academicJournals

Vol. 7(4), pp. 45-54, May, 2013
DOI: 10.5897/AJBR13.0680
ISSN 1996-0778 ©2013 Academic Journals
http://www.academicjournals.org/AJBR

African Journal of Biochemistry Research

Full Length Research Paper

Hot water and molybdenum dips: The case of antioxidant assays in lemon flavedo during cold storage

Nhlanhla Mathaba[1,2]* and Isa Bertling[1]

[1]Horticultural Science, School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, P/Bag X01, Scottsville, 3209, South Africa.
[2]Institute for Subtropical and Tropical Crops, Agricultural Research Institute, Private Bag X11208, Nelspruit, 1200.

Accepted April 16, 2013

Antioxidants are part of the plant's defense system, protecting tissue against oxidative stress. In citrus fruit, oxidative stress can occur during extended cold storage ultimately result in chilling injury. Citrus fruit contain certain rind antioxidants which occur in different forms (lipophilic and hydrophilic). Previous studies demonstrated a synergistic relationship between hot water dips (HWD) and molybdenum dips (Mo) in mitigating chilling injury in citrus fruit. Therefore, the aim of this study was to investigate the potential of HWD and Mo to alleviate chilling injury by enhancing antioxidant capacity in lemon flavedo of fruit stored at -0.5°C. Fruit from different sources, commonly displaying chilling injury and other seemingly resistant to the development of chilling symptoms, were preconditioned for 2 min with HWD (47 or 53°C) and thereafter soaked in 1 or 10 µM Na_2MoO_4 solution for 30 min. Fruit were subsequently stored at -0.5°C for 7 or 28 days, moved to ambient temperature for a week and then evaluated for chilling injury symptoms. Chilling susceptible lemon fruit showed low Trolox equivalent total antioxidant capacity (TEAC), phenolics, and flavonoids concentration and high lipid peroxidation. Hot water dips 53°C, as well as 1 and 10 µM Mo plus HWD 53°C treatments mitigated chilling injury by enhancing total antioxidant capacity, and total phenolics and flavonoids concentrations in the flavedo. However, the ability of these treatments to alter lipophilic and hydrophilic antioxidants was fruit source dependent.

Key words: Lemon (*Citrus limon*), chilling injury, antioxidant assays, hot water dips, molybdenum, cold storage.

INTRODUCTION

A large portion of the world's citrus produce is transported over long distances and often requires cold sterilization as a phytosanitary requirement against fruit fly for importing countries such as Japan and US. However, long-term cold storage may result in chilling injury (McLauchlan et al., 1997). Within the citrus fruit family, lemons are the second most chilling susceptible fruit after grapefruit (Chalutz et al., 1985). Chilling symptoms appear as sunken lesions, discolouration of peel and pitting, severely reducing fruit marketability (McLauchlan et al., 1997; Shellie and Mangan, 2002). Citrus fruit have a wide array of antioxidants, which play major roles in the defense against oxidative damage including chilling injury (Abeysinghe et al., 2007).

Oxidative stress results from damage to membrane lipids, DNA and protein caused by reactive oxygen species (ROS) ultimately leading to cell death (Huang et al., 2007). Reactive oxygen species such as the the superoxide

*Corresponding author. E-mail: mathaban@gmail.com. Tel: +27 13 753 1062. Fax: +27 13 752 3854

radical (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^•$) are unstable molecules and, therefore, rapidly attack bio-molecules; under stress the effect of ROS is even more severe (Tomizawa et al., 2005). Plants have developed a well-integrated antioxidant defense system to scavenge ROS and keep them below damaging levels (Huang et al., 2007). Recent research has revealed ROS as not only damaging molecules but also as signaling molecules (Foyer and Shegeoka, 2011). Therefore, methods to mitigate oxidative stress need to consider ROS as both, damaging and signaling molecules in order to balance the cellular redox homeostasis and signaling (Foyer and Shegeoka, 2011, Foyer and Noctor, 2005).

Previous research has shown that molybdenum (Mo) postharvest dips also alleviate chilling symptoms in citrus fruit (Mathaba et al., 2008). Molybdenum, as a co-factor enzyme xanthine dehydrogenase (XDH), has been associated with oxidative stress (Yesbergenova et al., 2007, Hesberg et al., 2003) as the activity of XDH is increased by salinity (Sagi et al., 1998) and decreased by cold (Hesberg et al., 2003). Therefore, the effect of XDH activity during stress is still not well understood. However, Mo remains an essential element in plant metabolism and plays an important role in balancing redox homeostasis (Mendel and Schwarz, 1999). There is a growing need for non-chemical methods to mitigate postharvest disorders in citrus fruit and hot water dips have been found to be an effective alternative (Irtwange, 2006). Hot water treatments can enhance production and activation of heat shock protein (HSPs) (Rorat et al., 2002; Rozenzvieg et al., 2004) some of which act as chaperones, assisting in protein folding assembly and transport, as well as in directing damaged proteins towards proteolysis (Rozenzvieg et al., 2004). Furthermore, these HSPs prevent protein aggregation at higher temperatures (Pavoncello et al., 2001) and presumably also at low temperatures.

The counteraction of fruit to temperature stress can be affected by an array of antioxidants which make up a full defense mechanism in plant cells against oxidative damage (Huang et al., 2007). As the nature of antioxidants is diverse, various assays are used to estimate plant antioxidants (Re et al., 1999). The increase in the number of total antioxidant assays used to determine antioxidants is due to the varying efficacy of assays to estimate in different tissue. Several assays are commonly used to estimate total antioxidant capacity of a certain tissue; these include the 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{•+}$) assay (Pellegrini et al., 2003), the 1, 1'-diphenyl-2-picrylhydrazyl ($DPPH^•$) assay (Wang et al., 2005) and the ferric reducing power (FRAP) assay (Li et al., 2006).

The citrus flavedo contains an array of lipophilic and hydrophobic antioxidants which constitute a comprehensive defense system against oxidative stress. Therefore, an antioxidant assay that will be able to give a holistic antioxidant measure is required to fully understand the antioxidant strength of citrus flavedo.

The aim of this work was to investigate the ability of hot water dips (HWD) and molybdenum (Mo) dips to mitigate chilling injury and to identify the role played by antioxidants present in the flavedo during cold stress. Moreover, the efficacy of different antioxidant assays in quantifying the antioxidant capacity of lemon flavedo treated with hot water and molybdenum was studied.

MATERIALS AND METHODS

Standards and chemicals

Naringin (naringenin-7-rhamnosidoglucosidose), hesperidin (hesperetin-7-rutinoside), Trolox® (6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid), 1,1'-diphenyl-2-picrylhydrazyl ($DPPH^•$)), (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) $ABTS^{•+}$, 2,4,6-tripyridyl-s-triazine (TPTZ), and α-tocopherol (vitamin E), β-carotene, ascorbic acid (vitamin C), rutin, chlorogenic acid, Folin-Ciocalteu reagent, ferric chloride ($FeCl_3.6H_2O$) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, Mo, USA).

