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Evaluation of Fruit Positions on Tree, Chilling Injury Sensitivity, and Antioxidant Enzyme Activities of (*Mangifera indica* L. 'Ewas') Mangos during Cold Storage

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ABSTRACT

Mango fruits have a high commercial income to being viewed as susceptible to chilling injury for the long storage duration. Fruits are stored at low temperatures many physiological changes that will demonstrate the side impacts of cold storage on fruits. The results are losing fruits and lower profits. The study aimed to find correlation between three fruit positions on the tree, i.e. sunny side (SUN; fruit exposed to the sun most of the day), shadow side (SHA; fruit grows on the shady side of trees), and inside the canopy (INS; fruit grows inside tree canopy), and cold storage stress (4 ± 1 °C and RH% 96%) for 35-days during two growing seasons (2015-2016). Obviously: the harvested fruit from INS presented more tolerance to chilled temperature compared to other fruit locations for 35 days. Through the results obtained the harvested fruit from INS showed significantly the highest activities of ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD) than other fruit locations. Reflecting the lack of chilling injury symptoms on fruits, unlike other fruit positions. In addition, contents of $O_2^{\bullet-}$ and H_2O_2 decreased in INS fruit throughout storage duration. Consequently, the cell membrane compositions were maintained, especially, low quantity in both malondialdehyde (MDA) and protein carbonyl group (PCG) throughout storage duration in days. These results pointed out that the fruit positions can be considered at harvesting for classifying of fruits before cold storage.

Keywords: Mango; Cold storage; Chilling injury; Antioxidant enzymes

INTRODUCTION

Mango is cultivated in numerous regions of the world, for example, Latin America (Brazil - Chile - Venezuela), Africa (Egypt and South Africa) and Asia (Philippines - Malaysia - India) (Yasunaga *et al.*, 2018). Also, it is the favorite fruit for the consumer and of great economic incomes, which is exported to numerous regions of the world. Internationally, mango marketing has recently increased for two main reasons, one is the increase in the number of cultivated varieties worldwide especially the belt of tropical and sub-tropical. Additionally, another reason is the interest in farm management programs during the production phases (Sivakumar *et al.*, 2011). Historically, the mango was planted initially in Egypt during the reign of Muhammad Ali Pasha since 1825. Currently, according to statistics by FAO, the harvested area constituted about 121,304 ha, which produces 1,307,869 tons (FAO, 2018).

However, environmental circumstances i.e. heat, and light were discussed their influences on the quality of fruits or vegetables at harvest, the study of these aspects of their behavior throughout storage or shelf-life are not clear in these respects (Moretti *et al.*, 2010). One of the negative effects of high temperatures is to increase the rate of air temperature surrounded the fruits by which increase water vapor during the fruit development stages, which reduces the amount of crop or stimulates the fruit ripening process (Henson, 2008). Prolonged exposure to temperatures in the field induces too many natural and physiological changes of fruit tissue prior to harvest (Kays, 1997). It also influenced the hardness of the fruits in the effect on the activity of cell wall decomposition enzymes for example cellulase and polygalacturonase activities (Woolf *et al.*, 1999). These variations occur when the temperature rises more than 40 degrees Celsius with the development of symptoms such as sunburn, water core, and more rapid softening on pear fruit (Ferguson *et al.*, 1994). One of the elements of light is the ultraviolet rays (UV), increasing the amount during fruit growth stages lead to the occurrence of biochemical modifications of fruit tissues such as a reduction in fruit firmness, increasing the characteristic color of the variety, and decreasing in levels of vitamin C and E. Additionally, increasing UV levels reduce the biosynthesis of pigments i.e. carotene and lycopene (Mditshwa *et al.*, 2017). At high concentrations, UV also destroys DNA, protein (Prinsze *et al.*, 1990), lipids (Kramer *et al.*, 1991), and plasma cell membranes (Hollósy, 2002). Also, UV affects the capacity of photosynthetic pigment i.e. chlorophyll (Marwood and Greenberg, 1996), and Carotenoids (Pfündel *et al.*, 1992).

Mangoes are also categorized as tropical fruits, which mature or deteriorated very quickly after harvesting or during frequent trading (Barman and Asery, 2014). Therefore, the usage of the low temperature during storage mango reduces fruit losses and biological processes in fruits especially after harvesting. However, cold storage for a long time drives to gain of chilling injury incidence,

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which reduces the value of fruit and quality during handling or trading (Nair and Singh, 2003). When mango stored blow temperature 13ºC (Barman *et al.*, 2014). Consequently, low-temperature forces metabolic changes by which causes changes in cell membranes structure under storage (Shewfelt and Del Rosario, 2000), Also minimizing in ethylene gas production and respiratory rates (Nair and Singh, 2009). Chilling injury symptoms (CI) appeared as a small patch on the surface of the fruit due to die the cells around the lentils. Afterward, it develops to combine to form large black spot areas of fruit skin (Mittler, 2002). Numerous investigations confirmed that CI generates the reactive oxygen species (ROS), which react indiscriminately with different molecules of the cell causing what is known as oxidative stress (Junmatonga *et al.*, 2015). Some studies focused on alleviating CI by different treatments using organic acids such as oxalic acid at 20 mM (Peiyan *et al.*, 2015), or applying methyl jasmonate and methyl salicylate (Wang, 2006). Using low-temperature conditioning (Rodeo and Esguerra, 2013).

The mango industry faces a problem when harvesting as producers often harvest mangoes once and at different ages. In addition to the weather conditions to previously listed, the developing fruits on trees are exposed to heat and ultra-violet radiation stress (UV) to varying degrees during day hours, which will decrease the activity of antioxidants throughout cold storage. Therefore, our opinion is the fruit locations influence its chill sensitivity during cold storage.

