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INCREASING THE STORAGE ABILITY OF ZIBDA MANGOES

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ABSTRACT

Mango (*Mangifera indica* L.), Zibda cultivar is considered as a sensitive cultivar to low storage temperature. Fruits were from Zibda CV harvested at three different maturity stages and stored at 4ºC (85–90% RH) for 35 days after treatment with ascorbic acid (AA) as antioxidants. Chilling injury (CI) was observed initially as a black spot and loss of peel color by end of the storage time pulp color changed into the seed area. In the case of mango, CI begins at 4ºC. The storage, handling and transport potential of fruits is limited by susceptibility to diseases, and sensitivity to low storage temperatures. To increasing the tolerant of Zibda fruits under cold storage may be achieve by minimizing the oxidative reactions during storage using addition antioxidants such as ascorbic acid (AA) to fruits at different immersing time. Normally, at storage temperature of 4ºC, CI appeared after 10 days, thereafter developed more rapidly with passing storage period. Using addition antioxidant delays CI up to 25 days. Delaying CI appearance of sensitivity cultivar may be useful for marketing Zibda fruits to long distance.

INTRODUCTION

Mango (*Mangifera indica* L.) is one of the most commonly eaten fruits specially in tropical countries. Large quantities of different varieties are produced annually. However, a greater proportion of these fruits are wasted during the season due to inadequate harvesting, handling, and storage (Falade*, et al.*, 2004). Moreover, it is considered a climacteric fruit and ripens rapidly after harvesting. The storage, handling and transport potential of fruits is limited by susceptibility to low storage temperatures below 12ºC (Acosta*, et al.*, 2000), and perishability due to chilling temperature sensitivity (Acosta, *et al.*, 2000.González-Aguilar*, et al.*, 2000). The most common visual symptoms of chilling injury (CI), are dark skin, scald–like discoloration, and pitting or sunken lesions on the peel during storage at low temperature (Nair*, et al.*, 2003). These visual symptoms are as results of the disruption of normal physiological activity, resulting in metabolic imbalances. It seems that the metabolic imbalance to involve changes in membrane organization such as decreases in the un-saturation fatty acid of membrane lipids. Additionally, oxidative reaction damage is associated with chilling injury, which implies that chilling treatments produce a disturbance in the cellular redox homeostasis of susceptible plant cells (Foyer and Noctor, 2000), in response to low storage temperature treatments. The antioxidants capacity of tissue frequently increases, and is consistent with the homeostasis model of Foyer and Noctor (2000). The generation of active oxygen species (AOS), may be casually related to alternations in membrane function (Foyer and Noctor, 2003), and the AOS are generated in different microsomal membranes from stressed tissue (Nair, *et al.*, 2003). Plant cell has mechanism to defense against AOS by using three antioxidants groups (water and lipid-soluble antioxidants and

antioxidant enzymes), even fruits and vegetables contain different amount from them. They are considered to protect tissue against AOS (Hodges*, et al.*, 2004).

The physiological importance of ascorbic acid (AA) in the plant cell is reverted to its functions as a precursor for oxalate and tartaric acid synthesis. It initiate in many processes, including photosynthesis, photo-protection, the cell cycle, cell-wall growth and cell expansion, synthesis of ethylene, gibberellins, anthiocyanine and hydroxyproline, and resistance to environmental factors. The most important role of AA is scavenge directly/indirect of AOS in plant cell (Smirnoff and Wheeler, 2000), and it may rest the relative stability of the monodehydroascorbate radical. Moreover, AA also has another important photoprotective function because of its antioxidant capacity. It is one of two major soluble antioxidants in chloroplasts (Foyer and Harbinson, 1994) and it assumed to protect α-Tocopherol and recycle it (a major lipid-soluble antioxidant) from α-Tocopheroxyl radical (Munné**-**Bosch and Alegre, 2002).

The aim of this paper was to increase the chilling tolerance of Zibda mango fruit under low storage temperature by treating with AA prior commencement of storage. Directly, the exploitation of physiological roles of AA in fruit cell metabolism which is associated with the developmental changes of fruit parts, in order to, delay chilling injury during long storage period at low temperature.

MATERIALS AND METHODS

Fruit harvesting and storage condition

Fruits of mangoes cv. Zibda were harvested on 11th August 2006, 2007 and 2008 from trees more than 20 years old growing in a sandy soil of commercial orchard. It was located in Sharkia province East Egypt (30.35 *N* and 31.30 *E*). Zibda cultivar was harvested at three different harvest maturity stages from shadow side of trees when the average field temperature 38^{o}C. The maturity stages were classified as: immature (M1), half-mature (M2) and fully mature (M3). This classification was based on the morphological development of fruits shoulder: in M1 fruits, the shoulder is below the stem end, in M2 fruits the shoulder is at the same level as the stem end, and in M3 fruits the shoulder is above the stem end (Lo'ay, 2009). Fruits were washed with the cold water at 10-13^{o}C to reduce the field temperature and to remove the microbial load on the fruit surface.

Experiment I: ascorbic acid mapping in mango fruits

Thirty fruits were harvested on August 2006 at different maturity stages (M1, M2 and M3). 10 fruits were divided into main parts stem and calyx end of fruit in order to understand the variation of AA content in between fruits maturation and parts. Three fruit parts of each main part were separated into sub-main parts peel, outer pulp (1cm from peel) and inner pulp (1cm from seed), in which measured AA content (mg 100 g^{-1} FW) was determined according to (A.O.A.C., 1980) to set out the AA map in fruit.