Plant materials

Lemon (c.v. Eureka) fruit were obtained from Ukulinga Research Farm (29°39'48.82"S; 30°24'19.89"E) and Sun Valley Estate Farm (28°51′00″S; 30°04′00″E) during the 2007 harvest season and from Ukulinga Research Farm and Eston Estate Farm (29°47'00"S; 29°27'00"E) during the 2008 harvest season. Fruit were soaked for 30 min in a 1 µM $Na_2MoO_4.2H_2O$ solution followed by a 2 min HWD 53°C. Treated fruit were waxed (15 fruit per box and three boxes per treatment), left to air-dry, weighed and stored at -0.5°C for up to 28 days, and sampled after 0 and 28 days for chilling injury evaluation. A second evaluation was carried out five days after withdrawal from cold storage, fruits were peeled and the peel freeze-dried, milled using mortar and pestle and stored at -21°C for further physiological analyses.

Estimation of chilling injury

The method of Sala (1998) was used to evaluate chilling injury five days after fruit were removed from cold storage. The method is based on a rating scale of surface browning intensity (0, sound fruit; 1, less than 10%; 2, 10 to 20%; 3, 30 to 40% and 4, more than 50% pitting). The chilling injury index (CI), which expresses the severity of cold damage, was calculated by adding the products of the number of fruit in each category by the value assigned to this category in the rating scale and dividing the sum by the total number of fruit evaluated.

$CI = \Sigma$ (number of fruit with chilling injury x score of severity) / Total number of fruit evaluated.

Total antioxidant capacity

Extraction of total antioxidants

Total antioxidants were extracted according to Halvorsen et al. (2002) with minor modifications. Freeze-dried lemon flavedo (0.1 g) was mixed with 5 ml 1 eq/L perchloric acid and homogenized using an Ultra-Turrax (Model: T25D, IKA-Germany) for 30 s. The homogenate was centrifuged at 10,000 *g* for 10 min at 4°C; the resulting supernatant collected and stored at 4°C until further analysis.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was carried out using a modified method of Li et al. (2006). The method is based on the reduction of the ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+}-TPTZ) to the ferrous form (Fe^{2+}-TPTZ) by a reductant, thereby determining the combined antioxidant power of lipophilic and hydrophilic antioxidants. The FRAP reagent was prepared freshly by mixing 300 mM sodium acetate buffer pH 3.6, 10 mM Fe^{2+}-TPTZ in 40 mM HCL and 20 mM $FeCl_3.6H_2O$ (10:1:1); 1000 µL FRAP reagent was mixed with 30 µL sample and the absorbance read at 593 nm after 10 min reaction time.

2, 2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\bullet+}$) assay

The $ABTS^{\bullet+}$ assay was carried out according to Pellegrini et al. (2003), with slight modifications. The method is based on the ability of antioxidant molecules to quench the long-lived $ABTS^{\bullet+}$, a blue-green chromophore with a characteristic absorption at 734 nm, compared with that of Trolox. The addition of antioxidant standard or sample acting as the radical cation reduces $ABTS^{\bullet+}$ and results in a decolorization. A stable stock solution of $ABTS^{\bullet+}$ was produced by reacting 7 mM aqueous $ABTS^{\bullet+}$ solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark for 6 to 12 h prior to use. The $ABTS^{\bullet+}$ stock solution was diluted daily before adjusting to an absorbance of 0.7±0.5 with ethanol. Immediately before conducting the assay, standard or sample (10 µl) was added to the diluted $ABTS^{\bullet+}$ working solution and the decrease in absorbance monitored at 734 nm; the antioxidant concentration was expressed as µmol Trolox equivalent per gram of tissue.

$DPPH^{\bullet}$ free radical scavenging capacity

The antioxidant capacity of lemon flavedo and standards was also determined using the 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) free radical according to Wang et al. (2005). Briefly, a 0.1 mM $DPPH^{\bullet}$ solution in methanol was prepared. The initial absorbance of $DPPH^{\bullet}$ in methanol was measured at 515 nm. An aliquot (50 µl) of the extract was added to 3 ml of methanolic $DPPH^{\bullet}$ and the change in absorbance measured after 30 min. The antioxidant capacity based on the $DPPH^{\bullet}$ free radical scavenging ability of the extract was expressed as µmol Trolox equivalents per gram of lemon flavedo.

Total phenolic extraction

Phenolic compounds were extracted according Abeysinghe et al. (2007) with minor modification. Briefly, 0.5 g ground lemon peel was weighed in a screw-capped test tube. Phytochemicals were extracted with 5 ml 50% DMSO, (50% 1.2 M HCl in 80% methanol/water) and vortexed for 1 min. Samples were then heated at 90°C for 3 h, with vortexing every 30 min. After samples had cooled to room temperature, they were diluted to 10 ml with methanol and centrifuged at 10, 000 g for 5 min to remove the solid fraction. The supernatant was used for determination of total phenolics and total flavonoids.

Determination of total phenolics

Total phenolic of the flavedo extract were measured using a modified Folin-Ciocalteu method (Abeysinghe et al., 2007). Distilled water (4 ml) and 0.5 ml diluted flavedo extract were placed in a glass test tube. Folin-Ciocalteu reagent (0.5 ml) was reacted with the sample for 3 min, the reaction neutralized with 1 ml saturated sodium carbonate and absorbance determined at 760 nm after 3 h. Chlorogenic acid was used as a standard; data were expressed as mg chlorogenic acid equivalent (CAE)/100 g dry matter DM.

Determination of total flavonoids

Total flavonoids were determined using a modified colorimetric method (Abeysinghe et al., 2007). Diluted flavedo extract (0.5 ml) was added to a glass test tube containing 3.5 ml of ethanol. After addition of 4 ml 90% diethylene glycol and thorough mixing, the reaction was initiated by adding 0.1 ml 4 M NaOH. The absorbance was read at 420 nm after 10 min incubation at 40°C. Rutin was used as a standard and the total flavonoids concentration was expressed as mg rutin equivalent (RE)/100 g DM).

Membrane lipid peroxidation

The malondialdehyde (MDA) concentration in flavedo tissue was determined by the thiobarbituric acid (TBA) reaction according to Heath and Packer, (1968). Freeze-dried lemon flavedo tissue (0.5 g) were homogenised in a cooled mortar using a pestle with 10 ml of ice cold 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 20 000 *g* for 10 min at 4°C. An aliquot (1 ml) of the supernatant was thoroughly mixed with 4 ml 20% TCA containing 0.5% TBA. The mixture was incubated at 95°C for 30 min and then quickly cooled in an ice bath. After centrifugation at 20 000 *g* for 10 min at 4°C, the absorbance of the supernatant was read at 532 nm and corrected for non-specific absorbance at 600 nm. The MDA concentration was calculated using an extinction coefficient of 155 mM cm^{-1}.

Total MDA (nmol 100 g^{-1} DM) = (Amount of extraction buffer (ml) x amount of supernant (ml) x [(Abs 532 – Abs 600)/155] x 103)* Amount of sample $(g)^{-1}$

Statistical analysis

Statistical comparison of mean (n = 5) values was performed by analysis of variance (ANOVA) using Genstat Version 14 (VSN International, UK). Means were separated using Duncan's Multiple Range Test at the 5% level of significance. Furthermore, data were subjected to principal component analysis (PCA) using an Unscrambler Version 9.8 (Camo Process AS, Oslo, Norway).