MATERIALS AND METHODS

1- Fruit materials and experimental setup

Fruits of mangoes cv. Ewas were harvested in August 2015 and 2016 from trees 10-years old which planted at 8 × 10 meters in the sandy soil of commercial orchard. The biological differences experiments were performed on mango trees that cultivated in Sharkia province East Egypt (30.5765° N, 31.5041° E). The weather measurements were obtained in terms of average temperatures, the number of light hours and the intensity of radiation on the region according to the Egyptian Meteorological Authority (Figures 1, 2, and 3).

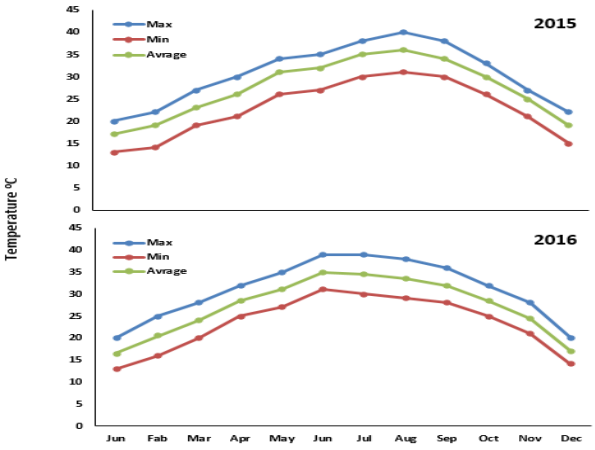

Figure 1. draws the maximum, minimum, and the average temperature during two seasons 2015 and 2016

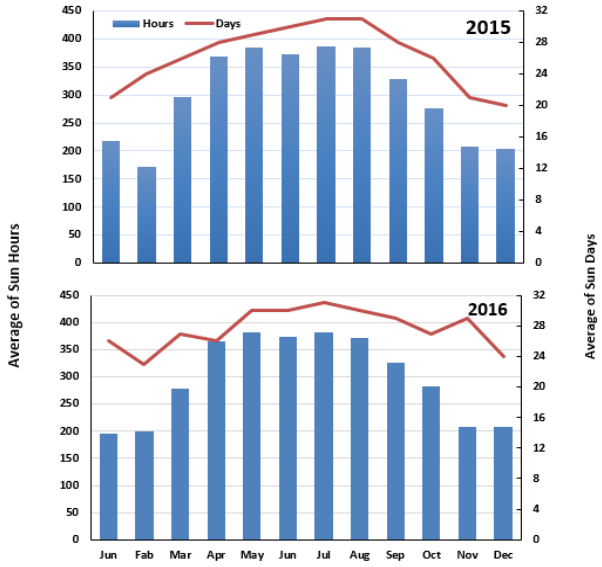

Figure 2. illustrates the average of sun hours and days during two seasons 2015 and 2016

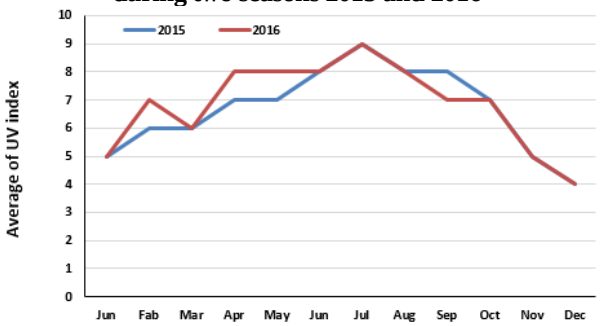

Figure 3. presents the average of UV index during two seasons 2015 and 2016

The experiment aims to study the effect of the fruit locations on trees and its relation with chilling injury symptoms phenomenon under cold storage stress. It was carried out on 360 fruits were picked and selected from three various locations, which are Sunny (SUN), Shadow (SHA), and Inside (INS) of the tree on mango trees at half mature (the shoulder is at the same level as the stem end) fruit stage (Majeed and Jeffery, 2002). Fruits were split into two main batches only each contains 180 fruits. One batch for physical measurements and another for chemical assays. Both batches were distributed on three replicates. Fruits were stored at 4 ± 1ºC and RH 96% ± 2 for 35 days. Whereas, it is known that the fruits were exposed during the summer months to high temperatures (36 – 38 ± 1ºC) and very high UV radiation (UV index 8 on average).

2- Chilling injury index

A visible evaluation of outside injuries on fruit peel, such as pitting, water-soaked areas, and decay is often used to assess the CI (Chaplin *et al.*, 1991). The scores of visual symptoms rating were classified into five levels: 1= no injuries, 2= light, 3= moderate, 4= severe, and 5= very severe injury. The CI-incidence was computed by the equation:

$$Chilling\ injury\ Index = \sum_{1=1}^{5} \frac{(Chilling\ injury\ level) * (Number\ of\ fruits\ at\ this\ level)}{Total\ number\ of\ fruits}$$

3- Antioxidant enzyme activities assays

Fruit pulp samples (10 g) was picked and homogenized with 25 mL of ice-cold extraction buffer and 0.5 g poly-vinyl-polypyrrolidone (PVPP) with a Kinematica tissue processor (Crl-6010, Kriens-LU, Switzerland). For Catalase (CAT) and Superoxide dismutase(SOD)examines, the extraction buffer was 50 mmol L^{-1} sodium phosphate (pH 7.8). Then the supernatants were utilized presently for testing both CAT and SOD enzymes (Wang *et al.*, 2005).