Experiment II: ascorbic acid absorption

This experiment was carried out on August 2006 in order to understand how the initial AA content was affected when the fruits were treated by solutions of AA. Mango fruits were treated (ambient air, water and 5.6 mM of AA) for 24 hours at 4°C. Fully mature green mango fruits (135) were harvested. Fruits were divided into 3 batches. Each one composed of 45 fruits into group (each group contains 16 fruits extra for distructive measurements). The first 46 fruits were treated with AA (5.6 mM), 45 fruits with water (as a control) and 45 for ambient air conditions treatment. The fruits were stored at 4°C for 35 days to observe the CI incidence.

Experiment III: ascorbic acid and increase chilling tolerance

The 250 fully mature fruits were used for non-destructive measurements (chilling injury index); 25 fruits were treated by AA solution 5.6 mM at different immersion time (0. 6, 12, 18 and 24 hr at 4°C), thereafter, they were stored at 4 and 10°C. Chilling injury index was measured at 5 days intervals up to 35 days. 160 fruits more for distructive measurements: only 16 fruits at each immersing time in AA, afterword they were stored for 35 days.

Measurements

Chilling injury index

A visual assessment of external damage, such as pitting, water soaked areas, and decay is often used to assess chilling injury. In order to attempt to relate the more objective measurements of electrolyte leakage to the visual assessment of injury and the method of (Chaplin*, et al.*, 1991) was used to score the symptoms. The CI index is then calculated using the following formula:

$$\text{Chilling injury index} = \sum_{1\rightarrow 5}^{5} \frac{(\text{chilling injury level}) * (\text{Number of fruits at the level})}{\text{Total number of fruit}}$$

Ion leakage percentage

The samples of five disks (7 mm diameter) of peel and pulp tissues were cut using a cork-borer from five different parts of each fruit every 5-days. The disks were washed three times in demineralized water and placed in 10 ml 0.4 M mannitol in demineralized water at 24°C for 3h (Hakim, 1999). Electrical conductivity of the aqueous phase was measured using a conductivity meter, after which the tissue samples were killed by heating in water bath at 100°C for 20 minutes. This cooking process allows the release of all electrolytes from the tissue. Once cooled to room temperature the conductivity was re-measured and the relative electrolyte leakage from the uncooked pulp and peel samples was calculated as follows:

$$\text{IL}\ (\%) = \frac{\text{Conductivity}_{\text{after 3 h}}}{\text{Conductivity}_{\text{after boiling}}} \times 100$$

Chlorophyll content

Fruit peel samples were stored and freezed at –20°C in dark. To avoid photo-oxidation of chlorophylls all preparations were carried out in the dark.

The 0.8 g of ground powder, 5 ml *N,N*-dimethylformamide (DMF) was added. The extraction chlorophyll method of (Mónica*, et al.*, 1994) was modified by using DMF instead of acetone (Lo'ay, 2005). Samples were stored at 4^{o}C for 16 hours to allow the DMF to leach the pigments from the sample (Minguez-Mosquera*, et al.*, 1991). Finally, samples were centrifuged for 5 min at 16000 rpm, then samples were determined for wavelengths 618 and 665 nm for chlorophyll *a* and *b* and.

Protein carbonyl (PCG) assay

Protein carbonyl group was measured according to (Levine*, et al.*, 1994). Precisely weighed mango samples (peel) of about 2.5 g. The spectrum was measured spectrophotochemically against the complementary blank in case of cured (without sample) samples or against water in case of purified proteins. The carbonyl content of the protein was calculated from the absorbance of the dinitrophenylhydrazone measured at 390nm and assuming an extinction coefficient of 22000 M-1 cm-1.

Lipid per-oxidation (MDA)

Exactly weighed 2.5 g mango (peel) samples according to (Iturbe-Ormaetxe*, et al.*, 1998). The calibration curves made by measuring 1,1,3,3-tetraethyoxypropane (Sigma) in the range 0-2 mM (TBARS) which was equivalent to 0-1 mM malondialdehyde (MDA). Tetraethyoxypropane is stoichiometrically converted into MDA during the acid-heating step of the assay

Statistical analysis

Data for evaluation of parameters in time were analyzed using analysis of variance (ANOVA). The means were compared using the least significant differences (L.S.D.) at p≤0.05 level of probability. The statistical software package GenStat Ver. 11(Lawes Agriculture Trust, Rothamsted Experimental station, UK) was used.

RESULTS AND DISCUSSION

Experiment I: Variations of AA content

Figure 1 shows the changes in AA (mg per 100g^{-1}FW) content in Zibda mango fruits were harvested at three different maturity stages. Ascorbic acid shows a significant interaction (P<0.001) when the developmental change factors such as fruit maturities (M1, M2 and M3), main fruit parts (stem and calyx end) and sub-main parts (peel, outer and inner pulp). Immature fruits have a three-fold AA content in calyx end of fruit than half and fully mature fruit and it is higher compared with fruit parts in stem end of the fruit. Ascorbic acid amount is higher in peel parts of fruit than outer and inner pulp parts (sub-main parts). Where as, half mature fruits have AA content slightly higher than fully mature fruit in both main and sub-main parts of fruit.