RESULTS

Effect of HWD and Mo dips on chilling injury symptoms during cold storage

Fruit were sourced from different farms with different growing and climatic conditions. Symptoms of chilling injury were detected on lemon fruit sourced from Sun Valley Estates during the 2007 harvest season (Table 1). However, during the year 2007 and 2008 harvest seasons, respectively, fruit sourced from Ukulinga and Eston Estates did not show any chilling symptoms. Treating fruit with hot water dips 47°C was not as effective in mitigating chilling injury symptoms for fruits harvested from Sun

Table 1. Chilling index of lemon fruit from different growing environment during the 2007 and 2008 harvest season, chilling index evaluated after 28 days cold storage plus 5 days shelf-life.

Treatment[a]	Ukulinga 2007[b]	Sun Valley Estates[c] 2007	Ukulinga 2008[b]	Eston Estates 2008[d]
Water dip (25°C)	No symptoms	0.13[a]	No symptoms	Below detection
HWD 47°C	No symptoms	0.18[a]	No symptoms	Below detection
HWD 53°C	No symptoms	0.09[a]	No symptoms	Below detection
1µM Mo	No symptoms	0.04[ab]	No symptoms	Below detection
1µM Mo + HWD 53°C	No symptoms	0.02[ab]	No symptoms	Below detection
10µM Mo + HWD 53°C	No symptoms	0.00[b]	No symptoms	Below detection

[a]Treatment effect after 28 days cold storage plus 5 days shelf-life. [b]Ukulinga lemon fruit showed no chilling symptoms during 2007 and 2008 harvest season. [c]Sun Valley lemon fruit showed chilling symptom expressible in chilling index and means followed by the same letter were not significantly different ($p < 0.05$). [d]Eston Estates lemon did show chilling symptoms but below detection and chilling index calculation.

Valley Estates during 2007 compared with HWD 53°C resulting in chilling indices of 0.18 and 0.09, respectively (Table 1). Molybdenum dips were effective at 1 µM Mo (compared with 5 and 10 µM Mo) reducing the chilling index to 0.04; 1 µM Mo plus HWD 53°C reduced the chilling index to 0 (Table 1).

Effect of fruit origin on total antioxidant capacity, phenolics, flavanoids and lipid peroxidation in lemon flavedo

The total antioxidant assays used (FRAP, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$) showed significantly lower antioxidant capacity for chilling-susceptible fruit from Sun Valley Estates compared with non-chilled fruit from Ukulinga and Eston Estates (Figures 1A, B and C). The $DPPH^{\bullet}$) assay also showed the antioxidant capacity of lemon fruit from Eston Estates to be not significantly different to those from Sun Valley (Figure 1A) with the $ABTS^{\bullet+}$ assay providing similar trends (Figure 1B).

Total phenolics as well as total flavanoids showed a significantly lower concentration of these antioxidants in the flavedo of Sun Valley Estates lemon fruit compared with other sites (Figures 1E and F). However, total phenolics and flavanoids showed different results to the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays (Figures 1A and B), respectively, with Eston Estates fruit containing the highest concentration of total phenolics and total flavonoids (Figures 1E and F).

The flavedo membrane damage (expressed as malondialdehyde (MDA)) of Sun Valley Estates lemons was significantly higher than that of Eston fruit, while Ukulinga fruit, in both seasons, displayed higher membrane damage than Eston fruit but less than Sun Valley fruit (Figure 1D).

The contribution of individual antioxidants to the "overall antioxidant strength" varied; specific antioxidants showed different activity in different assays. While, for all assays (FRAP, $ABTS^{\bullet+}$ and $DPPH^{\bullet}$), ascorbic acid was the most potent antioxidant (Figures 2A, B and C), the antioxidant strength with FRAP assay was vitamin C (ASE) > phenolics (CAE) > vitamin E (ATE) > flavonoids (RUE) > (hesperidin (HE) ≥ naringin (NE) ≥ β-carotene (BEC) (Figure 1B). While using the $ABTS^{\bullet+}$ assay, the order differs: vitamin C > β-carotene > phenolics > flavonoids > vitamin E > hesperidin > naringin (Figure 2C). With the $DPPH^{\bullet}$ assay a completely different result with antioxidant strength was obtained: vitamin C = flavonoids = vitamin E > phenolics > hesperidin > naringin > β-carotene (Figure 2A).

Principal component analysis

Application of principal component (PCA) on all data led to a reduction of variation of 68% with the variation explained by principal component one (PC1) and principal component two (PC3) (Figure 3A). PC1 explained the majority of variation (65%) associated with $DPPH^{\bullet}$ and FRAP; PC3 represented 3% of variation and was mainly associated with total phenolics (Figure 3A).

When grouping data, Eston Estates (cluster 2) and Ukulinga 2007 (cluster 3) fruit were similar and chilled fruit from Sun Valley showed different cluster compared with Ukulinga 2008 fruit (Figure 3B).

Effect of HWD and Mo dips on total antioxidants, total phenolics, total flavonoids and lipid peroxidation in lemon flavedo during cold storage

Postharvest HWD 53°C significantly increased total flavedo antioxidants, as shown by FRAP 0 days into cold storage, compared with control or HWD at 47°C treatments for Ukulinga 2007 and Sun Valley 2007 fruit, respectively. Moreover, total antioxidant capacity ($ABTS^{\bullet+}$ assay) of HWD 53°C increased 28 days into storage for Ukulinga 2007 and Eston Estates 2008, respectively (Table 2). In addition, sole HWD 53°C significantly decreased total flavanoids for chilled fruit from Sun Valley Estates; HWD 53°C significantly increased total flavonoids for all non-chilled fruit 21 days into cold storage (Tables 2 and 3). Ukulinga (2007) and Sun Valley Estates fruit showed decreased membrane integrity 0 days into

Table 2. Effect of fruit source, cold storage time, hot water and molybdenum dips on total antioxidant capacity (FRAP, ABTS$^{\bullet+}$and DPPH$^{\bullet}$), phenolics, flavonoids and lipid peroxidation of lemon flavedo during 2007 harvest season.