For CAT measurement, the response mixture composed of 2.8 mL H_2O_2 (40 mM, in 50 mmol L^{-1} sodium phosphate buffer, pH 7.0) and 0.2 mL enzyme extract. The decay of H_2O_2 (substrate) was estimated by the decrease in absorbance at 240 nm on spectrophotometer during the 120s. The activity was reported as Unit g^{-1} FW, where one unit of catalase equal the changes over one Mol of H_2O_2 per mass of fruit per min at 30°C

For SOD activity assay, the response for the composite performed about 3 ml volume includes 65 mM sodium phosphate cradle (pH 7.8). The methionine 13 mmol L^{-1}, nitro-blue tetrazolium 75 µmol L^{-1} (NBT), plus 10 µmol L^{-1} of EDTA, with 2 µmol L^{-1} riboflavin including to 0.1 ml of the enzyme extraction were finally combined. The combinations were lighted up by light (60 µmol m^{-2} s^{-1}) for 10 min and the absorbance was then determined at 560 nm on the spectrophotometer. The reaction solution takes some moments to be cleared. The reaction was expressed as unit g^{-1} FW, where one unit was characterized as the measure of chemical that caused the 50% reductions of the SOD inhabitable NBT diminishment per mass every hour.

Ascorbate peroxidase (APX) reaction was conducted on fruit pulp samples (Zhang *et al.*, 2013). It identified as one unit of APX activity as the measure of catalyst that it oxidized 1 µmol ascorbate per min at 30°C. The change in enzyme activity was set as Unit g^{-1} FW.

Peroxidase (POD) activity was measured at 30°C and concluded by monitoring the increase rate of absorbance at 398 and 460 nm. POD activity was represented as unit g^{-1}FW, where one unit was displayed as an increased rate of the absorbency per mass of fruit samples per min (Tian *et al.*, 2005).

4- Lipid peroxidation, protein oxidation, cell membrane leakage, and ascorbic acid content

Malondialdehyde (MDA) is a termination of lipid peroxidation. Exactly weighed 2.5 g of mango fruit for MDA extraction (Iturbe-Ormaetxe *et al.*, 1998). The calibration curves were performed by using and measuring, 1,3,3-tetraethyoxypropane (Sigma) in the range 0-2 mM (TBARS), which was equivalent to 0-1 mM MDA. Tetraethyoxypropane is stoichiometrically converted into MDA during the acid-heating step of the assay.

Protein carbonyl group (PCG) assay as a terminal of protein oxidation was estimated (Levine *et al.*, 1994). Precisely 2.5 g of mango samples weighed. Spectrophotometrically, the spectrum was measured against the complementary blank in case of cured (without sample) samples or against water in the case of purified proteins. The PCG of the protein was calculated from the absorbance of the dinitrophenylhydrazone estimated at 390 nm which was assumed an extinction coefficient of 22000 M^{-1} cm^{-1}.

Analyses of cell membrane leakage percentage were performed on five disk samples (7 mm diameter) from fruit tissues which were cut utilizing a stopper borer from five distinct parts of each fruit each 5-days intervals. The disks were washed three times with demineralized water and put in 10 mL of 0.4 M mannitol alcohol in demineralized water at 24ºC for 3h (Hakim *et al.*, 1999). The electrical conductivity of the sample was estimated utilizing a conductivity meter, after which the tissue tests were heated in a water bath at 100ºC for 30 min. Thereafter, both fruit pulp samples were placed at room temperature to cool until measuring afterword. Then the conductivity was measured again to calculate the ion leakage percentage in fruit pulp. **The permeability % was estimated by the formula following:**

$$Ion\ leakage\ \% = \frac{Conductivity_{\ after\ 3\ hours}}{Conductivity_{\ after\ boiling}}$$

The assessment of the ascorbic acid (AA) content of mango fruits was harvested from different locations on the tree. The AA measured utilizing the titrimetric method by 2,6-dichlorophenol indophenol reagent pigment and 6% oxalic acid as a substrate (A.O.A.C., 1995).

5- $O_2^{\bullet-}$, H_2O_2 production rates, and DPPH reduction

Fruit pulp samples (1.0 g) were compounded with 3 mL of chilly 50 mmol L^{-1} K_3PO_4 buffer (pH 7.8) including 1% (w/v) poly-vinyl-pyrrolidone (PVP), afterward centrifuged at 8,000 rpm at 4°C for 20 min. The $O_2^{\bullet-}$ generation rate was dictated by watching the nitrite generation from hydroxylamine in the presence of $O_2^{\bullet-}$ (Yang *et al.*, 2011). A standard bend including NO_2 was utilized to calculate the $O_2^{\bullet-}$ creation rate from the response condition of $O_2^{\bullet-}$ with hydroxylamine. The $O_2^{\bullet-}$ formulation rate was represented as a nmol min^{-1} g^{-1} FW.

The assay of H_2O_2 was investigated (Xu *et al.*, 2012), fruit pulp samples (1.0 g) were added to 6 mL of 100% C_3H_6O, then it was centrifuged at 8000 rpm for 20 min at 4°C. The distinct supernatant (1 ml) was joined with 0.1 mL of 5% Ti $(SO_4)_2$ in addition to 0.2 ml of a concentrated NH_4OH arrangement. The titanium-peroxide complex reacted with the residue, which was added up into 4 ml of 2M H_2SO_4 after centrifugation at 8000 rpm for 10 min. The range was estimated at 415 nm. The H_2O_2 content was processed from a standard bend arranged comparatively and showed as mmol g^{-1} FW.

The DPPH reduction % was measured of the antioxidant activity of mango and the dismutation of radical activity, the method was performed. The outcomes of DPPH radicals were exhibited as a percentage. Consequently, samples were assessed on a spectrophotometer at wavelength absorbance (517nm). The quenching of the DPPH radical was calculated utilizing the formula according to the method (Yen and Chen, 1995).