The differences between maturity in fruit parts and response of AA to maturation is important with respect to the storage of mango at low temperature or/and increase chilling tolerance of the fruits.

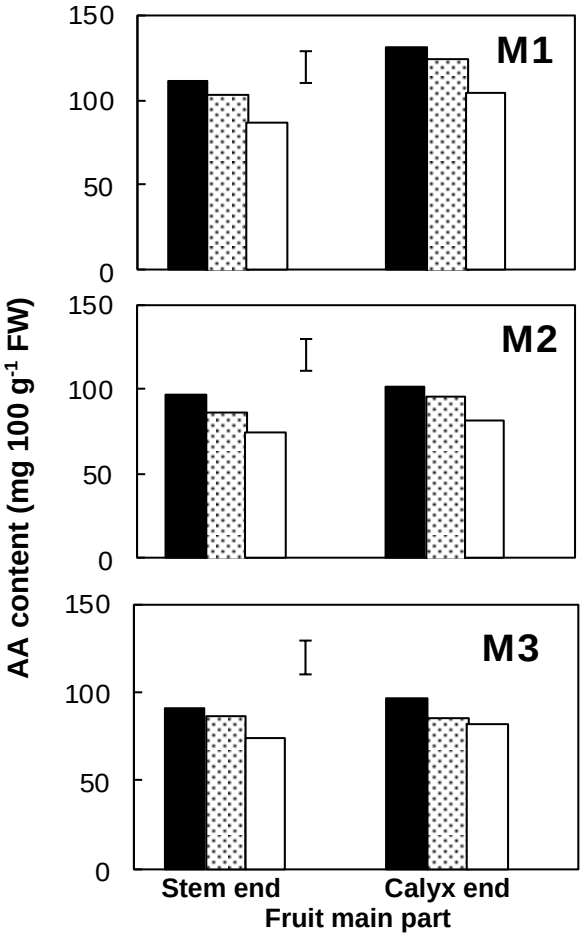


Figure 1: Diagram showing the interaction of developmental differences of AA contents at different fruit maturity stages (M1, immature fruit; M2 half mature and M3, Full mature fruit) from two main fruit parts (stem and calyx end of fruit) and different sub-main fruit parts (peel, outer-pulp and inner-pulp). The vertical bar represents the L.S.D. at *P*= 0.05% level of profanity.

The various AA contents between main and sub-main fruit parts and fruit maturities might be related to fruit development or genetic aspects. The decreases of AA in fully mature fruits may be related with fruit ripening processes (Kader, 2002). Generally, there are developmental changes in AA content among mango fruit maturities and they found at harvest time even among the same cultivar maturity stages. Zibda cv presented strongly these developmental changes of AA content among fruit maturity stages which has been presented by. (Lee and Kader, 2000)

Experiment II: Ascorbic acid application

The concerned results of this part of investigation are illustrated in Figure (2). It was clear that the fruit were pre-storing immersing in AA solution 5.6 mM for 24 hour then stored at 4ºC. Moreover, CI symptoms were minimized with fruits pre-storing immersing in AA solution which reached at end of experiment (1.533 index) compared with pre-storing immersing in water (4.433 index) and ambient conditions (3.733 index). In fact, the AA concentration of pre-storing immersing treatment was chosen so as to be in the range of AA content of the mango peel. The delay of initial appearance of CI symptoms by pre-storing immersing of AA treatment is considered as a very important improvement in the ways by which sensitive fruits such as mangoes are stored.

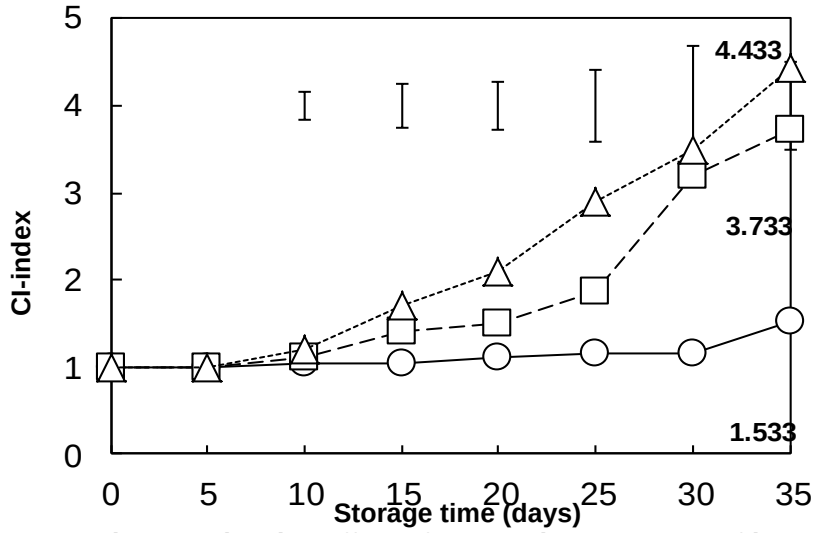


Figure 2: Diagram showing effect of pre-storing treatment of immersing in water (-△-) or in AA solution (-○-) and storing in ambient conditions (-□-) on chilling injury incidences in fully mature Zibda fruits stored at 4ºC for 35 days. CI-index expressed as a mean (n=3) .The vertical bars indicated to L.S.D. at p=0.05

The idea behind this experiment was to compensate AA consumed as antioxidant during the chilling conditions of fruits by quenching free radicals(Hodges, *et al.*, 2004). Natural antioxidants such as AA could be consumed by oxidative processes in stressed mango fruits under prolong storage time. The development of visual CI symptoms depends on the consumption rate of AA in fruits. It was believed that plant tissues act as permeable membranes. This suggested that when fruit were immersed in solutions containing AA (5.6mM) either diffusion of water from the surrounding solution in/out the tissue membrane may occur depending on the concentration gradient in or out the fruit tissue.

