Hot Water Dipping or Calcium Nitrate Treatments Alleviated Chilling Injury and Enhanced Quality of 'White Sukkary' and 'Zebda' Mangoes

M. Bassal* and M. El-Hamahmy**
*Horticulture Department, Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt
**Botany Department, Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt
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Abstract: Effect of hot water dipping (HWD), (47±1 ◦C/20 min, 53±1 ◦C/5 min) in 'White Sukkary'; (49±1 ◦C/30 min, 53±1 ◦C/8 min) in 'zebda' and $Ca(NO_3)_2$ (4 and 6% /15min) in both cultivars during cooling storage period (30 and 42 days) and subsequent shelf period (3 and 6 days) in 'White Sukkary' and 'Zebda', respectively were investigated. Generally, in both cultivars, 47(49)±1 ◦C/20-30 min HWD treatment was more potential to minimize chilling injury (CI) % with enhancing peroxidase(POX) and catalase (CAT) activity by 2-5 times in fruit peel and bulb. Also, low heat treatment increased calcium content in peel and bulb which significantly correlated with peel firmness. Moreover, fruit treated with 4% $Ca(NO_3)_2$ showed minimum weight loss% and maximum vitamin c (VC) content due to decrease ascorbic acid oxidase activity. TA%, SSC, reducing sugars and free phenols were also estimated.

Keywords: *Mangifera indica*, chilling injury, fruit quality, Peroxidase, Catalase, Ascorbic acid oxidase.

INTRODUCTION

Mango is considered one of the most important tropical fruit in the world in terms of production and consumer acceptance (FAO STAT, 2005). Mango as a climacteric fruit is very susceptible to post-harvest losses, due to fast softening, reach to respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature due to sudden ethylene generation (Narayana *et al.*, 1996). The short shelf life of mango varies among its varieties, depending on storage conditions, ranging from 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13°C (Carrillo *et al.*, 2000). This short period seriously limits the long distance commercial transport of this fruit (Gomer-Lim, 1997). Fruit of tropical origin exhibit a physiological disorder known as 'chilling-injury' when they are exposed to a temperature of 7-13°C, depending upon ripeness and variety, which is manifested by greyish scald-like discoloration of the skin, skin pitting, uneven ripening, carotenoids, aroma and flavour reduction during ripening and susceptibility to fungal decay, which seriously reduces the fruit marketability (Thomas and Oke, 1983). CI symptoms increased after fruit transportation to normal conditions; depend on varieties, degree of maturity, time and temperature of exposure and environmental conditions during storage (Mohammed and Brecht 2002, Phakawatmongkol *et al.*, 2004). Using of controlled atmosphere, UV-C irradiation, methyl jasmonate application and HWD prior to storage were more effective to alleviate CI of mango fruit (Gonzalez-Aguilar *et al.*, 2001; Pesis *et al.*, 2000).

Hot water quarantine treatment protocols (China: 48°C for 60 min; Iran: 45°C for 75 min) for fruit fly disinfestations, before export marine shipments was used in many countries for mangoes (Abdul Jabbar *et al.*, 2011).

It is widely accepted that symptoms of CI are a consequence of oxidative stress in the tissues (Sala, 1998) occurring when active oxygen species (AOS) such as hydrogen peroxides, superoxides and hydroxyl radicals are in excess of the scavenging capacity of fresh tissue (Hodges *et al.*, 2004). Involvement of antioxidant enzymes in regulation of AOS can be followed by measuring Guaiacol Peroxidase (EC 1.11.1.7) and Catalase (EC 1.11.1.6) activities during postharvest storage (Sala, 1998). Preconditioning treatments of fruit with hot water may induce chilling tolerance by modulating antioxidant systems that would prevent the accumulation of AOS (Sala and Lafuente, 2000).

The role of calcium in the physiology of plant tissue is well established. Addition of calcium rigidifies cell wall, obstructs enzymes such as polygalacturonase from reaching active sites (John, 1987, Lara *et al.*, 2004), involved in stabilization of cell membranes (Picchieni *et al.*, 1998), cell turgor pressure (Mignani *et al.*, 1995) and decreasing fruit softening. Calcium salts have also been used to preserve the quality of minimally processed commodities. Fresh-cut 'Kensington' mango evidenced a smaller softening rate when treated with 3% $CaCl_2$ however, combining calcium application with low oxygen atmosphere was found to be the most effective treatment for extending the shelf-life of mango slices (De Souza *et al.*, 2006). Calcium chloride combined with heat treatment was effective in preserving the quality of fresh-cut mango cubes (Trindade *et al.*, 2003).

The objective of this study was to evaluate the effect of HWD and Ca $(NO_3)_2$ treatments on alleviating chilling injury and maintaining quality of mango fruit. The physiological and biochemical changes associated with these treatments were also studied.

MATERIALS AND METHODS

Plant Material and Handling

During 2008 season, green mature fruit of *Mangifera Indica* L.cv 'White Sukkary' and 'Zebda' were harvested randomly from Suez Canal University Farm, Ismailia Governorate, Egypt (Latitude, 30°36' N; longitude, 32°14' E; Altitude, 10 m above sea level). Fruit were directly transported to postharvest lab. at Hort. Dept., Fac., Agric., Suez Canal Univ., and then sorted to eliminate defects. Samples of fruit of uniform

size and appearance were washed by chlorine solution (100 ppm/15 min), air dried and held for 24h at room temperature.

In the next day, fruit were randomly divided into five treatment groups, each of 21 'White Sukkary' or 'Zebda' fruit. The first group was used as the control dipped in water 15 min(28 °C), the second group was subjected to HWD at 47±1°C/ 20 min for 'White Sukkary' (43°C in bulb was recorded), 49±1°C for 30 min for 'Zebda'(42.5°C in bulb was measured); the third group were subjected to HWD at 53±1°C for 5 min for 'White Sukkary'(41.5°C inside bulb) and 8 min for 'Zebda' (37°C inside bulb). HWD treatments were performed in a water bath constantly maintained within ±1°C of the required temperature by a thermostat; the forth group were subjected to 4% $Ca(NO_3)_2$ and the final group were subjected to 6%$Ca(NO_3)_2$. Fruit of 'White Sukkary' and 'Zebda' were stored 30 and 42 days, respectively at 13°C (75 % RH). After cooling period all groups were left in shelf life conditions (32±2day/24± 2night°C and 35-40% RH) for 3 days in 'White Sukkary' and to 6 days in 'Zebda'.

Each treatment group was packed in 3 foam plates or 3 replications (7 fruit each) put in perforated polyethylene pages. Fruit plates were used for physical properties assessments (weight loss%, CI%) and chemical characteristics (soluble solids content (SSC), total acidity (TA), peel pigments, calcium content, ascorbic acid (VC), reducing sugars, free phenols, POX activity, CAT activity and Ascorbic acid oxidase (ASAO).