6- Statistical analysis

Data of means from the experiments for evaluating the influence of fruit locations on the antioxidant activities

and chilling injury symptoms occurrence of 'Ewas' mango fruits under cold storage stress. The statistical analysis of factorial design was applied. Means of all studied treatments were compared using Duncan's Multiple Range Test at P ≤ 0.05 using the Co-Stat software package, Version 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93940, USA).

RESULTS AND DISCUSSION

Chilling injury symptoms incidences

The chilling injury symptoms index, as a function of the cold storage period in days for all the fruit locations, is shown in Figure 4. CI-index shows a significant impact at P≤0.05 when fruit locations on trees were considered. Considering, the fruits were harvested from inside trees (INS), is presented more resistant to damage of low-temperature stress compared to other fruits locations. At the storage temperature of 4ºC, there were no CI indications before the 10th day of storage in all fruit locations. The first sign of CI that observed on the 10th day with fruits on SUN (1.91 CI-index, almost slight injured), on the 15th day with fruit on SHA (1.15 CI-index, low injured), and on the 20th day with fruit on INS (1.03 CI-index, trace injured). Though an increase in storage period up to the 20th day, the degree of damage that developed between the 10th and the 20th day of storage was slight symptoms. While CI was the more rapid differential between the 20th up to the 35th day of cold storage. The degree of CI was, in general, less severe with fruit were harvested from INS trees on the 35th day (1.66, CI-index below slight injuries), compared to SHA (2.25, CI-index slight injuries), and SUN (4.60 CI-index almost very severe injury). Accordingly, the change in visual appearance results of CI-symptoms on "Ewas' mango fruit according to the effect of three different biological factors. It seems that the change of CI could be related to exposure to solar heat and rays stress during fruit development. Especially, fruits located on the SUN side displays more CI-symptoms compared to fruits from other locations SHA and INS of tree side less stress. Consequently, sunny fruits were more sensitive to chilling temperature due to enhancing the physiological changes in cell membrane compartments under cold stress, which is not reversible (Leshem, 1992).

Furthermore, the increases in the degree of CI are independently due to the fruit maturity stages. This would appear to be an important biological factor when cold storage is required for mango fruit (Lo'ay, 2005). Also, the difference of CI-symptoms among fruit locations could be explained that fruit from INS has more antioxidant activities than ROS generation compared to SHA and SUN during cold storage (Foyer *et al.*, 2017). These increases provide fruit more resistance to chilling damage throughout cold storage duration (Nair and Singh, 2003). Fruits are exposed to high temperatures during the growing season, which leads to the activation of antioxidants during storage (Galletta and Bringhurst, 1990). However, prolonged exposure may lead to the reduction of vitamin C levels and thus reduce the action of antioxidant enzymes during cold storage (McKeon *et al.*, 2006). However, the CI of fruits harvested from different locations could have different content of antioxidants and also ROS production (Usha *et al.*, 2015).

Therefore, our results concerning the concentrations of ascorbic acid (Figure 6) and antioxidant enzyme activities (Figure 5) confirm that the fruits inside the trees are presented at the highest level compared to other fruit locations during storage duration.

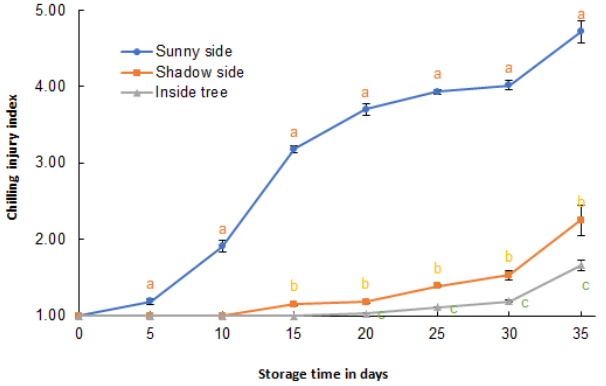

Figure 4. presents a chilling injury index (CI-index) of fruits harvested from different locations (the sunny side of trees, shadow, and inside of trees) on 'Ewas' mango trees during cold storage at 4ºC. The vertical bars presented to ± SE (n=3) the alphabet letters referred to the significant of treatments according to Duncan's Multiple Range Test at 5%.

Antioxidant enzyme activities (AEAs)

Figure 5 illustrates the variation of the antioxidant enzyme activities (unit g^{-1} FW) as a function of storage time in days for the biological differences of 'Ewas' mango under low temperature. Clearly, the antioxidant enzymes exhibit a significant interaction at P≤ 0.05 when the storage time and fruit locations were considered. Initially, there are different values in antioxidant enzyme activities at harvest time overall enzymes. Also, APX enzyme increase in activity to reach the maximum on the 20th day. However, the POD,CAT and SOD enzymes reach the peak up to the 15th day of cold storage. Obviously, fruits on the INS side of trees exhibit almost more stability in antioxidant activities with all enzymes compared to fruit from SHA and SUN side during cold storage. However, fruits were picked from the SUN side, which showed highly decline in antioxidant enzyme activities after the 20th day until to end of cold storage period. These observed differences are due to the oxidative stress reaction. It is occurred according to a consequence of the relationship between the formation and scavenging of ROS as known by hemostasis (Foyer *et al.*, 2017). Through the results of the experiments, it is clear, that fruit locations have a significant impact on the activity of antioxidants during the cold storage period. Thus, it can be argued that increasing the activity of antioxidants until the 15th day imposes the protection of fruits against free radicals generated by low-temperature storage stress. This is could be reflected in the protection of cellular walls from the oxidation reactions and hence the absence of symptoms injuries on fruits. It is clear especially with fruit located on INS trees. Also, it suggested that the INS fruits have more antioxidant activities for the quenching of ROS during long-term cold storage. Besides that, It presents less CI-

symptoms compared to other fruit locations (Usha *et al*., 2015). So, more CI-symptoms appeared on fruits that harvested from SUN locations on trees due to the decreases in antioxidant activities.