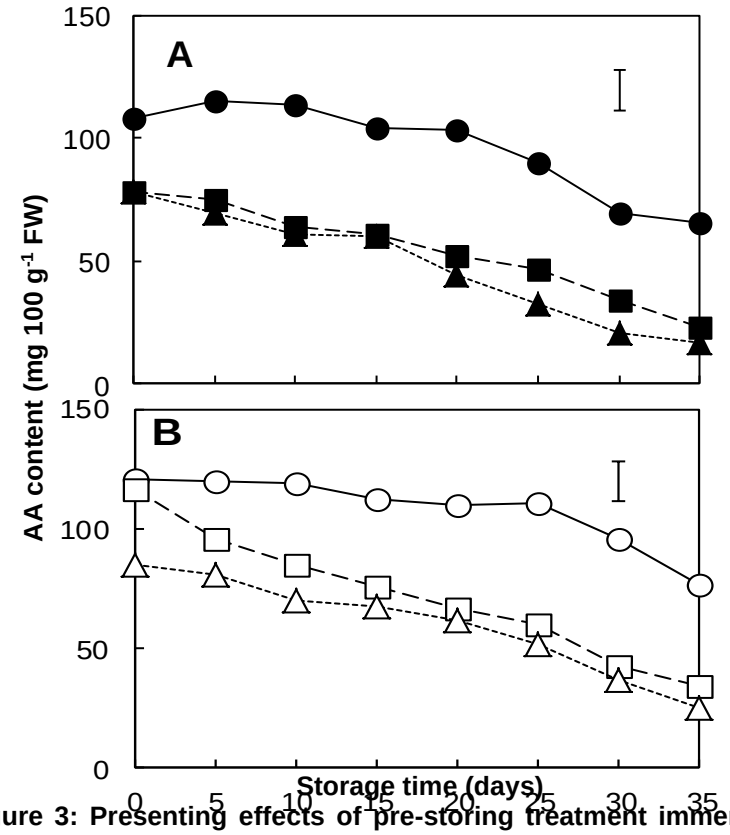


Figure 3: Presenting effects of pre-storing treatment immersing in AA solution 5.6 mM, water and storing in ambient conditions on AA content in fully mature Zibda mango fruits stored at 4ºC for 35 days. AA content expressed as a mean (n=3) .The vertical bars indicated to L.S.D. at p=0.05

This process may strongly occur when fruit pre-storing immerged in water for 24 hr. It may be a dilution of the AA content in the fruit tissue. It is observed from Figure (3) that the continuous AA measuring during storage period of stressed fruit in different parts (peel and pulp), when fruits were treated by pre-storing immersing in AA solution (5.6 mM), water and in ambient conditions for 24 hr. Peel and pulp of fruits were immersed in AA solution have a high amount of ascorbic acid up to 25 days of storage time. Whereas, fruits stored in ambient condition have moderate amount compared with fruits immersed in water at the storage time. The delay of visual CI symptoms on mango fruits immersed in AA solution may be according to the physiological role of AA. Its reaction as antioxidant in plant cell and it may rest the relative stability of the monodehydroascorbate radical to ascorbic acid (Smirnoff, 2000). It assumed to protect α-Tocopherol (α-TOC) and recycle α-Toc (a major lipid-soluble antioxidant; vit E) from α-Tocopheroxyl radical (Munné-Bosch and Alegre, 2002).

Experiment III: increase chilling tolerance

Ascorbic acid application and fruit tolerance

CI-index as function of storage time in days for all the storage temperatures (4 and 10ºC) of Zibda variety at different time in AA solution immersing is shown in Table 1. CI-index shows a significant interaction at $P \leq 0.05$ when storage time, temperature, time immersing in AA solution were considered. It is clear that the differences between both storage temperature and time up to 35 days. At storage temperature 10ºC, there was no evidence for CI damage during storage period even in all immersing times in AA solution (0, 6, 12, 18 and 24 hr). In the case of fruits stored at 4ºC, no injury was detected before 10 days of storage in all the immersing period. The first symptoms that appeared after 10 or 20 days were black spots on the peel of the fruit. The degree of damage that developed between 10-20 days was slight at different times of immersing in AA solution. Thereafter, there was only one week of storage to show damage development. After 20 days of storage CI developed more rapidly in fruits immersed in AA solution for 0 and 6 hours showing more damaging than 12 and 18 hours. However, at 24 hours immersing in AA solution, the degree of injury was less and no injury symptoms developed at the end of storage time.