Evaluation of Firmness and Fruit Weight Loss

Weight loss % was calculated every 5 days in 'White Sukkary' and every 7 days intervals in 'Zebda' fruit during cooling period storage and after 3 and 6 days at the end of subsequent shelf period in 'White Sukkary' and 'Zebda', respectively. Total number of fruit manifesting CI symptoms (lenticels prominence and abundance, peel discoloration, fruit softening, brown spots on peel and bulb browning) was determined and expressed as the CI %. Fruit firmness was measured using a penetrometer (Fruit Tester) fitted with a 6 mm diameter flat probe and results were expressed as kg cm^{-2}.

Chemical Analysis of Juice

At zero time, at end cooling and subsequent shelf life periods, fruit samples were taken for determination of the chemical properties. SSC was measured by refractometer; TA% and VC were determined according to AOAC (1985).

Free phenols and reducing sugars

Ethanolic extract (96% ETOH) of juice were prepared according to Abdel-Rahman *et al.*, (1975), then the free phenols were determined spectrophotometricaly by using (Beckman DK-2 Spectrophotometer) at 650 nm with Folin-Ciocalteu reagent according to AOAC (1985). Reducing sugars were determined with alkaline copper and arsenomolybdate reagents spectrophotometricaly at 540 nm according to Moore (1974).

Peel Pigments Determination

0.5 g carotenoid-free peel was ground with 10ml aceton 85% and then filtered. Optical density was measured at 662,644 and 440.5 nm using a Beckman DK-2 Spectrophotometer. Concentration of Chl a, Chl b and carotenoids as mg per g FW were calculated according to Fadl and Sari Eldeen (1978).

Calcium Content

0.5 g dried peel or bulb was digested with 10ml H_2SO_4 overnight, then digestion was completed with H_2O_2 and adjusted to final volume to 50 ml dionized H_2O. Calcium was determined by titration against versenate solution (Na-EDTA) and 0.1 M sodium hydroxide (pH 12-13) according to Chapman and Pratt (1961).

Preparation of Enzyme Extract in Juice and Peel

At zero time and after shelf life period fruit samples were taken for the enzymes assay. 0.5 g fresh juice or peel were homogenized by using a mortar and pestle with 0.1 M phosphate buffer (pH 6.5) at 4°C and stirred for 20 min. The suspension obtained was filtered through one layer of muslin cloth and then centrifuged at 18000g for 15 min, 4°C. The supernatant was used to determine activity of antioxidant enzymes (Urbanek *et al.*, 1991) as follow:

Peroxidase (POX) [EC 1.11.1.7] assay

The reaction mixture consisted of 3.5 ml of 0.1 M phosphate buffer (pH 6.5), 0.3 ml of 0.1 % o-dianisidine solution, 0.2 ml of enzyme extract and 0.2 ml of 0.2 M hydrogen peroxide solution (Urbanek *et al.*, 1991). The reaction mixture was incubated at 30°C for 10 min and the oxidation of o-dianisidine measured by changes in optical density at 430 nm (Beckman DK-2 Spectrophotometer). Corrections were done for the oxidation rate of o-dianisidine in the absence of H_2O_2 in the reaction mixture. The activity of POX was expressed as optical density per milligram of protein per minute. One unit of POX activity (AU) was taken as the change of 1.0 unit of optical density per minute.

Catalase (CAT) [EC 1.11.1.6] assay

The reaction mixture consist of 0.01 ml enzyme extract and 2.99 ml hydrogen peroxide-phosphate buffer (pH 6.8) prepared after dilution of 0.16 ml of H_2O_2 (10% w/v) to 100 ml phosphate buffer (Urbanek *et al.*, 1991). The oxidation of H_2O_2 was measured by changes in optical density at 240 nm in 30 sec. intervals for 5 min (Beckman DK-2 Spectrophotometer). The unit of CAT activity was defined as the amount of enzyme, which decomposes 1 mmol H_2O_2 per minute at 25°C.

Ascorbic acid oxidase (ASAO) [E.C. 1.10.3.3] assay

The reaction mixture consist of 0.1 ml enzyme extract and 2.9 ml ascorbic acid–phosphate buffer (pH 5.6) prepared as 8.8 mg ascorbic acid dissolved in 300 ml phosphate buffer. The oxidation of ascorbic acid was measured by changes in optical density at 265 nm in 30 sec. intervals for 5 min (Beckman DK-2 Spectrophotometer). The unit of ASAO activity was defined as the amount of enzyme, which decomposes 1 mmol ascorbic acid per minute at 25°C. Protein content of the extracts was determined according to Bradford

(1976), using bovine albumin serum (BSA) as a standard.

Statistical Analyses

All data were statistically analyzed as randomized complete blocks design (Steel *et al.*, 1997); using the MSTAT-C statistical package (M-STAT, 1990) and means were separated by LSD test, $P \leq 0.05$.

RESULTS AND DISCUSSION

CI%

CI is one of the main factors limiting refrigeration in several horticultural commodities of subtropical and tropical origin such as mango (Mitra and Baldwin, 1997). In general, CI symptoms were wide-ranging and extensive in both 'White Sukkary' (Figure 1) and 'Zebda' (Figure 2) fruit ; appeared in form of fruit softening, brown spots on peel and bulb browning in 'Zebda' but characterized in 'White Sukkary' by abundance and prominence lenticels, peel discoloration, brown spots on peel and bulb browning.

The analysis of variance indicated that, minimum CI% was (14%) recorded in 'White Sukkary' fruit treated with both HWD treatments stored for 30 days at 13°C. CI% maintained with low percentage (14%) during subsequent shelf period for 3 days only in fruit treated with 53±1°C/5min HWD. In the same trend, 'Zebda' fruit treated with 49±1°C/30min HWD only showed minimum CI% (23 and 30%) after 42 days storage at 13°C and subsequent shelf period for 6 days, respectively compared to control and other treatments. Results cleared that, high temperature for short time in small fruit as 'White Sukkary' was more effective to minimize CI, in contrary to large fruit as 'Zebda' necessitated low heat treatment for long phase.

