and EL 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.

Previous studies have revealed that growing conditions have great effect on phenolic and flavonoid content of fruit. In this study, Ukulinga fruit had higher phenolic and flavonoid levels when compared with Ithala fruit (Figures 4 and 5). 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 between the localities.

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 work of Agarie et al. (1998), Liang et al. (2008) and 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. 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 reported 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., 2007). The high fruit weight loss and electrolyte leakage at high Si concentration (150 and 250 mg L^{-1} K_2SiO_3) was possibly caused by water channels caused by concentrated Si.

Increased flavonoid and phenolic content in plants after silicon application has been reported (Maksimovic et al., 2007). In this study, both flavonoid and phenolic content increased with potassium silicate concentration. These results are in line with an earlier study by Bekker et al. (2007) that showed increased phenolic content on avocado fruit 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 indicate that lower concentration of postharvest Si dips reduced the incidence of chilling injury while higher concentration of 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. The impaired visual fruit quality after postharvest silicon dips suggests that preharvest silicon applications should be considered as a method to reduce postharvest chilling injury.

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