Table 1: Effect of pre-storing fruit treatments of immersing in water or in AA solution (5.6 mM) for different immersing time (0, 6. 12, 18, and 24 hr) on chilling injury symptoms in Zibda mango fruits stored at 4 and 10ºC for 35 days

Storage temperature	Time Immersing hours	Storage time (days)							
		0	5	10	15	20	25	30	35
Immersing in Ascorbic acid 5.6 mM									
4ºC	0	1.00	1.00	1.20	1.70	2.10	2.93	3.50	4.43
	6	1.00	1.00	1.10	1.40	1.50	1.86	3.20	3.73
	12	1.00	1.00	1.03	1.06	1.10	1.33	2.00	2.76
	18	1.00	1.00	1.03	1.03	1.06	1.16	1.23	1.53
	24	1.00	1.00	1.00	1.00	1.00	1.06	1.16	1.43
10ºC	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	18	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Immersing in water									
4ºC	0	1.00	1.00	1.00	1.16	1.66	1.93	3.66	4.55
	6	1.00	1.00	1.06	1.23	1.73	1.86	3.13	3.56
	12	1.00	1.00	1.13	1.43	1.83	2.06	3.06	4.20
	18	1.00	1.00	1.26	1.63	1.93	2.40	3.16	4.20
	24	1.00	1.00	1.26	1.80	3.10	3.56	3.76	4.66
10ºC	0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	6	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	12	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	18	1.00	1.00	1.00	0.70	1.00	1.00	1.00	1.00
	24	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
LSD at 5%				0.07	0.23	0.29	0.29	0.28	0.25

The tolerance of Zibda fruits to injury was independent of time immersing in AA solution. Whereas, the tolerance of fruit increased as the time immersing in AA solution more up to 24 hours before storage. Injury due

to exposure of fruit to chilling temperature has been frequently attributed to oxidative stress (Hodges, *et al.*, 2004). So, increasing chilling tolerance of fruit when immersed in AA solution for 24 hours before storage 35 days at 4°C may keep the balance between the formation of AOS and their destruction (Hodges, 2003).

Also it is clear from Photograph 1 that the fruits immersed in AA solution for 24 hours presented less damage than fruit immersed for 0 hours at day 30 of storage time. However, fruit stored at 10°C presented no injury except for the change in chlorophyll content.

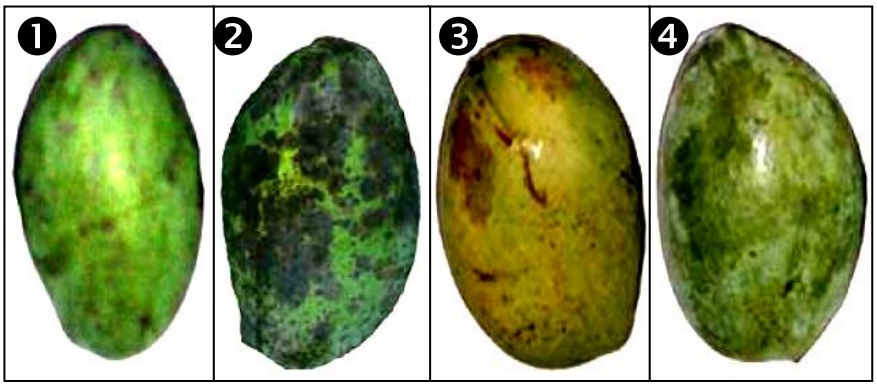
Ion leakage (IL %)

Table 2 shows the ion leakage percentage plotted as function of storage time (days) at two storage temperature. It is appeared that un-immersed fruit has more IL% compared with fruit immersed in AA solution (5.6 mM) and water. In very broad terms, it is clear that IL% increases with storage period, but when considered in more detail there are some interesting differences between storage temperatures and immersing time in AA solution. After 20 days of storage time the condition of fruits became so injured Table 1.

Table 2: Effect of pre-storing fruit treatments of immersing in water or in AA solution (5.6 mM) for different immersing time (0, 6. 12, 18, and 24 hr) on ion leakage percentage in Zibda mango fruits stored at 4 and 10oC for 35 days.

Storage temperature	Time immersing hours	Storage time (days)							
		0	5	10	15	20	25	30	35
Immersing in Ascorbic acid 5.6 mM									
4°C	0	22.29	24.72	27.66	57.87	68.91	93.19	94.63	98.76
	6	22.29	25.68	34.61	37.08	40.43	48.99	88.09	96.01
	12	22.29	23.38	30.65	33.63	39.08	45.21	83.74	90.81
	18	22.29	23.08	29.09	30.43	36.02	38.16	61.01	78.91
	24	22.29	22.57	26.76	26.66	33.00	35.01	52.24	62.93
10°C	0	22.29	24.12	33.37	56.37	64.99	67.23	69.36	79.91
	6	22.29	24.14	39.26	44.36	46.95	57.22	62.54	65.37
	12	22.29	26.69	46.11	53.05	60.81	65.44	66.60	68.69
	18	22.29	33.99	42.56	54.43	60.26	71.69	76.70	81.92
	24	22.29	37.29	53.32	61.61	62.81	68.24	76.93	85.77
Immersing in water									
4°C	0	22.29	23.16	34.48	37.08	40.43	47.71	61.01	74.30
	6	22.29	21.59	22.99	26.03	29.47	31.94	53.60	65.42
	12	22.29	23.40	27.67	29.31	31.27	35.32	68.84	94.07
	18	22.29	26.29	29.22	31.74	33.67	40.27	78.87	98.93
	24	22.29	28.23	30.67	34.49	39.80	59.26	85.29	99.83
10°C	0	22.29	24.12	33.37	56.37	64.99	67.23	69.36	79.91
	6	22.29	33.71	46.56	60.02	65.79	66.72	68.37	80.60
	12	22.29	47.56	50.28	59.43	67.65	73.31	78.29	86.02
	18	22.29	57.38	61.85	66.74	72.66	78.99	86.46	89.00
	24	22.29	66.00	60.86	68.88	71.84	75.99	82.10	94.13
	LSD at 5%		4.997						