In line to the findings of Farooqi and Sattar (1985) who found that varietal response to CI disorder is specific, 'Sen sation' (USA) mangoes developed more skin symptoms due to CI than 'Samar Bahisht' (Pakistan) ones when stored at 6, 9, and 12±1° C for 0, 4, 8, 12, and 16 days and then transferred to room temperature (25-28°C) for ripening. Moreover, according to Pesis et al., 2000, CI symptoms at 10°C appear as red spots on the peel, pitting and black spots appear at lower temperatures in 'Tommy Atkins' and 'Keitt' mangoes. Temperature conditioning by gradually decreasing the temperature from 20°C to 17°C or 14°C during 2 days after harvest, before storage at 9°C, reduced the number of red spots. This reduction in CI symptoms was accompanied by an increase in fruit softening after 3 weeks at 9°C. Heat treatment for 48h at 38°C in 100% RH, or at 18°C for 48h in ethanol vapour or a low-oxygen atmosphere prior to cold storage reduced the CI symptoms that developed at 5°C. Also, Immature 'Tommy Atkins' mangoes succumbed to CI after 18 days at 5°C, with symptoms increasing in severity upon warming (1-3 days/20°C). While mature fruit had no CI symptoms, they were overripe and fruit decay incidence was 26.6%, compared to half-mature fruit which had no decay, a trace of CI symptoms and possessed the best overall quality. The CI index of 'Wacheng' mangoes treated at 0°C for 4 h was 59.7% lower than that of the control fruit directly stored at 2°C, 85–95% RH. Some attributes were assayed in the fruit treated at 0 °C for 4 h. Ion leakage of the cold-shock fruit at 0°C for 4 h was 16% or 10% lower than that of the control on day 9 or day 12 of storage, respectively. Malondialdehyde content of the cold-shocked fruit was 70% or 50% lower than that of the control on day 6 or day 12 of storage at 2°C, respectively (Zhao *et al.*, 2006). Furthermore, both methyl jasmonate (10^{-4}M) and diphenylamine (12mM) treatments had the ability to reduce the CI symptoms in 'Tommy Atkins' mangoes stored at low temperatures (1 and 10°C). Decay was reduced during subsequent ripening. The overall quality of methyl jasmonate and diphenylamine treated fruit was good with less surface pitting and scalding compared to the control treatment. The best results were obtained at storage temperatures of 7 and 10°C (Tasneem *et al.*, 2004).

Weight Loss %

Fruit shriveling was significantly higher at the end of storage period, as shown in fruit deformation data. This increase in water loss during storage may be due to the artificially higher humidity environment of fruit bagged with plastic, which may therefore influence structure and/or composition of the cuticle and/or lenticels (Hofman *et al.*, 1997; Joyce *et al.*, 1997).

Figure 3 (a and b) showed that fruit lost their weight under cooling circumstance, with stationary rate in both two cultivars. Maximum percentage of weight loss was recorded after 10 and 15 days in 'White Sukkary' fruit (1.7 and 1.6%, respectively) under 47±1°C/20min HWD treatment and after 20, 30 days (1.6 and 1.9 %, respectively) under 53±1°C/5min during cooled storage. Within subsequent shelf periods, fruit treated with 47±1°C/20min HWD and $Ca(NO_3)_2$/6% treatments showed maximum weight loss % (6.1% and 5.7%, respectively). In general, $Ca(NO_3)_2$/4% treatment was more effective to minimize weight loss % during cooling and subsequent shelf periods in 'White Sukkary' fruit. The same trend was found in 'Zebda' fruit, which exhibited maximum weight loss % (4.5% and 4.9%) after 14 and 35 days, respectively during cooling period and 9.7% in subsequent shelf periods under 53±1°C/8min HWD treatment compared to control and other treatments. Minimum level of weight loss % (1.65%) was recorded in fruit treated with 49±1°C/30 min HWD after 28 and 35 days in cooling period.

The rate of fruit weight loss in Eureka' lemons stored at 1°C for 28 or 42 days and received a hot water immersion treatment of 25°C for 42 min during 7 days at 20°C was significantly lower in the treated fruit than control. Hot water dips at 47-53°C for 1-3 min significantly reduced the chilling injury (McLauchlan *et al.*, 1997). In addition, Tasneem *et al.*, 2004 reported that, 'Tommy Atkins' fruit treated with methyl jasmonate and stored at 7, 10°C had lower mass loss, brighter colored skin, and higher total soluble solids (TSS) than the control treatment.

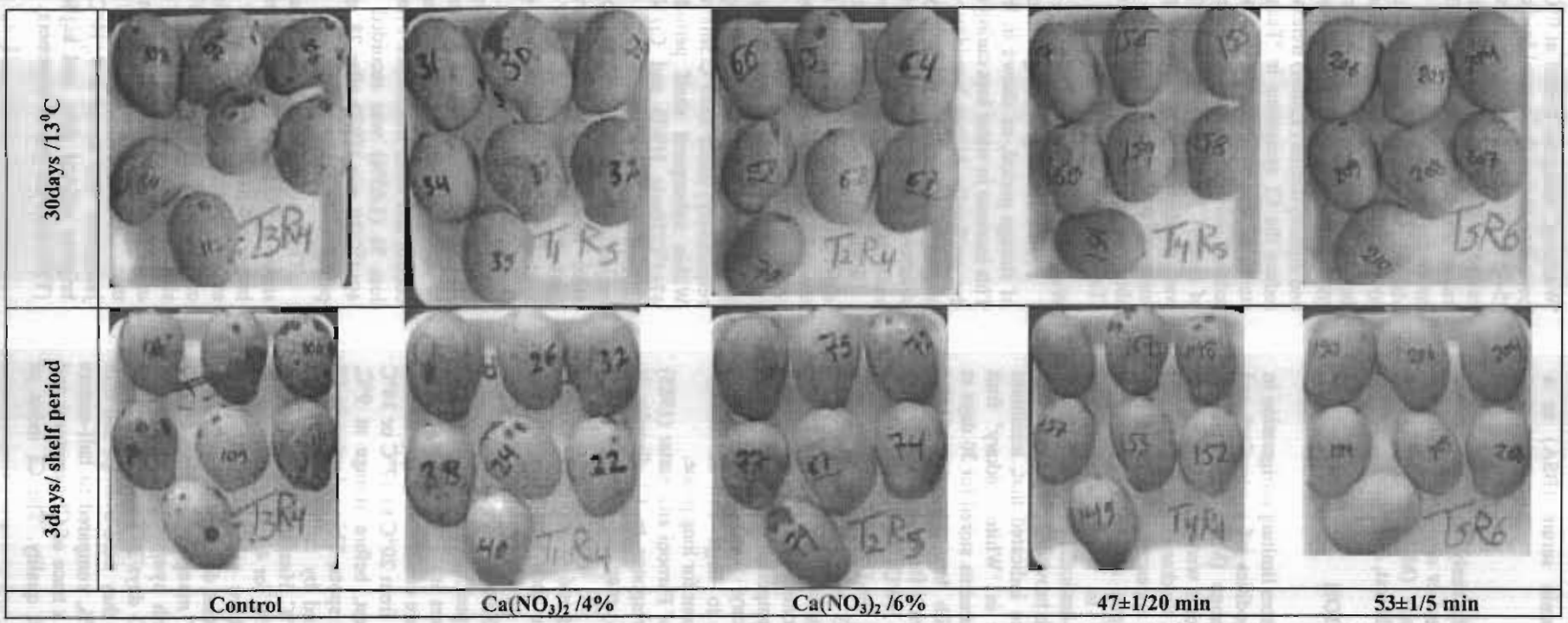

Figure.1.Chilling injury symptoms in 'White Sukkary' fruits under Calcium nitrate and HWD treatments during 30 days at 13°C then subsequent 3 days as shelf life.