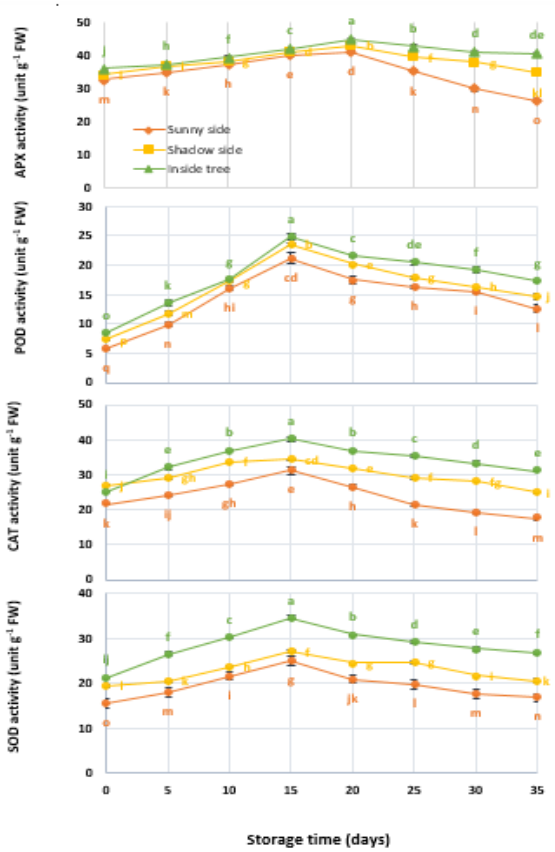

Figure 5. presents antioxidant enzymes activities of fruits harvested from different locations (the sunny side of trees, shadow, and inside of trees) on 'Ewas' mango trees during cold storage at 4ºC. The vertical bars presented to ± SE (n=3) the alphabet letters referred to the significant of treatments according to Duncan's Multiple Range Test at 5%.

Lipid peroxidation, protein oxidation, ion leakage%, and ascorbic acid content

Figure 6 depicts the variations in lipid peroxidation (MDA), protein oxidation (PCG), cell membrane leakage (IL%), and ascorbic acid content (AA) during 35-days of cold storage. In fact, the oxidize cell membrane compartment presents a significant interaction at P≤0.05 when the storage factors such as time and fruit locations were considered. Initially, the results of MDA, PCG, IL%, and AA were obtained at harvest time are the different overall of the experiments. It is clear that cellar degradation increased gradually up to the end of cold storage period. Regarding, fruit locations present a significant variance between the fruit location on trees. The obvious results were found that the fruit location (INS) shows less accumulation of MDA,PCG, low IL%, and the highest amount of AA. However, fruits from SUN and SHA presented more rapid accumulation in both MDA nd PCG. Also, they had an increase in cell membrane permeability and decreases in AA content until up to the end of the cold storage period. Likewise, fruits that were harvested from the sunny side of trees are more rapid accumulation in MDA, PCG, and IL%. However, it presented more rapid degredation in AA content during 35 days of cold storage.

The change in lipid and protein of cell membranes due to cold storage stress terminated to MDA and PCG accumulation. Consequently, dysfunction in the cell membrane results in increased cell permeability up to the end of the experiment. Chilling injury symptoms appearing because of exposure of fruit to low-temperature has been often associated with oxidative stress processes (Hodges, 2003). Consequently, the oxidative stress reaction occurs as a result of the imbalance between the production of ROS and antioxidant levels (Hodges *et al.*, 2004). So, the cumulative of MDA equivalent and protein carbonyl is due to the impact of ROS or oxidative reaction processes in stressed tissue (Lo'ay, 2005). So that it would be expected that the fruit on the SUN side of the tree might be stressed before harvesting (heat and rays of the sun). Hence, it could be more sensitive to low storage temperature and more CI-symptoms incidence (Usha *et al.*, 2015). This is the knowledge that the growth of mango fruits under the sun prolong exposure before harvesting may lead to increased activity of decomposing enzymes, such as cellulose and polygalacturonase (Woolf *et al.*, 1999), thus increasing fruit softening before storage (Hicks *et al.*, 1983). However, the accumulation of MDA and PCG appears to be largely independent on fruit locations. Fruits harvested from INS presents the lowest CI-symptoms compared to other SHA and SUN locations. It could be due to the fruit located in INS contains more balance between ROS and quenching antioxidants (Foyer and Noctor, 2003; Foyer *et al.*, 2017).