Cold storage time (days)	Treatment	Ukulinga 2007						Sun Valley Estates 2007					
		TEAC						TEAC					
		FRAP[1]	ABTS$^{\bullet+}$ [2]	DPPH$^{\bullet}$ [3]	TP[4]	TF[5]	MDA[6]	FRAP[1]	ABTS$^{\bullet+}$ [2]	DPPH$^{\bullet}$ [3]	TP[4]	TF[5]	MDA[6]
0	Water dip (25°C)	139.5^{d}	43.4^{d}	491.0^{ab}	140.0^{bcd}	77.8^{a}	10.47^{b}	217.6^{a}	27.9^{b}	503.2^{a}	95.2^{c}	47.9^{e}	10.15^{e}
	HWD 47°C	158.5^{cd}	55.8^{cd}	442.3^{b}	144.8^{bc}	80.8^{a}	10.34^{b}	213.0^{a}	40.0^{ab}	432.5^{bcd}	94.7^{c}	49.5^{de}	12.26^{b}
	HWD 53°C	177.1^{bcd}	90.5^{abcd}	463.9^{ab}	157.1^{ab}	79.8^{a}	9.02^{d}	228.4^{a}	33.9^{ab}	478.2^{ab}	98.4^{ab}	56.7^{b}	10.30^{de}
	1 µM Mo	128.7^{d}	105.2^{abc}	412.8^{b}	157.1^{ab}	76.9^{a}	8.20^{f}	200.0^{ab}	26.8^{b}	442.6^{abc}	95.8^{bc}	46.8^{ef}	12.15^{bc}
	1 µM Mo + HWD 53°C	258.9^{a}	116.1^{ab}	507.7^{ab}	174.9^{a}	78.8^{a}	8.85^{d}	209.6^{a}	51.1^{ab}	431.2^{bcd}	100.2^{a}	55.1^{bc}	13.35^{a}
	10 µM Mo + HWD 53°C	231.0^{abc}	73.5^{bcd}	319.5^{b}	135.3^{cde}	81.3^{a}	11.65^{a}	166.7^{abc}	33.5^{ab}	424.5^{bcd}	96.3^{bc}	58.1^{ab}	11.99^{c}
28	Water dip (25°C)	307.9^{a}	50.7^{d}	454.2^{b}	117.4^{e}	80.5^{a}	9.44^{c}	89.2^{d}	45.3^{ab}	436.9^{bcd}	91.2^{d}	42.2^{g}	8.94^{g}
	HWD 47°C	216.4^{a}	59.0^{cd}	495.0^{ab}	124.9^{cde}	84.4^{a}	9.64^{c}	163.1^{abcd}	35.9^{ab}	393.5^{cd}	91.0^{d}	52.4^{cd}	9.86^{f}
	HWD 53°C	294.8^{a}	76.4^{bcd}	507.5^{ab}	117.4^{e}	77.0^{a}	8.50^{e}	126.9^{bcd}	38.2^{ab}	452.8^{abc}	89.0^{d}	43.5^{fg}	7.87h
	1 µM Mo	255.2^{a}	129.1^{a}	481.0^{ab}	120.2^{de}	80.5^{a}	8.28^{f}	88.2^{d}	52.6^{ab}	412.2^{bcd}	91.7^{d}	48.8^{de}	10.30^{e}
	1 µM Mo + HWD 53°C	295.4^{a}	118.8^{ab}	591.9^{a}	123.0^{de}	84.2^{a}	8.51^{e}	109.8^{cd}	39.8^{ab}	373.6^{d}	91.6^{d}	57.0^{b}	10.23
	10 µM Mo + HWD 53°C	245.9^{a}	135.7^{a}	501.5^{ab}	130.5^{cde}	77.0^{a}	11.73^{a}	127.9^{bcd}	69.1^{a}	403.5^{cd}	91.5^{d}	61.4^{a}	10.44^{d}

[1,2,3]TEAC$_{(FRAP,\ ABTS^{\bullet+}\ ,\ DPPH^{\bullet})}$- nmol/g DW. [4]Total phenolics- µg chlorogenic acid equivalent (CAE)/100 g DW. [5]Total flavanoids- µg rutin equivalent (RUE)/100 g DW. [6]Total MDA- nmol/g DW. *Treatments mean (±SE) obtained from 5 replication for TEAC assays, total phenolics, flavonoids and lipid peroxidation. **Cold storage time mean within same column having different letters indicates significant difference ($p<0.05$).

cold storage due to HWD 53°C treatment; however, membrane recovery significantly increased 28 days (Tables 2 and 3).

Treatment with a low concentration Mo dip (1 µM Na_2MoO_4) significantly increased total antioxidant capacity (FRAP) in flavedo of Ukulinga (2007 and 2008) and Sun Valley Estates fruit during cold storage and maintained a high flavedo antioxidant capacity up to 28 days into cold storage (Tables 2 and 3). In addition, 1 µM Mo dips significantly increased total antioxidant capacity (DPPH$^{\bullet}$) as well as total phenolics and flavanoids of Sun Valley Estates fruit during cold storage, thus significantly increasing membrane integrity between 28 days (Tables 2 and 3).

The 10 µM Mo plus HWD 53°C dips had a greater effect on total antioxidants (FRAP, ABTS$^{\bullet+}$ and DPPH$^{\bullet}$) 28 days into cold storage compared with 1 µM Mo plus HWD 53°C for Ukulinga and Sun Valley Estates fruit (Tables 2 and 3). The higher total antioxidants concentrations in Ukulinga and Sun Valley Estates flavedo observed between 28 days cold storage was aligned with decreased MDA levels in response to 10 µM Mo plus HWD 53°C treatment; Mo plus HWD 53°C did not significantly reduce MDA levels in Eston Estates fruit (Table 4).

DISCUSSION

Bioactive compounds with antioxidant properties are potential markers for estimating the severity of oxidative stress; exposure to oxidative stress could affect post-harvest fruit quality hence, several assays have been developed to estimate total antioxidant capacity (Szőllősi and Varga, 2002). Recently, total antioxidant capacity has been expressed as Trolox (a vitamin E analogue) equivalent antioxidant capacity (TEAC) (Brezová et al., 2009; Art et al., 2001). Studies have shown that TEAC assays have drawbacks and can be biased towards either lipophilic (α-tocopherol, β-carotene and lycopene) or hydrophilic (ascorbic acid and phenolic groups) compounds (Sies and Stahl, 1995; Pérez-Jiménez et al., 2008). Our results confirm that the $TEAC_{FRAP}$ assay mainly detected hydrophilic antioxidants (particularly ascorbic acid and phenolic groups), while the $TEAC_{ABTS^{\bullet+}}$ assay mainly identified lipophilic antioxidants (β- carotene). The $TEAC_{DPPH^{\bullet}}$, however, detected both, hydrophilic and lipophilic antioxidants (Figure 1). Brezová et al. (2009)

Table 3. Effect of fruit source, cold storage time, hot water and molybdenum dips on total antioxidant capacity (FRAP, $ABTS^{\bullet+}$, $DPPH^{\bullet}$), phenolics, flavonoids, and lipid peroxidation of lemon flavedo during 2008 harvest season.