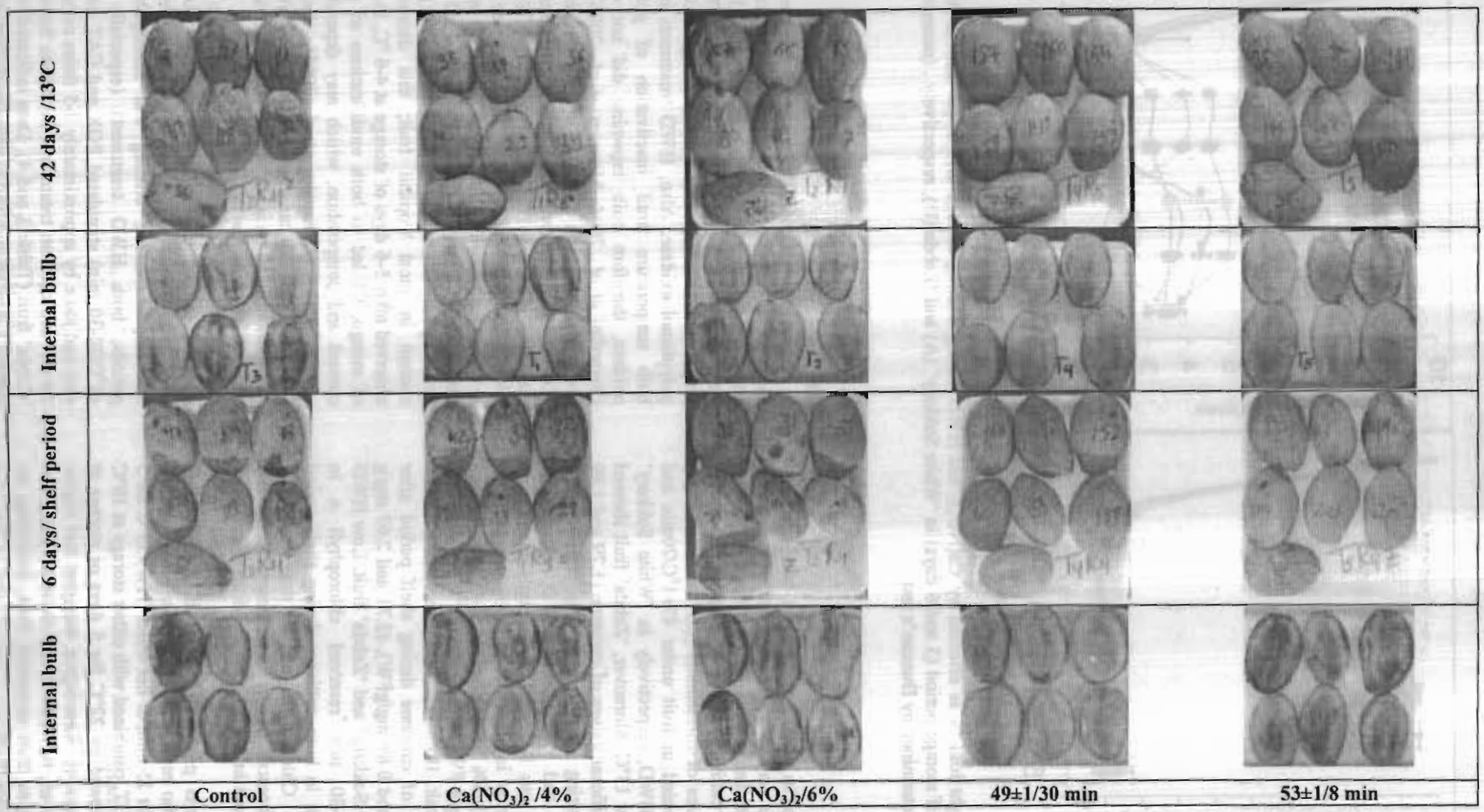

Figure.2. Chilling injury symptoms in 'Zebda' fruits under Calcium nitrate and HWD during 42 days at 13^0C then 6 days subsequent shelf life.

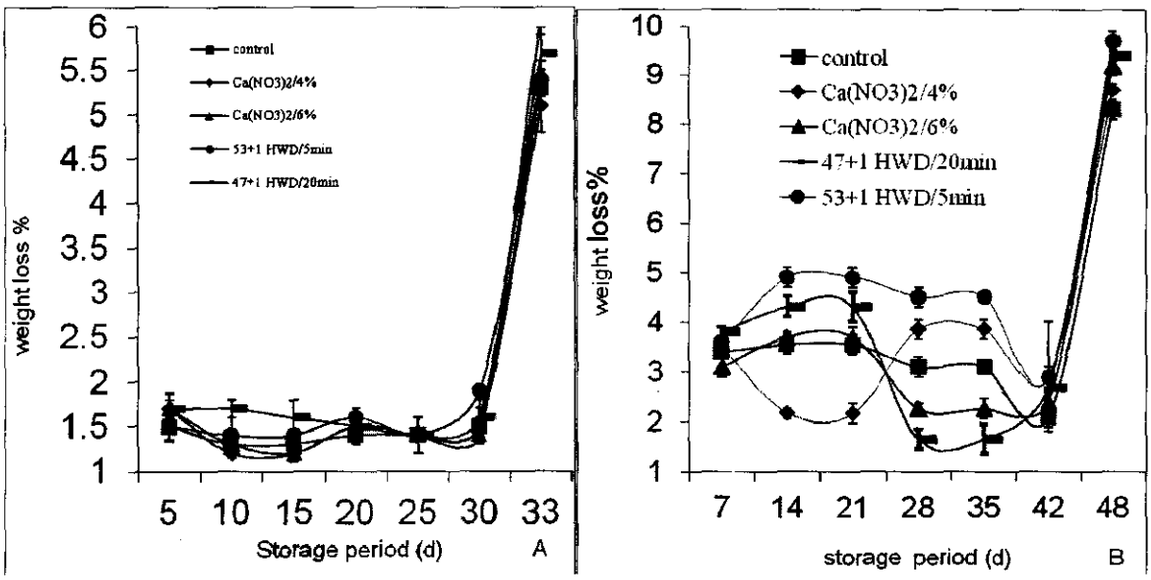

Figure.3. Weight loss % as affected by $Ca(NO_3)_2$ and HWD during cooling storage at13°C (30 and 42 days) and subsequent shelf storage period (3 and 6 days) in 'White Sukkary' (A) and 'Zebda'(B), respectively . (means±SE, n = 4). P≤0.05 as determined by Duncan's test.