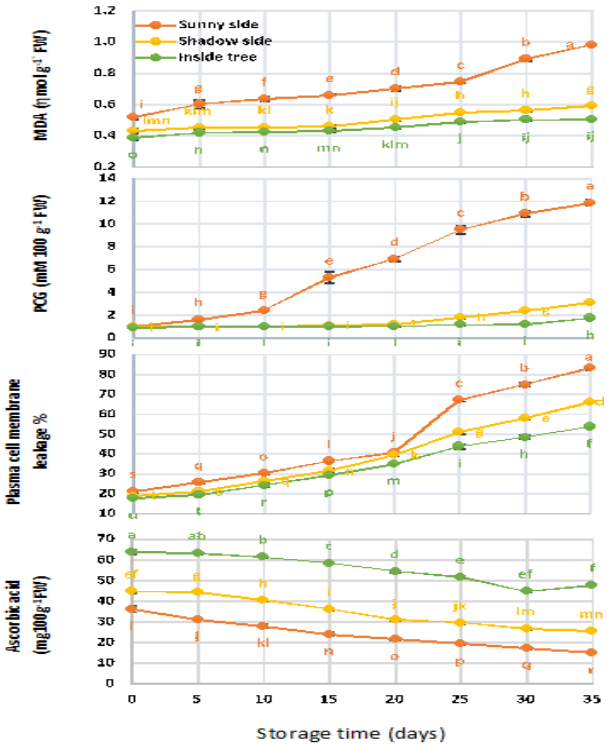

Figure 6. presents lipid peroxidation (malondialdehyde, MDA), protein oxidation (protein carbonyl group, PCG), cell membrane leakage percentage, and ascorbic acid content of fruits harvested from different locations (the sunny side of trees, shadow, and inside of trees) on 'Ewas' mango trees during cold storage at 4ºC. The vertical bars presented to ± SE (n=3) the alphabet letters referred to the significant of treatments according to Duncan's Multiple Range Test at 5%.

$O_2^{\bullet-}$, H_2O_2 production rates and DPPH reduction

Figure 7 presents the variations between $O_2^{\bullet-}$, H_2O_2 production rates and DPPH reduction as a function of the cold storage period in days. The $O_2^{\bullet-}$, H_2O_2 production rates, and DPPH presented a significant interaction at $P \leq 0.001$, when cold storage time (days) and fruit locations on trees were considered. Initially, there are different values of $O2^{\bullet-}$ and H_2O_2 production rates at harvest time between fruit locations. Generally, the $O_2^{\bullet-}$ and H_2O_2 production increased gradually during 15 days of cold storage overall fruit locations.

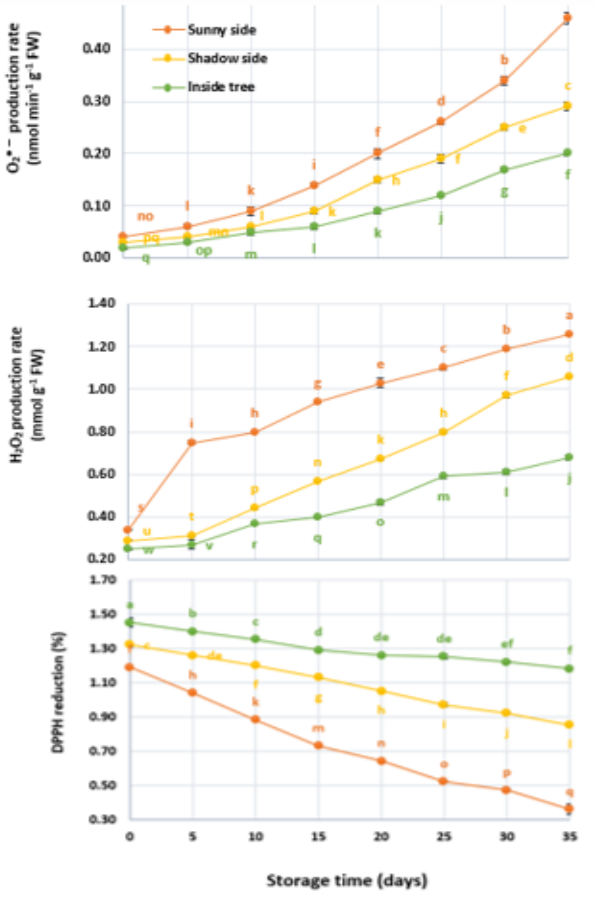

Figure 7. presents $O_2^{\bullet-}$ production rate (nmol min^{-1} g^{-1} FW), H_2O_2 production rate (mmol g^{-1} FW), and DPPH reduction % of fruits harvested from different locations (the sunny side of trees, shadow, and inside of trees) on 'Ewas' mango trees during cold storage at 4ºC. The vertical bars presented to ± SE (n=3) the alphabet letters referred to the significant of treatments according to Duncan's Multiple Range Test at 5%.

Thereafter, both $O_2^{\bullet-}$ and H_2O_2 production increased independently according to fruit locations during 35 days of cold storage. Fruits were harvested from the INS side of trees are the lowest in both $O_2^{\bullet-}$ and H_2O_2 production (0.200 nmol min^{-1} g^{-1} FW and 0.680 mmol g^{-1} FW). However, other locations SUN (0.460 nmol min^{-1} g^{-1} FW and 1.260 mmol g^{-1} FW) and SHA (0.290 nmol min^{-1} g^{-1} FW and 1.060 mmol g^{-1} FW) exhibit much more production at the 35th day of cold storage.

The highest amount of $O_2^{\bullet-}$ and H_2O_2 production in all experiments could be due to other physiological processes such as respiration (Tang *et al.*, 2012). So, the increase in antioxidant enzyme activities such as APX, POD, CAT, and SOD could be also enhanced fruit tissues immunities against $O_2^{\bullet-}$ generation by quenching it during storage period at chilled temperature stress. Consequently, increasing antioxidant activities would participate strongly in scavenging $O_2^{\bullet-}$ and H_2O_2 production during cold stress (Yang *et al.*, 2011). These results explained that fruits in the INS side of trees are more tolerated to chilled temperature. Also, they have more increases in the antioxidant enzyme activities, which proved a protection against chilling damage. Moreover, the increases in antioxidant levels scavenge $O_2^{\bullet-}$ and H_2O_2 generation during cold stress (Foyer *et al.*, 2017).