Cold storage time (days)	Treatment	Ukulinga 2008						Eston Estates 2008					
		TEAC						TEAC					
		FRAP[1]	$ABTS^{\bullet+}$ [2]	$DPPH^{\bullet}$ [3]	TP[4]	TF[5]	MDA[6]	FRAP[1]	$ABTS^{\bullet+}$ [2]	$DPPH^{\bullet}$ [3]	TP[4]	TF[5]	MDA[6]
0	Water dip (25°C)	312.2^{bcde}	47.2^{cd}	628.8^{cd}	122.8^{a}	95.7^{ab}	13.51^{b}	249.2^{c}	40.2^{c}	395.0^{a}	127.7^{d}	104.5^{bc}	8.52^{e}
	HWD 47°C	408.2^{abc}	45.2^{cd}	658.5^{bc}	129.5^{a}	97.2^{a}	10.49^{ef}	345.7^{abc}	54.7^{bc}	366.2^{a}	135.2^{bcd}	104.5^{bcd}	8.13^{ef}
	HWD 53°C	356.0^{abcd}	44.1^{cd}	563.7^{d}	116.2^{a}	65.0^{c}	10.23fg	318.0^{abc}	59.8^{bc}	354.2^{a}	130.5^{cd}	87.5^{e}	9.88^{cd}
	1 µM Mo	291.3^{cde}	70.6^{cd}	616.7^{cd}	122.8^{a}	65.6^{c}	8.17i	359.3^{abc}	36.4^{c}	407.1^{a}	135.2^{bcd}	92.2^{cde}	8.02^{ef}
	1 µM Mo + HWD 53°C	318.3^{abcd}	79.0^{c}	581.2^{cd}	120.0^{a}	61.7^{c}	10.53^{e}	246.6^{c}	34.6^{c}	393.7^{a}	140.0^{abc}	92.2^{cde}	7.07g
	10 µM Mo + HWD 53°C	427.3^{ab}	84.1^{c}	570.7^{cd}	119.7^{a}	63.9^{c}	10.09g	316.8^{abc}	40.7^{c}	369.7^{a}	144.7^{ab}	89.5^{e}	7.77f
28	Water dip (25°C)	322.6^{bcde}	29.0^{d}	777.7^{a}	127.0^{a}	81.8^{b}	14.61^{a}	292.4^{bc}	97.6^{ab}	360.9^{a}	144.7^{ab}	114.7^{ab}	8.52^{e}
	HWD 47°C	377.3^{abcd}	214.6^{a}	769.4^{a}	123.5^{a}	88.0^{ab}	11.81^{c}	368.2^{abc}	74.4^{abc}	394.9^{a}	139.4^{a}	119.7^{a}	11.27^{a}
	HWD 53°C	224.9^{e}	80.2^{c}	748.6^{a}	131.5^{a}	87.2^{ab}	9.66h	511.9^{a}	37.1^{c}	386.3^{a}	149.4^{a}	123.7^{a}	10.45^{b}
	1 µM Mo	455.5^{a}	65.2^{cd}	726.9^{ab}	123.5^{a}	87.2^{ab}	13.34^{b}	469.1^{ab}	24.2^{c}	340.8^{a}	150.3^{a}	119.7^{a}	9.40^{d}
	1 µM Mo + HWD 53°C	403.8^{abc}	65.8^{cd}	753.4^{a}	127.8^{a}	87.2^{ab}	10.21fg	444.9^{abc}	118.4^{a}	371.3^{a}	152.4^{a}	123.7^{a}	10.33^{bc}
	10 µM Mo + HWD 53°C	272.3^{de}	168.5^{b}	737.2^{ab}	119.5^{a}	83.7^{ab}	11.31^{d}	503.8^{a}	40.7^{a}	362.2^{a}	169.1^{a}	111.8^{a}	10.59^{b}

[1,2,3] TEAC $_{(FRAP,\ ABTS^{\bullet+},\ DPPH^{\bullet})}$- nmol/g DW. [4]Total phenolics- µg chlorogenic acid equivalent (CAE)/100 g DW. [5]Total flavanoids- µg rutin equivalent (RUE)/100 g DW. [6]Total MDA- nmol/g DW. *Treatments mean (±SE) obtained from 5 replication for TEAC assays, total phenolics, flavonoids and lipid peroxidation. **Cold storage time mean within same column having different letters indicates significant difference ($p<0.05$).

found $TEAC_{DPPH\bullet}$ to detect the antioxidants strength of lipophilic as well as hydrophilic nature in different coffee cultivars.

None of the various TEAC assay has previously been correlated with chilling occurrence in citrus fruit. Previous investigations in other plant species have reported a strong positive correlation between the $TEAC_{ABTS\bullet+}$ and the $TEAC_{DPPH\bullet}$ assay when using a Pearson correlation coefficient (r = 0.906) (Dudonné et al., 2009) in different exotic fruit and a Spearmans-Rho coefficient correlation (ρ = 0.949) in different fruit and vegetables (Floegel et al., 2011). In this study, chilling stress resistance was more related to $TEAC_{DPPH\bullet}$ and total phenolics than to $TEAC_{ABTS\bullet+}$ or $TEAC_{FRAP}$ (Figure 3A); such correlation has been confirmed for different agricultural produce (Floegel et al., 2011).

Previous studies have reported differences in chilling susceptibility within citrus cultivars, with "Lisbon" lemons being more susceptible than "Eureka" lemons (Underhill et al., 1999); as well as coastal lemons being more susceptible than desert lemons (Aung et al., 1999); differences in chilling susceptibility have also been attributed to different micro-climates and farms practices (Mathaba et al., 2008). Differences in chilling susceptibility were further attributed to variations in antioxidant composition due to differences in fruit ripeness using $TEAC_{FRAP}$ assay (Huang et al., 2007). Our results further attribute differences in chilling injury to the low flavedo $TEAC_{DPPH\bullet,\ ABTS\bullet+}$ and FRAP (Figures 2A, B and C) due to low phenolics (Figure 2F) and flavonoids concentrations (Figure 2E).

Antioxidant assays quantify the ability to protect a certain tissue against ROS membrane damage. The damage caused by ROS, particularly to poly-unsaturated membrane fatty acids (MDA), makes membrane-bound proteins intercross and conjugate, leading to oxidative stress(Campos et al., 2003). A high TEAC combined with low membrane lipid peroxidation (Figure 2D) was previously observed in *Hypoxi rooperi* ("African potato") (Laporta et al., 2007) as well as in different parts of cucumber seedlings (Jian-yong et al., 2008). Cold storage appeared to have induced alterations in TEAC, phenolics and flavonoids concentration. Such a phenomenon was previously observed in different orange segments stored after 12 days at 4°C ($TEAC_{DPPH\bullet}$) (Plaza et al., 2011);

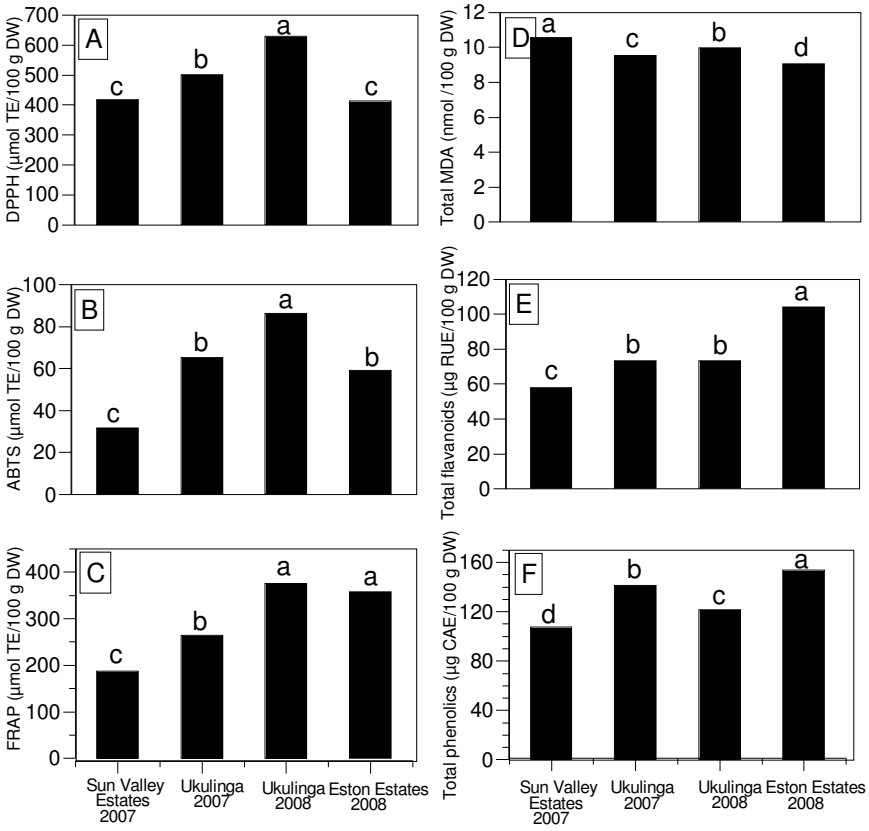

Figure 1. Evaluation of authentic equivalent bioactive compound standard over different trolox equivalent antioxidant (TEAC) assays. **(A)** FRAP, **(B)** $ABTS^{\bullet+}$, **(C)** $DPPH^{\bullet}$.