Peel Pigments

Data represented in Table 1 showed that, biosynthesis of carotenoids was very slow during cooled storage period in all treatments and control during 30 and 42 days in both mango cultivars. In this respect, maximum significant values of carotenoids (0.37 and 0.44 mg/g FW) was recorded in fruit under 47±1°C/20min and 53±1°C/5min HWD, respectively in 'White Sukkary' after 30 days at 13°C. Moreover, 'Zebda' fruit showed maximum significant values of carotene (1.79 and 1.89 mg/g FW) during 42 days cooling period under $Ca(NO_3)_2$/6% and 53±1°C/8min HWD, respectively. Chlorophyll a was increased significantly under cool postharvest period in all treatments compared to zero ones. $Ca(NO_3)_2$/6% recorded the maximum chl.a content (2.5 mg/g FW) in peel of both 'White Sukkary' and 'Zebda' fruit. Both HWD treatments showed the highest values of carotene during shelf period after cooling (0.98 and 0.89 mg/gFW), (2.71 and 2.67 mg/g FW) in 'White Sukkary' and 'Zebda' fruit. Low HWD 47(49)±1°C/20-30 min reserved chlorophyll a in maximum level in both cultivars during shelf period after cooling. Chlorophyll b did not show any significant differences among all treatments under any conditions, except in 'White Sukkary' fruit after 3 days shelf period.

This supports previous findings by Jacobi and Giles 1997 who reported that vapour heat treatment (VHT) /47°C for 15 min, or hot water (HW)/53°C for 5 min prior to VHT, combined with either storage at 10°C for 5 days followed by 22°C for 5 days or storage at 22°C for 10 days of 'Kensington mangoes had higher skin colour ratings, reflectance and chroma values, and lower hue angles than untreated fruit, indicating an enhancement of ripening.

The Antioxidants

Degradation of phenolics during cooling period was found to be very low in both cultivars in all treatments and control compared to zero ones (Table 2, 3). Mango and banana flesh have very low concentration of polyphenols in bulb, correlated to high activity of polyphenol oxidase. Also, HWD treatments increased bulb temperature until inactivation of polyphenol oxidase, therefore bulb browning did not occurred (Berardini *et al.*, 2005; Marques *et al.*, 2006). High levels of free phenols was found in 'White Sukkary' and 'Zebda' fruit under both HWD treatments and $Ca(NO_3)_2$/6%. Low HWD 47(49) ±1°C/20-30 min in 'White Sukkary' and 'Zebda' maintained free phenols to maximum significant levels (74.2 and 72.9 mg/g FW, respectively) during shelf life periods. Cold storage generally resulted in a marked decrease in total phenolic content in most tropical fruit; this decrease was observed after 5-6 days of storage at 4-6 °C, in the peel of mango. CI led to both modifications of phenolic content and composition, which may depend on the species or variety (Kondo, *et al.*, 2005). Peroxidase (POD, EC 1.11.1.7) can also oxidize phenolic substrates into quinones and be involved in browning reactions in fruit bulb (Marques, *et al.*, 2006).

POX and CAT were induced in peel and juice of mango fruit under all treatments and postharvest conditions compared to zero time (Table 2 and 3). In this respect, during cooling and subsequent shelf periods, both HWD treatments (especially 47(49) ±1°C/20-30 min) stimulated POX and CAT activity in peel and juice by approximately 2-5 times compared to control and calcium treatments in 'White Sukkary and 'Zebda' fruit (Table 3 and 4). Data indicated that POX and CAT may be involved in immune system which

Table 1. Impact of $Ca(NO_3)_2$ and HWD on Chl a , b and carotene content in mg /g FW of 'White Sukkary' and 'Zebda' fruit peel.

Parameters	'White Sukkary'						Zebda					
	A			B			C			D		
Treatments	Chl.a	Chl.b	Carotein	Chl.a	Chl.b	Carotein	Chl.a	Chl.b	Carotein	Chl.a	Chl.b	Carotein
Zero time	1.3	1.8	0.5				3.3	1.2	0.98			
Control	1.53 b	0.38 a	0.29 b	0.55 b	0.45 a	0.66 b	2.16 b	0.73 a	1.67 b	0.62 c	0.71 a	2.38 b
$Ca(NO_3)_2$/4%	1.8 b	0.35 a	0.22 b	0.42 c	0.36 ab	0.79 a	2.2 b	0.89 a	1.19 c	1.08 a	0.63 a	2.13 b
$Ca(NO_3)_2$/6%	2.5 a	0.37 a	0.34 b	0.54 b	0.44 a	0.99 a	2.55 a	0.86 a	1.79 a	1.21 a	0.58 a	2.32 b
HWD 47(49)±1°C/20-30 min	1.53 b	0.33 a	0.37 a	0.64 a	0.31 ab	0.98 a	1.38 c	0.76 a	1.27 c	1.30 a	0.58 a	2.71 a
HWD 53±1°C/5(8) min	1.79 b	0.23 a	0.44 a	0.37 c	0.17 b	0.89 a	1.42 c	0.73 a	1.89 a	0.86 b	0.53 a	2.67 a
L.S.D 0.05	0.29	ns	0.11	0.05	0.17	0.24	0.29	ns	0.11	0.19	ns	0.21

(A): 30 days cooling storage period/13°C in 'White Sukkary', (C):42 days in Zebda; (B): 3days subsequent shelf periods in 'White Sukkary '(D): 6 days in Zebda. Values are the means of 7 fruit per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 2. Chilling injury % and antioxidants levels in 'White Sukkary' fruits as affected with $Ca(NO_3)_2$ and HWD.

	Chilling injury %		Free phenols mg/g FW		POX activity unit/mg protein.min				CAT activity unit/mg protein.min			
					peel		bulb		Peel		bulb	
	A	B	A	B	A	B	A	B	A	B	A	B
Zero time	0.0		92.4		0.02		0.04		0.12		0.09	
Control	100 a	100 a	58.3 c	38.03 d	0.07 c	0.22b	0.52 c	0.58 e	0.31c	0.45c	0.64 d	0.92 d
$Ca(NO_3)_2$/4%	42 b	57 c	64.2 b	33.9 d	0.36 b	0.12c	0.63 bc	0.91 d	0.44 c	0.45c	0.91 c	1.4 c
$Ca(NO_3)_2$/6%	30 c	71 b	86.5 a	49.6 c	0.37 b	0.24b	0.81 b	1.18 c	0.92 b	0.45c	1.27 b	1.54 c
HWD 47±1°C/20 min	14 d	42 d	92.8 a	74.2 a	0.65 a	0.42a	1.46 a	2.8 a	1.47a	1.12b	2.33 a	3.64 a
HWD 53±1°C/5 min	14 d	14 e	90.7 a	56.5 b	0.63 a	0.42a	0.79 b	1.4 b	1.38a	1.53a	2.27 a	2.25 b
L.S.D 0.05	1.9	3.1	5.4	5.9	0.21	0.11	0.2	0.19	0.13	0.03	0.23	0.31

(A): 30 days cooling storage period/13oC, (B): 3 days subsequent shelf periods. Values are the means of 7 fruit per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

elevate the tolerance ability of mango fruit against chilling stress.