Generally, IL% has less percentage with increasing immersing period in AA solution. It may be that fruits immersing in AA (5.6 mM) for 24 hour quite enough than other immuring period to stabilize cell membrane under cold stress (Leshem, 1992) by quenching AOS formation (Hodges, *et al.*, 2004).Then fruits stored at 4ºC IL% have an overall liner increase with time. Immersed fruits in AA for 24 hours have less IL% compared with the immersing periods. However, immersing fruit in water treatment presented more IL% compared with AA and non-treated fruits.



Photograph 1: Photograph presents a comparison between fruit at day 30 of storage time immersed in AA solution at 5.6 mM for 24 hours before storage 35 days at two storage temperatures (4 and 10oC). Fruit stored at 4oC (1), fruit stored at 4oC without AA treatment (2), fruit stored at 10oC (3), fruit stored at 10oC without AA treatment (4),

Lipid pre-oxidation (MDA) and Protein carbonyl group (PCG)

Table 3 shows the changes of lipid peroxidation expressed as the concentration of malondialdehyde equivalent (MDA) and protein carbonyl group (PCG) in fully mature Zibda mango fruit. In fact, the MDA and PCG show a significant interaction at $P \leq 0.001$ when storage time, temperatures and immersing periods were considered. Considering first changes in fruit stored at low temperature at 4ºC, the rate of MDA and PCG accumulation increased at 4 than 10ºC as to untreated fruit at both storage temperatures

The accumulation of MDA and PCG increased in fruits immersing in water for 24 hours and ambient air than fruit immersed in AA solution for 24 hours. It could be explained that the fruits immersed in AA for 24 hours quenched directly AOS during low storage temperature stress. Consequently, the balances between AOS generation and scavenging those, in other word it control cell death (Linster and Clarke, 2008) . So, AA maintained the cell plasma membranes structure (lipid and protein). Therefore, less oxidative reactions accumulations (Purvis, 2004). However, fruits immersed in water presented more accumulations of MDA and PCG during storage. It might be a kind of dialysis of AA into water during immersing period (24 hours). So, oxidative reaction occurred rapidly according to less content of AA with fruits.

Table 3: Effect of pre-storing fruit treatments of immersing in water or in AA solution (5.6 mM) for different immersing time (0, 6. 12, 18, and 24 hours) on total chlorophyll content (mg 100 g^{-1} FW) in Zibda mango fruits stored at 4 and 10ºC for 35 days

Storage Temperature	Time immersing Hours	Total chlorophyll *AB* (mg 100 g^{-1} FW) content during Storage time (days)							
		0	5	10	15	20	25	30	35
4ºC	0	2.72	2.97	2.36	2.56	2.34	2.10	1.99	1.65
	6	2.74	3.26	2.95	2.66	2.52	2.45	2.51	2.18
	12	2.95	3.35	2.97	2.71	2.61	2.45	2.51	2.21
	18	3.09	3.36	2.99	2.73	2.68	2.47	2.52	2.23
	24	3.14	3.42	3.08	2.80	2.71	2.67	2.56	2.45
10ºC	0	2.72	2.60	2.31	2.03	1.86	1.67	1.61	1.27
	6	2.74	2.84	2.36	2.09	2.05	1.78	1.67	1.35
	12	2.95	2.84	2.40	2.27	2.20	1.88	1.81	1.38
	18	3.09	2.85	2.50	2.35	2.21	1.94	1.82	1.46
	24	3.14	2.97	2.56	2.56	2.34	2.10	1.99	1.65
L.S.D. at 5 %	0.145								

Total chlorophyll *AB* content and *A:B* ratio

Table 4 depicts the variation of total chlorophyll (Chl_{ab}) as a function of storage time at two storage temperature. The interaction ($P<0.005$) was significant among days, treatments and time of immersing. Chl_{ab} content was decreased with storage duration. However, the final Chl_{ab} content was largely the same among time of immersing, with the exception of the 10ºC fruits. The time of immersing on the Chl_{ab} content was significant. Even, the fruits were stored at ambient air lost chlorophyll rapidly than other time of immersing. However, fruits immersed in AA for 24 hours present more stable chlorophyll content than other times of immersing in both storage temperatures.