However, The measurement of antioxidant activities using DPPH reduction showed that the activity decreased independently and gradually overall fruit locations during storage intervals. The activities are the highest in fruits that are harvested from INS. It recorded 1.18 % linked with other fruit locations. However, SUN presented the lowest activities in DPPH reduction (0.36 %) on the 35th day of the experiment time. This could be explained that AA mitigated the lipid peroxidation process throughout cold storage by controlling antioxidants network through storage duration (Lo'ay and El-Khateeb, 2018). Also, due to the variability of ROS formation and antioxidant quenching content (Lo'ay and Taher, 2018). So, the presence of AA at high content in fruit of INS could be supported plasma cell membrane (lipid and protein) by quenching ROS throughout cold storage (Lo'ay *et al.*, 2019). Hence, maintaining membranes functions/integrity by recycling α-tocopheroxyl radical form at the cell membrane to α-tocopherol (stabilizer cell membrane) to keep satisfying $OH^{\bullet}$ radical composition (Linster and Clarke, 2008).

CONCLUSION

In conclusion, the specific location of fruit on tree (sunny and shadow side and inside the tree canopy) affects the chilling sensitivity of mangos during cold storage. It also affects the activity of specific antioxidant enzymes and consequently, on CI-symptoms incidences during storage. The result provides that the fruits were harvested from INS present less sensitivity to low-temperature storage and low CI-symptoms due to more activities in antioxidant enzymes. Also, we suggest that the location of mango fruits on trees should be taken into consideration for the harvest process.

REFERENCES

A.O.A.C. (1995). Association of Official of Analytical Chemist. (15th ed.), Published by the A.O.A.C., Washington., USA (1995).

Barman, K., and Asery, R. (2014). Salicylic acid pre-treatment alleviates chilling injury, preserves bioactive compounds and enhances shelf life of mango fruit during cold storage. J. Sci. Ind. Res., 73: 713-718.

Barman, K., Asrey, R., Pal, R.K., Jha, S.K., and Bhatia, K. (2014). Post-harvest nitric oxide treatment reduces chilling injury and enhances the shelf-life of mango (*Mangifera indica* L.) fruit during low-temperature storage. J. Hortic. Sci. Biotechnol., 89: 253–260.

Chaplin, G.R., Cole, S.R., Landrin, M., Nuevo, P.A., Lam, P.F., and Graham, G. (1991). Chilling injury and storage of mango (*Mangifera indica* L.) held under low temperatures. Acta Horticulturae, 291: 461-471.

FAO (2018). Food and Agriculture Organization of the United Nations, www.FAO.org.

Ferguson, I.B., Lurie, S., and Bowen, J.H. (1994). Protein synthesis and breakdown during heat shock of cultured pear (*Pyrus communis* L.) cells. Plant Physiol., 104:1429-1437.

Foyer, C.H., and Noctor, G. (2003). Redox sensing and signalling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. Physiologia Plantarum, 119: 355-364.

Foyer, C.H., Ruban, A.V., and Noctor, G. (2017). Viewing oxidative stress through the lens of oxidative signaling rather than damage. Biochem. J., 474: 877–883.

Galletta, G.J., and Bringhurst, R.S. (1990). Strawberry management. G.J. Galletta, R.S. Bringhurst (Eds.), Small Fruit Crop Management. Englewood Cliffs, Prentice-Hall (1990), pp. 83-156.

Hakim, A., Purvis, A.C., and Mullinix, B.G. (1999). Differences in chilling sensitivity of cucumber varieties depends on storage temperature and the physiological dysfunction evaluated. Post. Biol. and Technol., 17: 97-104.

Henson, R. (2008). The Rough Guide to Climate Change (2nd ed.), Penguim Books, London (2008) (p. 384).

Hicks, J.R., Manzano-Mendez, J., and Masters, J.F. (1983). Temperature extremes and tomato ripening. Proceedings Fourth Tomato Quality Workshop, 4: 38-51.

Hodges, D.M. (2003). Overview: oxidative stress and postharvest produce. In:D.M. Hodges (ed). Postharvest Oxidative Stress in Horticultural Crops. Food Products Press, New York., 1-12.

Hodges, D.M., Lester, G.E., Munro, K.D., and Toivonen, P.M.A. (2004). Oxidative stress: Importance for postharvest quality. HortScience 39, 924-929.

Hollósy, F. (2002). Effects of ultraviolet radiation on plant cells. Micron., 33: 179-197.

Iturbe-Ormaetxe, I., Escuredo, P.R., Arrese-Igor, C., and Becana, M. (1998). Oxidative damage in pea plants exposed to water deficit or paraquat. Plant Physiol. 116, 173-181.

Junmatonga, C., Faiyueb, B., Rotarayanonta, S., Uthaibutraa, J., Boonyakiatc, D., and Saengnila, K. (2015). Cold storage in salicylic acid increases enzymatic and non-enzymatic antioxidants of Nam Dok Mai No. 4 mango fruit. Science Asia, 41: 12–21.

Kays, S.J. (1997). Postharvest Physiology of Perishable Plant Products. AVI, Athens (1997) 532 p.

Kramer, G.F., Norman H.L., Krizek D.T., and Mirecki, R.M. (1991). Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. Phytochem., 30: 2101-2108.

Leshem, Y.Y. (1992). Plant Membranes: A Biophysical Approach to Structure, Development and Senescence. Kluwer Acadamic Publishers, Dordrecht. The Netherlands, ISBN : 0792313534, 1-266.

Levine, R.L., Williams, J.A., Stadtman, E.R., and Schacter, E. (1994). Carbonyl assay for determination of oxidatively modified proteins. Methods Enzymol., 233: 346-357.

Linster, C.L., and Clarke, S.G. (2008). L-Ascorbate biosynthesis in higher plants: the role of VTC2. Trends Plant Sci. 13, 567–573.

Lo'ay, A.A. (2005). Chilling Injury in Mangoes. Ph. D. Thesis, 2005, University; Wageningen, 1–224, (ISBN:90-8504-309-3).