The contents of catalase, ascorbate peroxidase, glutathione and phenolic compounds in 'Wacheng' mangoes treated for 3, 4 or 5 h at 0 °C, or treated for 8, 10 or 12 h at 4 °C, respectively, then transferred to 20 °C for 20 h prior to being stored at 2 °C, 85–95% RH, were all markedly enhanced by the cold-shock treatment, whereas activities of superoxide dismutase, glutathione reductase and content of ascorbic acid were slightly influenced by the cold-shock treatment (Zhao *et al.*, 2006). Exhibition of CI symptoms in Lemon fruit was associated with decreasing of CAT and SOD and increasing of POD activities during cold storage (1.5 °C /8 weeks). 53°C/3 min HWD and $CaCl_2$/1.5 % treatments similarly reduced CI and delayed the reduction of CAT and SOD and suppressed the increase in POD activities compared to control.CAT activity increased in treated fruit before cold storage than in the untreated fruit over the storage period (Safizadeh *et al.*, 2007). Furthermore, 'Zill' mangoes treated with oxalic and salicylic acid showed lower superoxide anion content, higher hydrogen peroxide content, lower lipoxygenase activity and higher activities of superoxide dismutase , catalase , guaiacol peroxidase, ascorbate peroxidase and glutathione reductase under 14 or 5 °C wasassociated with alleviating of CI compared to control (Ding *et al.*, 2007).

Fruit Quality

Fruit Firmness and Calcium Content

'White Sukkary' characterized by fine peel, showed higher significant values of peel firmness under both HWD treatments and $Ca(NO_3)_2$/6% during cooling and subsequent shelf periods (Table 5 and 6). Moreover, low HWD 49 ±1°C/30 min and low calcium concentration treatments (4%) recorded high significant levels of peel firmness of 'Zebda' fruit, which have thickened peel for the duration of cooling and subsequent shelf periods (Table 5). Calcium content was increased in peel and bulb during cooling and subsequent shelf periods, in both cultivars, under all treatments compared to zero point. 53±1°C/5min HWD increased Ca^{2+} levels (0.42 mg/g DW) in 'White Sukkary' peel but Ca^{2+} content found in high concentration in fruit bulb(0.15 mg/g FW) treated with 47 ±1°C/20 min and $Ca(NO_3)_2$/6% . Increase of calcium content may be attributed to the degradation of calcium-containing compounds during postharvest period. Furthermore, both calcium treatments and 49 ±1°C/30 min/HWD elevated calcium content in peel and bulb of 'Zebda' fruit through both cooling and subsequent shelf periods.

Le *et al.*, 2010 mentioned that, VHT (46.5°C for 40 min) maintained firmness and total soluble solid content at 3°C of storage time in 'Tuu Shien mangoes. Moreover, a combination of low oxygen and calcium allowed 'Kensington' slices to be held for at least 15 days at 3◦C (De Souza et al., 2006). HWD (45°C/25 min) induced a firming effect and avoid softening of kiwifruit slices, while calcium dips had a marginal effect on this parameter. A calcium loss was observed due to dip treatment, but this effect was minimized when treatment was conducted in 3% $CaCl_2$ solution. The firming effect provided was due to the activation of pectinmethylesterase while the presence of calcium in treatment solution reduces or inhibits enzyme activation (Costa *et al.*, 2008). Fruit subjected to extended hot air treatments of 38 or 40°C often soften slower than non-heated fruit, although disinfestation procedures for mangos and papaya of hot forced air for 4 h at 50°C led to faster softening after the treatment (Shellie and Mangan, 1994).

SSC, TA% and Reducing Sugars

Both 'White Sukkary' and 'Zebda' fruit showed no significant differences increasing SSC during both cooling and subsequent shelf periods. Both calcium treatments especially $Ca(NO_3)_2$/4% was more effective in SSC levels in both cultivars compared to HWD treatments. Mango soluble solids are not affected by an insect vapor heat treatment (Jacobi and Giles, 1997).

The major sugars in mango are glucose, fructose and sucrose, with sucrose predominating. The major acids are citric, malic, and sometimes tartaric at 0.1 to 0.4 % TA (Nairain, *et al.*, 1997) and 10 to 16 SSC (Baldwin *et al.*, 1999). During cooling period, 'White Sukkary fruit showed no significant differences in TA% but, in subsequent shelf periods fruit exhibited significant response to all treatments. Both 53 ±1°C/5 min HWD and $Ca(NO_3)_2$/4% showed maximum values of TA% (0.56%) in White Sukkary fruit. In contrary, 'Zebda' fruit showed no significant differences in TA% during subsequent shelf periods. Moreover, $Ca(NO_3)_2$/4% significantly increased TA% to maximum values (0.39 %) in 'Zebda' throughout the cooling period.

Mango as a climacteric fruit shows softening, increase in sugars contents and decrease in organic acid contents accompanied with ripening (Mitra and Baldwin 1997). Table 4 and 5 demonstrated that, reducing sugars was increased in both cultivars approximately 3 times in cooling period compared to zero time, in addition to 1.5 times after subsequent shelf period. Both 47 ±1°C/5 min HWD and $Ca(NO_3)_2$/4% showed highest values of reducing sugars in 'White Sukkary' fruit in cooling period. Moreover, 'White Sukkary fruit under all treatments showed no significant differences in reducing sugars during subsequent shelf periods. In addition, 'Zebda' fruit showed high significant levels of reducing sugars under 49 ±1°C/8 min HWD and $Ca(NO_3)_2$/4% during both cooling and subsequent shelf periods.

The development of chilling injury in mango peel, as in the pulp is marked by a significant decrease in the soluble sugar content (mainly sucrose), no significant change in total hexose content and less starch breakdown; in addition, invertase activity increases whereas that of amylase decreases. Mango invertase shows two temperature optima, one at 0°C and the other at 37°C (Chhatpar *et al.*, 1971). Low C_2H_4 production, poor colour development, minor changes to fruit composition, insipid flavour and poor aroma revealed that fruit ripening was insufficient to reduce CI compared to half mature and mature 'Tommy Atkins' mangoes. Half mature and mature fruit had higher C_2H_4

Table 3. Chilling injury % and antioxidants content in 'Zebda' fruits as affected with $Ca(NO_3)_2$ and HWD.