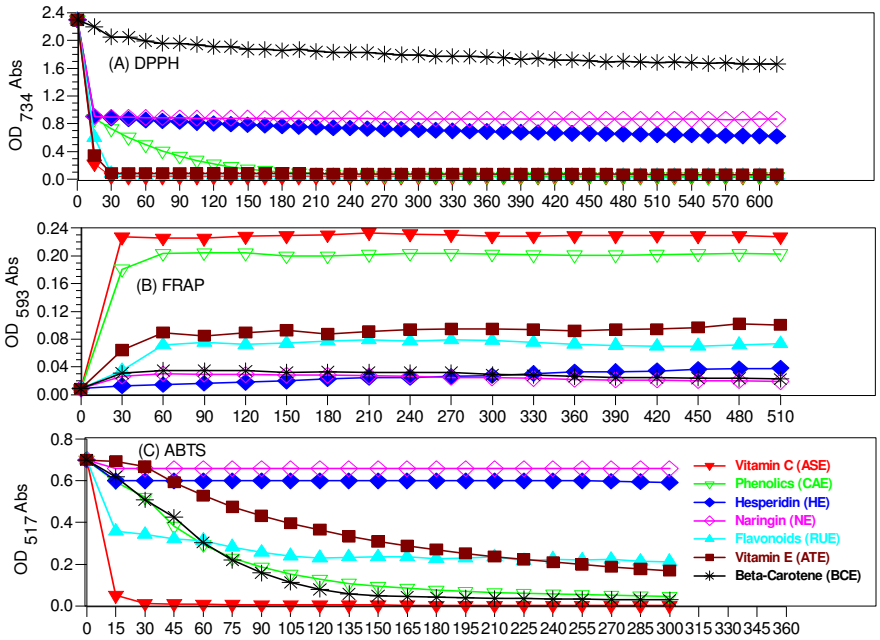

Figure 2. Effect of fruit source on lemon flavedo trolox equivalent total antioxidant capacity (TEAC), phenolics, flavonoids and lipid peroxidation. (A) $DPPH^{\bullet}$, (B) $ABTS^{\bullet+}$, (C) FRAP, (D) MDA, (E) total flavonoids and (F) total phenolics.

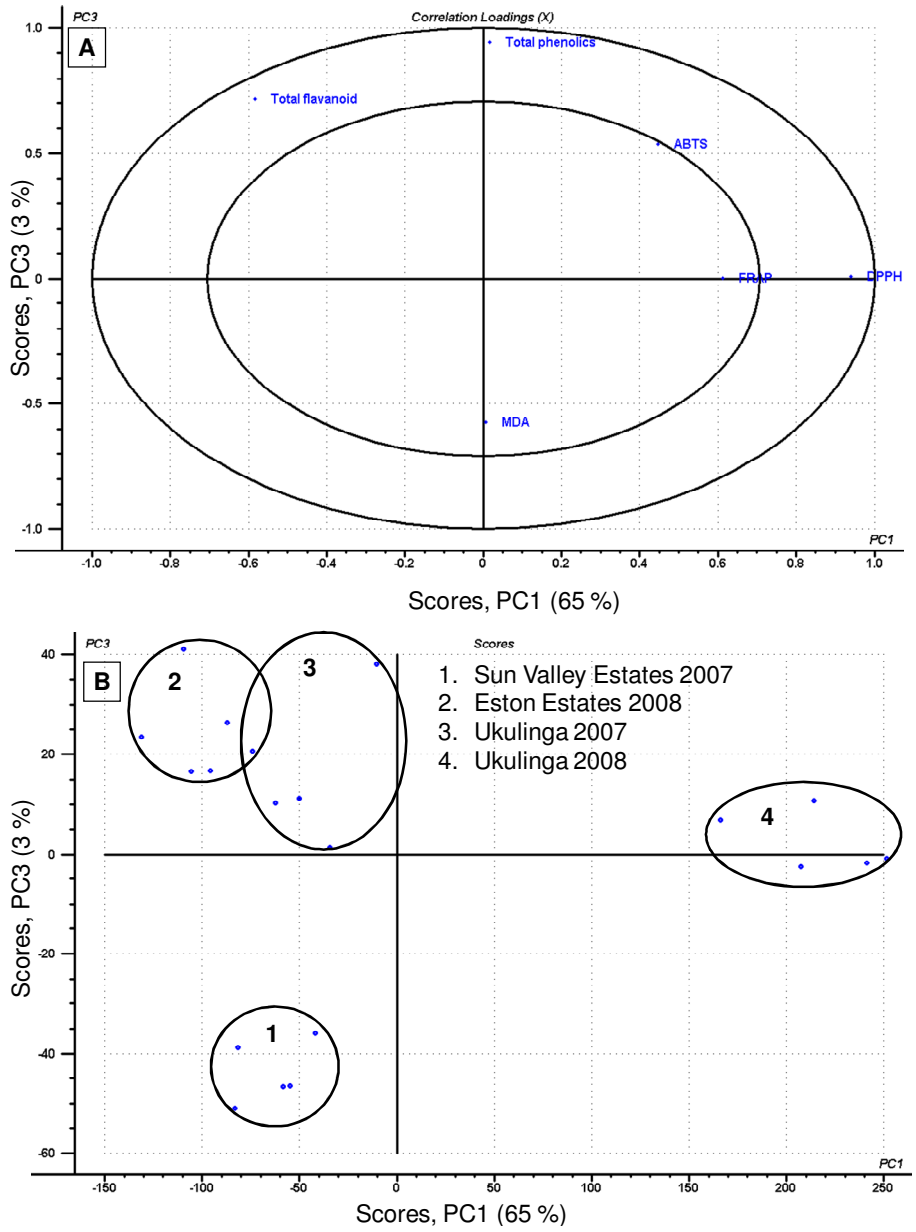

Figure 3. Principal component analysis (PCA) showing correlation loadings (A). Score plot lemon specific flavedo bioactive compounds (B). Score plot for the groups of the bioactive compounds for lemons from different source with difference in chilling susceptibility, PC1 explains 65 % and PC3 explains 3 % of total variation.

in broccoli florets stored at 2°C for up to seven ($TEAC_{DPPH}$. and total phenolic) days (Cogo et al., 2011); in grapefruit juice stored at 4 and 18°C for up to two months (total flavonoids) (Igual et al., 2011); in different cultivars of plums stored at 2°C for up to 35 days ($TEAC_{ABTS\bullet+}$ and total phenolics) (Díaz-Mula et al., 2009). Alterations in total antioxidant concentrations further confirm that various (lipophilics and hydrophilic) antioxi-dants are responsible for deactivating ROS accumulating during cold stress thereby possibly creating a cascade of physiological events as a holistic protection mechanism against any form of stress.