Table 4: Effect of pre-storing fruit treatments of immersing in water or in AA solution (5.6 mM) for different immersing time (0, 6. 12, 18, and 24 hours) on chlorophyll A:B ratio (mg 100 g^{-1} FW) in Zibda mango fruits stored at 4 and 10ºC for 35 days

Storage Temperature	Time immersing Hours	Total chlorophyll *A:B* ratio (mg 100 g^{-1} FW) during Storage time (days)							
		0	5	10	15	20	25	30	35
4ºC	0	0.72	0.74	0.66	0.58	0.73	0.64	0.64	0.68
	6	0.84	0.74	0.68	0.72	0.74	0.68	0.65	0.69
	12	0.87	0.79	0.76	0.74	0.77	0.68	0.69	0.75
	18	0.93	0.80	0.78	0.74	0.81	0.69	0.71	0.78
	24	0.98	0.84	0.90	0.75	0.81	0.71	0.72	0.79
10ºC	0	0.72	0.70	0.67	0.73	0.65	0.69	0.72	0.77
	6	0.84	0.73	0.70	0.74	0.69	0.74	0.72	0.79
	12	0.87	0.74	0.73	0.78	0.7	0.77	0.73	0.85
	18	0.93	0.77	0.78	0.79	0.71	0.82	0.78	0.97
	24	0.98	0.80	0.90	0.79	0.73	0.78	0.85	1.01
L.S.D. at 5 %	0.111								

Table 5 shows the chlorophyll a/b ratio ($Chl_{a/b}$) in function of storage time for both storage temperatures. The initial values are the same of both storage temperatures at all immersing periods.

However, during storage there are interesting differences between times of immersing which are independent of storage temperatures. The $Chl_{a/b}$ ratio was nearly unchanged during storage with immersing in AA for 24 hours in both storage temperatures. On the other hand, showed increases in the $Chl_{a/b}$ ratio after 20 days of both storage temperatures.

It seems reasonable to propose chlorophyll content measurement to assess chilling damage development during mango storage. Also, CI-index, IL% and change of chlorophyll content are a homogeneous group of measurements for chilling injury (Lo'ay, 2005). Also the $Chl_{a/b}$ ratio responds differently to chilling temperature at different time immersing in AA and the increasing $Chl_{a/b}$ ratio suggests a preferential breakdown of Chl_b containing chlorophyll binding proteins such as the LHC of photosystem I or II (Rosenqvist and van Kooten, 2003).

Conclusion

Normally, mango fruits are sensitive to low storage temperature below 10^{o}C (Acosta *et al.*, 2000). It generates the oxidative stress reactions by AOS which increased under prolong storage time. The processes, lipid peroxidation and protein oxidation of cell membrane structure are target to AOS under low temperature stress when antioxidants became low. In other word, unbalance between generation of AOS and antioxidant scavengers (Foyer and Noctor, 2003). Cell death becomes more noticed by losing cell membrane function resulting in more ion leakage and cell death. Finally, chilling injury symptoms become visible at short time of storage. So, to explore the biological difference between fruit maturities and per fruit (EXP I and II) as to different AA content between (Peel, outer and inner pulp) to use AA application at 5.6 mM for increasing chilling tolerance as well as fruits with longer storability. the problem with link between the loss of AA and membrane oxidation is response at 4^{o}C which show that AA level can decreased with an increased in MDA equivalent and PCG. It is possible, however, that low storage temperature is protecting cell membrane fatty acid and protein from oxidation by immersing fruit in AA for 24 hour to prevent the loss of AA during storage.

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زيادة القدرة التخزينية لثمار المانجو

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أجريت هذه الدراسة خلال اعوام ٢٠٠٦- ٢٠٠٧ على ثمار المانجو صنف الذبدة بمزرعة خاصة بحافظة الشرقية بهدف زيادة القدرة التخزينية للثمار تحت دراجات حرارة منخفضة لفترات طويلة من خلال معاملة الثمار بمحلول الاسكوربيك قبل البدء فى التخزين البارد على درجة حرارة ٤ درجة مئوى بغرض إطالة الفترة التخزين للثمار و تقليل اضرار البرودة. و قد تم البحث على العديد من التجارب

التجربة الاولى :

قد أظهرت النتائج وجود اختلافات بيولوجية بين المراحل المختلفة من إكتمال نمو الثمار حيث كان محتوى الثمار من حمض الاسكوربيكك على فى الثمار الغير مكتملة النمو بحوالى ٥٠% مقارنة بالثمار النصف مكتملة و المكتملة النمو، كما وجدت أختلافات فى العينات المأخذة من اجزاء الثمار حيث تحتوى منطقة الطرف الزهرى من الثمرة على محتوى عالى من حمض الاسكوربيك مقارنة بمنطقة الطرف الساقى للثمرة فى كافة الاعمار الثمار، و ان محتوى القشرة من حمض الاسكوربيك اعلى من كل طبقة اللحم الخارجى و الداخلى للثمار فى كل من المنطقتين الطرف الساقى و الزهرى.