	Chilling injury %		Free phenols mg/g FW		POX activity unit/mg protein.min				CAT activity unit/mg protein.min			
					peel		bulb		Peel		bulb	
	C	D	C	D	C	D	C	D	71	D	C	D
Zero time	0.0	0.0	89.1		42		0.11		0.19		0.13	
Control	61 a	67 b	39.3 c	35.1 b	0.32a	0.36d	0.71 d	0.87 e	0.22 b	0.63 c	0.79 e	0.86 e
$Ca(NO_3)_2$ /4%	45 b	67b	64.03 b	24.6 c	0.28 b	0.33 d	0.84 d	0.99 d	0.25 b	0.62 c	1.24 d	2.11 c
$Ca(NO_3)_2$ /6%	33 c	61 c	68.2 ab	44.4 b	0.35 a	0.47 c	0.99 c	1.35 c	0.34 a	0.84 b	1.64 c	2.32 b
HWD 49±1°C/30 min	23 e	30 d	87.6 a	72.9 a	0.26b	1.12a	1.46 a	2.89 a	0.21 b	0.88 b	2.55 a	3.18 a
HWD 53±1°C/8 min	29 d	85 a	80.8 ab	46.8 b	0.33a	0.95 b	1.17 b	1.55 b	0.31 a	1.45 a	2.3 b	1.55 d
L.S.D 0.05	1.6	1.6	15.9	9.65	0.03	0.04	0.14	0.11	0.03	0.05	0.19	0.09

(C): 42 days cooling storage period/13°C, (D): 6 days subsequent shelf periods. Values are the means of 7 fruit per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 4. Some quality parameters of 'White Sukkary' fruit as affected by $Ca(NO_3)_2$ and HWD .

Treatments	SSc		TA%		Firmness Kg/cm^{-2}		Calcium content mg/g DW				Reducing sugars mg/g FW	
							peel		bulb			
	A	B	A	B	A	B	A	B	A	B	A	B
Zero time	9.87		1.7		5.4		0.366		0.043		56.2	
Control	16.6 a	15.2 a	0.92 a	0.42 a	0.37 b	0.24 ab	0.29b	0.27c	0.13a	0.09b	177.8 a	274.1 a
$Ca(NO_3)_2$ /4%	17 a	15.9 a	0.86 a	0.56 a	0.4 b	0.21 b	0.44a	0.22c	0.16a	0.09b	173.8 a	259.8 a
$Ca(NO_3)_2$/6%	16.4 a	15.2 a	0.92 a	0.29 b	0.42 ab	0.27 a	0.43a	0.38b	0.16a	0.15a	129.5 b	195.2 a
HWD 47±1°C/20 min	15.5 a	15.3 a	0.91 a	0.46 a	0.48 a	0.22 b	0.44a	0.33b	0.17a	0.15a	163.4 a	261.9 a
HWD 53±1°C/5 min	16 a	15.9 a	0.82 a	0.55 a	0.42 ab	0.24 ab	0.42a	0.42a	0.15a	0.14a	106.2 b	220.5 a
L.S.D 0.05	ns	ns	ns	0.13	0.08	0.04	0.14	0.12	ns	0.06	23.6	ns

(A): 30 days cooling storage period/13°C, (B): 3 days subsequent shelf periods. Values are the means of 7 fruit per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

Table 5. Some quality parameters of 'Zebda' fruit as affected by $Ca(NO_3)_2$ and HWD.

Treatments	SSc		TA%		Firmness Kg/cm^2		Calcium content mg/g DW				Reducing sugars mg/g FW	
							peel		bulb			
	C	D	C	D	C	D	C	D	C	D	C	D
Zero time	11.8		0.61		7.5		0.863		0.057		78.3	
Control	16.3 a	16.5 a	0.25 ab	0.14 a	0.49 b	0.76 ab	1.03b	0.92a	0.43b	0.64a	151.9 a	245.9 a
$Ca(NO_3)_2$/4%	17.3 a	17.7 a	0.39 a	0.18 a	0.94 a	0.63 bc	1.22a	0.92a	0.83a	0.66a	156.3 a	199.5 b
$Ca(NO_3)_2$6%	17.6 a	16.5 a	0.32 ab	0.15 a	0.64 ab	0.55 c	1.12a	0.63b	0.82a	0.52a	111.7 b	162.1 c
HWD 49±1^{0}C/30 min	17 a	16.9 a	0.21 ab	0.18 a	0.93 ab	0.85 a	1.22a	0.91a	0.86a	0.73a	127.6 ab	212 9 b
HWD 53±1^{0}C/8 min	17.5 a	16.1 a	0.18 b	0.16 a	0.49 b	0.34 d	0.92b	0.72a	0.52b	0.66a	122.3 ab	162.4 c
L.S.D 0.05	ns	ns	0.19	ns	0.3	0.2	0.12	0.29	0.43	ns	28.2	24.8

(C): 42 days cooling storage period/13°C, (D): 6 days subsequent shelf periods. Values are the means of 7 fruit per replicate. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

production rates than immature fruit and ripened normally with acceptable flavour and aroma after 18 days at 5°C and 3 days at 20°C (Mohammed and Brecht 2002). Previously, Ram *et al.,* 1983 reported that HWT did not appreciably affect pH, ascorbic acid and total sugars in treated 'Deshehari' mangoes.

VC and ASAO Activity

In general, Figure 4 (a and b) showed that, 'White Sukkary fruit contain high level of VC compared to 'Zebda' fruit, it may be demonstrated that differences in VC represented genotypic character more than phenotypic one. Also, behavior of VC during postharvest was altered according to cultivar and postharvest conditions. Figure 4 cleared that VC was increased in 'White Sukkary' fruit during storage at 13°C, but in the same time decreased in 'Zebda' fruit. VC increased approximately 3-5 folds compared to zero points during cooling storage period, and then decreased by 0.5-2 times during subsequent shelf periods in the two cultivars.

Calcium treatments especially Ca(NO3)2/4% induced maximum significant content of VC in 'White Sukkary' (24.8 and 19.1 mg/ml juice during both cooling and subsequent shelf periods compared to HWD treatments. The same incidence was found in 'Zebda' fruit but without significant differences. ASAO convert ascorbic acid (Vitamin C) to dehydroascorbic acid and is therefore of interest to the mango industry.

Low content of VC was recorded in heat treated fruit especially at 53 ±10C/5(8) min contributed with higher activity of ASAO. 53 ±10C/5(8) min HWD treated Fruit in both 'White Sukkary and 'Zebda' showed maximum activity of ASAO (2.11 and 2.22 unit/mg protein.min) during 30 and 42 days at 13°C. Control fruit recorded maximum activity of ASAO during subsequent shelf period (3.63 and 3.23 unit/mg protein.min) in 'White Sukkary and 'Zebda' fruit, respectively. Maintaining of VC at high level under calcium treatments especially $Ca(NO_3)_2$/4% was correlated with lower activity of ASAO in both cultivars under cooling and subsequent shelf periods.