The success of HWD to maintain postharvest quality has been reported for various commodities (Koukounaras et al., 2009; Fallik, 2004); however, little research has successfully associated HWD with TEAC. In addition, HWD have been proven to increase total phenolics and flavonoids concentrations thus reducing occurrence of chilling injury in different crops. In 'Fortune' mandarin, stored at 16°C for 32 days, heat conditioning at 37°C prior to storage reduced chilling injury by increasing $TEAC_{DPPH\bullet}$, $TEAC_{ABTS\bullet+}$, the phenolic as well as flavonoid concentration (Lafuente et al., 2011). However, in 'Navel' and 'Valencia' oranges, HWD 50°C reduced free pheno-

lics with an increase in chilling resistance compared with HWD 41°C over 20 days cold storage (Bassal and El-Hamahmy, 2011); in rocket leaves, stored at 8°C for five to 10 days heat treatments (51°C) increased postharvest shelf-life by increasing $TEAC_{DPPH\bullet}$ and significantly decreasing total phenolics (Koukounaras et al., 2009). The ability of HWD to maintain fruit postharvest quality and to reduce postharvest physiological disorders and to extend shelf-life by increasing antioxidants (Tables 2 and 3), is supported by reduced lipid peroxidation in two banana cultivars stored at 4°C for 10 days (Promyou et al., 2008). According to Lamikanra and Watson, (2007) hot water dips not only induce heat shock proteins but also alter the $TEAC_{FRAP}$, $TEAC_{DPPH\bullet}$, $TEAC_{ABTS\bullet+}$, phenolics and flavonoids concentration depending on HWD temperature, time of application, cultivar and pre-harvest conditions.

Several chemical post-harvest dips have shown potential to mitigate post-harvest physiological disorders in different horticultural commodities. These chemicals have further shown positive, synergistic effect with different heat treatments; calcium plus post-harvest heat treatment reduced chilling disorders in plums stored at 2°C for up 28 days (Valero et al., 2002). While in peaches, hot air treatment plus methyl jasmonate alleviated internal browning when fruit were stored at 0°C for up to five weeks (Jin et al., 2008). Similarly, salicylic acid plus calcium chloride and heat treatment improved post-harvest quality of peaches and strawberries stored at 0°C and 2°C, respectively (Wang et al., 2006; Shafiee., 2010).

Our results show molybdenum post-harvest dips to have a synergistic effect with hot water treatments in alleviating chilling injury in lemons (Table 1). Hot water dips have been found to activate HSPs and molybdenum has been hypothesized to increase the production of ROS during cold storage (Yesbergenova et al., 2005; Hesberg et al., 2003; Sagi et al., 1998) probably to a threshold level that signals a cascade of defenses resulting in production of antioxidants. Reactive oxygen species have also been found to induce acclimation to stress (Foyer and Shigeoka, 2011; Foyer and Noctor, 2005); and therefore it is proposed that ROS also increase both lipid and hydrophilic antioxidants. In addition, pre-exposure of ginseng root to methyl jasmonate or salicylic acid was shown to significantly increase $TEAC_{DPPH\bullet}$, phenolics and flavonoids concentrations which reduced oxidative stress during germination of the roots (Ali et al., 2007).

In conclusion, antioxidants play an important role in mitigating oxidative stress during cold storage of lemon fruit. Furthermore, antioxidants differ in nature; the lipophilic component which is quantified mostly by using the $TEAC_{ABTS\bullet+}$ assay and hydrophilic antioxidants are best quantified by using the $TEAC_{FRAP}$ assay; while the $TEAC_{DPPH\bullet}$ assay is sensitive to both lipophilic and hydrophilic antioxidants. It is therefore important to use several assays to attain a complete understanding of total antioxidant activity of a certain tissue. Any post-harvest treatment is unlikely to affect different antioxidant concentrations in a similar manner during cold storage.

ACKNOWLEDGEMENTS

Mr. B. Beghin of Gateway Packhouse (Pty) is acknowledged for supplying fruit. This research was funded by the Citrus Academy, Citrus Research International (CRI) of the South Africa Citrus Growers Association (CGA) and Canon Collins Trust Scholarship.

REFERENCES

Abeysinghe DC, Li X, Sun C, Zhang W, Zhou C, Chen K (2007). Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of four species. Food Chem. 104:1338-1344.

Ali M, Eun-Joo H, Kee-Yoeu P (2007). Methyl jasmonate and salicylic acid induces oxidative stress and accumulation of phenolics in *Panax ginseng* bioreactor root suspension cultures. Mol. 12:607-621.

Aung LH, Jenner JF, Fouse DC, Houck LG (1999). Postharvest conditioning temperatures and fruit maturity on flavedo soluble sugars of coastal and desert lemons. J. Hortic. Sci. Biotechnol. 74(3):288-292.

Brezová V, Šlebodová A, Staško A (2009). Coffee as a source of antioxidants: An EPR study. Food Chem. 114:859-868.

Chalutz E, Waks J, Schiffmann-Nadel M (1985). A comparison of the responses of different citrus fruit cultivars to storage temperature. Sci. Hortic. 25:271-277.

Cogo SLP, Chaves FC, Schirmer MA, Zambiazi RC, Nora L, Silva JA, Rombaldi CV (2011) Low soil water content during growth contribute to preservation of green colour and bioactive compounds of cold-stored broccoli (*Brassica oleraceae* L.) florets. Postharvest Biol. Technol. 60:158-163.

Compos PS, Quartin V, Ramalho JC, Nunes MA (2003). Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of Coffea sp. Plants. J. Plant Physiol. 160:283-292.

Díaz-Mula HM, Zapata PJ, Guillén F, Martínez-Romero D, Castillo S, Serrano M, Valero D (2009). Changes in hydrophilic and lipophilic antioxidant activity and related bioactive compounds during postharvest storage of yellow and purple plum cultivar. Postharvest Biol. Technol. 51:354-363.

Dudonné S, Vitrac X, Coutiere P, Woillez M, Merillon JM (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using $DPPH^{\bullet}$, $ABTS^{\bullet+}$, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57:1768-1774.

Fallik E (2004). Prestorage hot water treatments (immersion, rinsing and brushing). Postharvest Biol. Technol. 32:125-134.

Floegel A, Dae-Ok K, Sang-Jin C, Sung IK, Chun OK (2011). Comparison of $ABTS^{\bullet+}$ /$DPPH^{\bullet}$ assays to measure antioxidant capacity in popular antioxidant-rich US foods. J. Food Compos. Anal. doi:10.1016/j.jfca.2011.01.008

Foyer CH, Noctor G (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. Plant Cell. 17:1866-1875.

Foyer CH, Shigeoka S (2011). Understanding oxidative stress and antioxidant function to enhance photosynthesis. Plant Physiol. 155:93-100.