التجربة الثانية:

كما وجد ان معاملة الثمار المكتملة النمو بحمض الاسكوربيك بتركيز ٥,٦ مللى مول لمدة ٢٤ ساعة و التخزين على درجة حرارة ٤ درجة مئوى قد اجل ظهور اعراض البرودة حتى اليوم ٢٥ من فترة التخزين وبذلك تكون المعاملة الثمار بحمض الاسكوربيك أدت الى زيادة من قدرة الثمار على تحمل التخزين على درجات الحرارة المنخفضة

التجربة الثالثة:

حيث ان الثمار المكتملة النمو التى عوملت بحمض الاسكوربيك بنركيز ٥,٦ مللى مول و و النقع على فترات مختلفة (٠ و ٦ و ١٢و ١٨و ٢٤ ساعة) بهدف معرفة افضل فترة نقع لتقليل وقت المستهلك فى المعاملة والتخزين على درجة حرارة ٤ درجة مئوى لمدة ٣٥ حيث أظهرت النتائج ان نقع الثمار فى حمض الاسكوربك لمدة ٢٤ ساعة افضل من فترات نقع الاخرى فى تقليل اعراض اضرار البرودة على الثمار و التى تقل معها نواتج الاكسدة الحيوية لكل من البروتينات و الدهون و بالتبعية انخفاض كبير فى نفاذية الخلايا مما يعد ان معاملة الثمار بحمض الاسكوربيك فعالة فى تقليل اعراض البرودة على الثمار المكتملة النمو مما يتيح الفرصة الى تصدير ثمار المانجو الى اسواق بعيدة و قد يمكن استغلال معاملة ثمار الذبدة بحامض الاسكوربيك على درجة حرارة ٤ مئوى لاحقا لزيادة قدرة الثمار على تحمل التخزين البارد لفترة طويلة.

قام بتحكيم البحث

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Table 5: Effect of pre-storing fruit treatments of immersing in water or in AA solution (5.6 mM) for different immersing time (0, 6. 12, 18, and 24 hr) on Lipid pre-oxidation (MDA) and Protein carbonyl group (PCG) content in Zibda mango fruits stored at 4 and 10°C for 35 days

Storage Temperature	Time Immersing hours	Storage time (days)															
		Lipid peroxidation (MDA)								Protein carbonyl group (PCG)							
		0	5	10	15	20	25	30	35	0	5	10	15	20	25	30	35
Immersing in Ascorbic acid 5.6 mM																	
4°C	0	0.31	0.50	0.64	0.66	0.74	0.84	1.28	1.38	22.29	24.72	27.66	57.87	68.91	93.19	94.63	98.76
	6	0.31	0.48	0.57	0.64	0.73	0.81	1.16	1.31	22.29	25.68	34.61	37.08	40.43	48.99	88.09	96.01
	12	0.31	0.47	0.52	0.61	0.71	0.78	1.02	1.26	22.29	23.38	30.65	33.63	39.08	45.21	83.74	90.81
	18	0.31	0.44	0.50	0.59	0.69	0.72	0.77	0.79	22.29	23.08	29.09	30.43	36.02	38.16	61.01	78.91
	24	0.31	0.34	0.48	0.56	0.63	0.67	0.70	0.72	22.29	22.57	26.76	26.66	33.00	35.01	52.24	62.93
10°C	0	0.31	0.41	0.47	0.56	0.63	0.92	1.13	1.36	22.29	23.16	34.48	37.08	40.43	47.71	61.01	74.30
	6	0.31	0.32	0.44	0.47	0.55	0.74	0.83	1.03	22.29	21.59	22.99	26.03	29.47	31.94	53.60	65.42
	12	0.31	0.34	0.46	0.53	0.60	0.79	0.90	1.25	22.29	23.40	27.67	29.31	31.27	35.32	68.84	94.07
	18	0.31	0.39	0.47	0.56	0.63	0.84	0.99	1.35	22.29	26.29	29.22	31.74	33.67	40.27	78.87	98.93
	24	0.31	0.46	0.47	0.60	0.73	0.93	1.15	1.41	22.29	28.23	30.67	34.49	39.80	59.26	85.29	99.83
Immersing in water																	
4°C	0	0.31	0.50	0.64	0.66	0.74	0.84	1.28	1.38	22.29	24.12	33.37	56.37	64.99	67.23	69.36	79.91
	6	0.31	0.39	0.44	0.56	0.69	0.82	0.88	1.00	22.29	24.14	39.26	44.36	46.95	57.22	62.54	65.37
	12	0.31	0.42	0.48	0.66	0.75	0.87	1.02	1.23	22.29	26.69	46.11	53.05	60.81	65.44	66.60	68.69
	18	0.31	0.63	0.69	0.76	0.80	0.97	1.18	1.38	22.29	33.99	42.56	54.43	60.26	71.69	76.70	81.92
	24	0.31	0.78	1.05	1.16	1.33	1.40	1.38	1.57	22.29	37.29	53.32	61.61	62.81	68.24	76.93	85.77
10°C	0	0.31	0.41	0.47	0.56	0.63	0.92	1.13	1.36	22.29	24.12	33.37	56.37	64.99	67.23	69.36	79.91
	6	0.31	0.36	0.43	0.44	0.56	0.68	0.78	0.83	22.29	33.71	46.56	60.02	65.79	66.72	68.37	80.60
	12	0.31	0.42	0.48	0.53	0.70	0.75	0.89	1.01	22.29	47.56	50.28	59.43	67.65	73.31	78.29	86.02
	18	0.31	0.52	0.58	0.69	0.80	0.89	1.07	1.37	22.29	57.38	61.85	66.74	72.66	78.99	86.46	89.00
	24	0.31	0.65	0.75	0.80	0.90	0.98	1.33	1.60	22.29	66.00	60.86	68.88	71.84	75.99	82.10	94.13
L.S.D. at 5%		0.071								4.997							