Champion *et al.* (2004) stated that ascorbic acid is oxidized when reacting with oxygen in the presence of air or with oxidant substance or with particular enzyme (ASAO). Combinations of $CaCl_2$/ 1%, ascorbic acid/ 2% and citric acid/ 2% treatment prevented loss of sugar and vitamin C of cubes during storage at 5°C in 'Ataulfo' mangoes with Shelf life extended to 21 days. There is a correlation between carotene and vitamin C content and its longer shelf life (Gonzalez-Aguilar et al., 2008).

The optimal pH for the browning is between 5-7 and if the pH is significantly below 3, the PPO will be inactivated. Decreasing of TA% in mango fruit during ripening was correlated with lowering of fruit pH, therefore, activity of PPO was decreased. Also, ASAO (optimum pH, 5.6) was inhibited and maintaining of VC was observed. Ascorbic acid prevents oxygen from reacting with the polyphenol oxidase (Marques *et al.,* 2006).

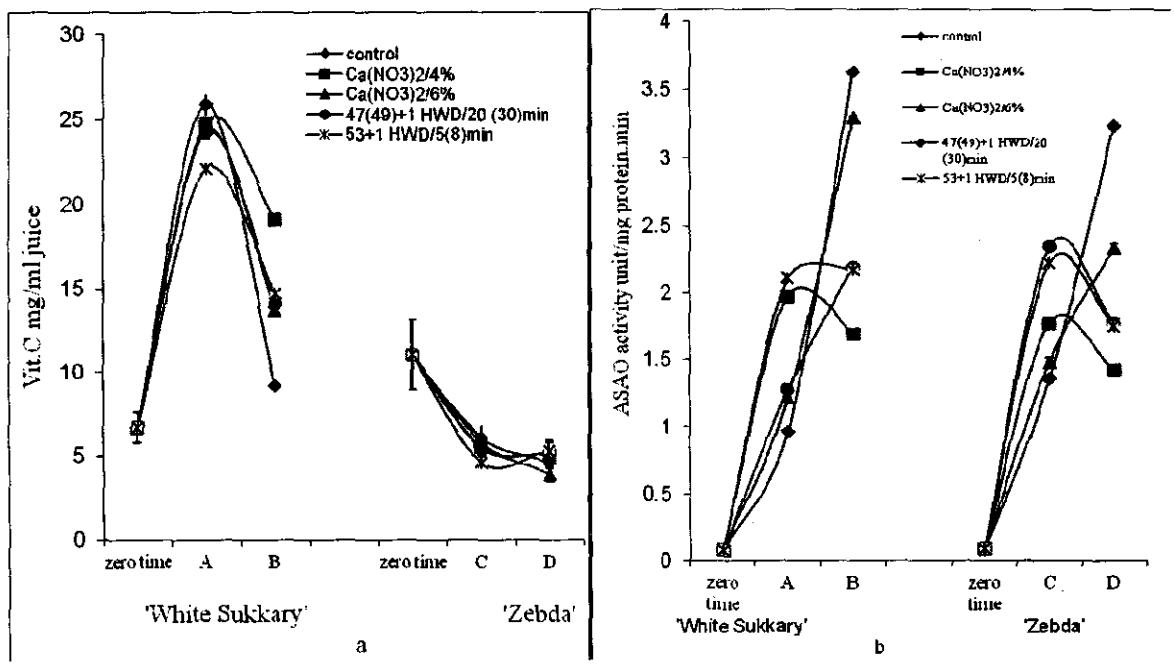

Figure.4. VC (a) and ASAO (b) activity in 'White Sukkary' and 'Zebda' as affected by $Ca(NO_3)_2$ and HWD during cooling storage at 13 °C and subsequent shelf storage periods. (A) after 30 days in 'White Sukkary,(C)42 days in Zebda, cooling period/13°C (B) subsequent shelf periods, 3days in'White Sukkary',(D)6 days in Zebda. (means±SE, n = 4). $P \leq 0.05$ as determined by Duncan's test.

CONCLUSIONS

47(49)±1 ◦C/20-30 min HWD treatment minimized chilling injury (CI) with enhancing POX and CAT activity by2-5 times in both two cultivars. Also, low heat treatment increased calcium content in peel and bulb which was significantly correlated with peel firmness. TA%, SSC and reducing sugars were also significantly increased and associated with low free phenol content under low hot water treatment. Moreover, fruit treated with 4% $Ca(NO_3)_2$ showed minimum weight loss% and maximum VC content due to decrease ASAO activity.

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انخفاض اضرار البرودة وتحسين جودة ثمار المانجو صنفى السكرى الابيض والزبدة اثناء التخزين البارد بعد المعاملة بالغمر فى الماء الساخن ونترات الكالسيوم

* مجدى على محمد بصل و ** محمد على محمود الحماحمى
*قسم البساتين – كلية الزراعة – جامعة قناة السويس- ٤١٥٢٢ الاسماعيلية - مصر
** قسم النبات الزراعى-كلية الزراعة –جامعة قناة السويس-٤١٥٢٢ الاسماعيلية-مصر

تم دراسة تاثير معاملة غمر الثمار فى الماء الساخن (٤٧±١ م لمدة ٢٠ دقيقة و ٥٣ ± ١ م لمدة ٥ دقائق) فى السكرى الابيض و (٤٩±١ م لمدة ٣٠ دقيقة و ٥٣±١ م لمدة ٨ دقائق) فى الزبدة وكذلك نترات الكالسيوم بتركيز ٤ و٦ % لمدة ١٥ دقيقة فى كليهما اثناء التخزين البارد على ١٣ م لمدة ٣٠ و ٤٢ يوم يليها فترة تخزين عادى لمدة ٣ و ٦ ايام فى كلا من الصنف السكرى الابيض والزبدة على التوالى . بصفة عامة اظهرت معاملة غمر الثمار فى الماء الساخن (٤٧(٤٩) ±١ لمدة ٢٠-٣٠ دقيقة) اعلى تاثير معنوى فى تقليل النسبة المئوية لاضرار البرودة مع زادة نشاط انزيم البيروكسيديز والكتاليز بمعدل من ٢-٥ مرات فى جلد ولب الثمار. كما ادت نفس المعاملة لزيادة تركيز الكالسيوم فى جلد وقشرة الثمار والذى ارتبط معنويا بصلابة الثمرة مقارنة بمعاملات نترات الكالسيوم والكنترول. فى حين اظهرت معاملة نترات الكالسيوم بتركيز ٤ % اقل معدل فى فقد وزن الثمرة وكذلك اعلى معدل لفيتامين ج والذى ارتبط بنشاط منخفض فى انزيم اسكوربيك اسد اوكسيديز. كذلك تم تقييم مستوى الحموضة الكلية والمواد الصلبة الكلية والسكريات المختزلة والفينولات الحرة وكلوروفيل أوب والكاروتنيدات.