Halvorsen BH (2002). The biochemistry and medical significance of the flavonoids. Pharmocol. Therapeut. 96: 67-202.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:180-198.

Hesberg C, Hansch R, Mendel RR, Bittner F (2003). Tendem orientation of duplicated xanthine dehydrogenase genes from *arabidopsis thaliana*. J. Biol. Chem. 279(14):13547-13554.

Huang R, Xia R, Hu L, Lu Y, Wang M (2007). Antioxidant activity and oxygen-scavenging system in orange pulp during ripening and maturation. Sci. Hortic. 113:166-172.

Igual M, García-Martínez E, Camacho MM, Martínez-Navarrete N (2011). Changes in flavonoid content of grapefruit juice caused by

thermal treatment and storage. Innov. Food Sci. Emerg. Technol. doi:10.1016/j.ifset.2010.12.010

Irtwange SV (2006). Hot water treatment: A no-chemical alternative in keeping quality during postharvest handling of citrus fruits. Agricultural Engineering International: the CIGR Ejournal. Invited Overview No. 5. Vol VIII. February

Jian-young L, Hai-xia T, Xin-guo L, Jing-jing M, Qi-wei H (2008). Higher chilling-tolerance of grafted-cucumber seedling leaves upon exposure to chilling stress. Agric. Sci. China. 7(5): 570-576.

Jin P, Zheng Y, Tang S, Rui H, Wang CY (2009). A combination of hot air and methyl jasmonate vapour treatment alleviates chilling injury of peach fruit. Postharvest Biol. Technol. 52:24-29.

Koukounaras A, Siomos SA, Sfakiotakis E (2009). Impact of heat treatment on ethylene production and yellowing of modified atmosphere package rocket leaves. Postharvest Biol. Technol. 54:172-176.

Lafuente MT, Ballester AR, Calejero J, González-Candelas L (2011). Effect of high-temperature-conditioning treatments on quality, flavonoid composition and vitamin C of cold stored 'Fortune' mandarins. Food Chem. doi:10.1016/j.foodchem.2011.03.129

Lamikanra O, Watson MA (2007). Mild heat calcium treatment effects on fresh-cut cantaloupe melon during storage, Food Chemi. 102:1383-1388.

Laporta O, Pérez-Fons L, Mallavia R, Caturla N, Micol V (2007). Isolation, characterization and antioxidant capacity assessment of the bioactive compounds derived from *Hypoxis rooperi* corm extract (African potato). Food Chem. 101:1425-1437.

Li BB, Smith B, Hossain MMd (2006). Extraction of phenolic from citrus peels II. Enzyme-assisted extraction method. Separation and Purification Technology. 48:189-196.

Mathaba N, Bower JP, Bertling I (2008). Influence of hot water and molybdenum dips on the production of total antioxidants during cold storage of lemon and their ability of such to scavenge reactive oxygen species. Proceed. Int. Soc. Citriculture. 2:1130-1314.

McLauchlan RL, Underhill SJ, Dahler JM, Giles JE (1997). Hot water dipping and low temperature storage of 'Eureka' lemons. Australian J. Exper. Agric. 37:249-252.

Pavoncello D, Lurie S, Droby S, Porat R (2001). A hot water treatment induces resistance to *Penicillium digitatum* and promotes the accumulation of heat shock and pathogenesis-related proteins in grapefruit flavedo. Physiol. Plantarum. 111:17-22.

Pellegrini N, Serafini M, Colombi B, Del Rio D, Salvatore S, Bianchi M, Brighenti F (2003). Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J. Nutr. 133(9):2812-2819.

Pérez-Jiménez J, Arranz S, Tabernero M, Díaz-Rubio ME, Serrano J, Goñi I, Saura-Calixto F (2008). Update mythology to determine antioxidant capacity in plant food, oils and beverages: Extraction, measurement and expression of results. Food Res. Int. 41:274-285.

Plaza L, Crespo I, de Pascual-Teresa S, de Ancos B, Sánchez-Moreno C, Muñoz M, Cano MP (2011). Impact of minimal processing of orange bioactive compounds during refrigerated storage. Food Chem. 124:646-651.

Promyou S, Ketsa S, van Doorn WG (2008). Hot water treatments delays cold-induced banana peel blackening. Postharvest Biol. Technol. 48:132-138.

Rorat R, Pavoincello D, Lurie S, Mccollum TG (2002). Identification of a grapefruit cDNA belonging to a unique class of citrus dehydrins and characterization of its expression patterns under temperature stress conditions. Physiol. Plantrum 115:598-603.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS$^{\bullet+}$ radical cation decolarization assay. Free Radical Biology and medicine. 26(9/10):1231-1237.

Rozenzvieg D, Elmaci C, Samach A, Lurie S, Porat R (2004). Isolation of four heat shock protein cDNAs from grapefruit peel tissue and characterization if their expression in response to heat and chilling temperature stresses. Physiol. Plantarum 121:421-428.

Sala JM (1998). Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. Postharvest Biol. Technol. 13:255-261.

Shafiee M, Taghavi TS, Babalar M (2010). Addition of salicylic acid to nutrient solution combined with postharvest treatments (hot water, salicylic acid, and calcium dipping) improved postharvest fruit quality of strawberry. Sci. Hortic. 124:40-45.

Shellie KC, Mangan RL (2000). Postharvest disinfestation heat treatments: response of fruit and fruit fly larvae to different heating media. Postharvest Biol.Technol. 2:51-60.

Sies H, Stahl W (1995). Vitamins E, C, β-carotene, and other carotenoids as antioxidants. Am. J. Clin. Nutr. 62:1315S-1321S.

Szőllősi R, Varga IS (2002). Total antioxidant power in some species of Labiatae (Adaptation of FRAP method). Proceedings of the 7th Hungarian Congress on Plant Physiology. S2-P24.

Tomizawa S, Imai H, Tsukada S, Simizu T, Honda F, Nakamura N, Nagono T, Urano Y, Matsuoka Y, Fukasaku N, Saito N (2005). The detection and quantification of highly reactive oxygen species using navel HPF fluorescence probe in a model of focal cerebral ischemia. Neurosci. Res. 53:304-313.

Underhill SJ, Dahler JM, McLauchlan RL, Barker LR (1999). Susceptibility of 'Lisbon' and 'Eureka' lemons to chilling injury. Australian J. Exper. Agric. 39:757-760.

Valero D, Martínez-Romero D, Serrono M (2002). The role of polyamines in the improvement of the shelf life of fruit. Trends Food Sci.Technol. 13:228-234.

Wang H, Hu SH, Liang ZC, Lee MY (2005). Antigenotoxicity of extracts from *Pleurotus citrinopileatus*. J. Sci. Food Agric. 85:770-778.

Wang L, Cheng S, Kong W, Li S, Archibold DD (2006). Salicylic acid pretreatment alleviated chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest Biol. Technol. 41:244-2512.

Yesbergenova Z, Yang G, Oron E, Soffer D, Fluhr R, Sagi M (2005). The plant Mo-hydroxylases aldehyde oxidase and xanthine dehydrogenase have distinct reactive oxygen species signatures and are induces by drought and abscisic acid. Plant J. 42: 862-876.