Lo'ay, A.A., and El-Khateeb, A.Y. (2018). Antioxidant enzyme activities and exogenous ascorbic acid treatment of 'Williams' banana during long-term cold storage stress. Scientia Horticulturae 234, 210-219.

Lo'ay, A.A., and Taher, M.A. (2018). Effectiveness salicylic acid blending in chitosan/PVP biopolymer coating on antioxidant enzyme activities under low storage temperature stress of 'Banati' guava fruit. Scientia Horticulturae 238, 343-349.

Lo'ay, A.A., Taha, N.A., and El-Khateeb, Y.A. (2019). Storability of 'Thompson Seedless' grapes: Using biopolymer coating chitosan and polyvinyl alcohol blending with salicylic acid and antioxidant enzymes activities during cold storage. Sci. Hortic. 249, 314-321.

Majeed, M., and Jeffery, K.B. (2002). Reduction of chilling injury in 'Tommy Atkins' mangoes during ripening. Sci. Horti., 95: 297-308.

Marwood, C.A., and Greenberg, B.M. (1996). Effect of supplementary UVB radiation on chlorophyll synthesis and accumulation of photosystems during chloroplast development in *Spirodela oligorrhiza*. Photobiol., 64: 664-670.

McKeon, A.W., Warland, J., and McDonald, M.R. (2006). Long-term climate and weather patterns in relation to crop yield: A minireview. Can. J. Bot., 84: 1031-1036.

Mditshwa, A., Magwaza, L.S., Tesfay, S.Z., and Mbili, N.C. (2017). Effect of ultraviolet irradiation on postharvest quality and composition of tomatoes: a review. J. Food Sci. Technol., 54: 3025-3035.

Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.

Moretti, C.L., Mattos, L.M., Calbo, A.G., and Sargent, S.A. (2010). Climate changes and potential impacts on postharvest quality of fruit and vegetable crops: A review. Food Res. Int., 43: 1824-1832.

Nair, S., and Singh, Z. (2003). Pre-storage ethrel dip reduces chilling injury, enhances respiration rate, ethylene production and improves fruit quality of 'Kensington Pride' mango. J. Food Agric. Environ., 1: 93-97.

Nair, S., and Singh, Z. (2009). Chilling injury during storage affects respiration rate and fruit quality in 'Kensington Pride' mango fruit. Acta Horticulturae, 820: 737–743.

Peiyan, L., Xiaolin, Z., Md. Golam Ferdous C., Kim, C., and Jeffrey, K.B. (2015). Prestorage Application of Oxalic Acid to Alleviate Chilling Injury in Mango Fruit. HortScience, 50: 1795-1800.

Pfündel, E.E., Pan, R.S., and Dilley, R.A. (1992). Inhibition of violaxanthin deep oxidation by ultraviolet-B radiation in isolated chloroplasts and intact leaves. Plant Physiol., 98: 1372-1380.

Prinsze, C., Dubbleman, T.M.A.R., and Steveninck. J.V. (1990). Protein damage induced by small amounts of photodinamically generated singlet oxygen or hydroxyl radicals. Biochim. Biophys. Acta Horticulturae, 1038: 152-157.

Rodeo, A.J.D., and Esguerra, E.B. (2013). Low temperature conditioning alleviates chilling injury in mango (*Mangifera indica* L. cv. Carabao) fruit. Philipp J. Crop Sci., 38: 24-32.

Shewfelt, R.L., and Del Rosario, B.A. (2000). The role of lipid peroxidation in storage disorders of fresh fruits and vegetables. HortScience, 35: 575–579.

Sivakumar, D., Jiang, Y., and Yahia, E.M. (2011). Maintaining mango (*Mangifera indica* L.) fruit quality during the export chain. Food Res. Int., 44: 1254-1263.

Tang, T., Huang, D.-W., Zhou, C.-Q., Li, X., Xie, Q.-J., and Liu, F.-S. (2012). Molecular cloning and expression patterns of copper/zinc superoxide dismutase and manganese superoxide dismutase in *Musca domestica*. Gene, 505: 211–220.

Tian, S.P., Li, B.Q., and Xu, Y. (2005). Effects of O_2 and CO_2 concentration on physiology and quality of litchi fruit in storage. Food Chem., 91: 659–663.

Usha, K., Thakre, M., Goswami, A.K., and Nayan, D.G. (2015). Fundamental of Fruit production, Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi,110 012, 1-245.

Wang, C.Y. (2006). Reducing chilling injury and maintaining quality of horticultural crops with natural products and their derivatives. Acta Hortic., 712: 285-290.

Wang, Y.S., Tian, S.P., and Xu, Y. (2005). Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruit during postharvest periods. Food Chem., 91: 99–104.

Woolf, A.B., Ferguson, I.B., Requejo-Tapia, L.C., Boyd, L., Laing, W.A., and White, A. (1999). Impact of sun exposure on harvest quality of 'Hass' avocado fruit. Rev. Chapingo Ser. Hortic., 5: 352-358.

Xu, M., Dong, J., Zhang, M., Xu, X., and Sun, L. (2012). Cold-induced endogenous nitric oxide generation plays a role in chilling tolerance of loquat fruit during postharvest storage. Post. Biol. and Technol., 65: 5-12.

Yang, H., Wu, F., and Cheng, J. (2011). Reduced chilling injury in cucumber by nitric oxide and the antioxidant response. Food Chem., 127: 1237–1242.

Yasunaga, E., Fukida, S., Nagle, M., and Spreer, W. (2018). Effect of storage conditions on the postharvest quality changes of fresh mango fruits for export during transportation. Environ. Control Biol., 56: 39-44.

Yen, G.C., and Chen, H.Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agr. Food Chem., 43: 27-32.

Zhang, X., Shen, L., Li, F., Meng, D., and Sheng, J. (2013). Arginase induction by heat treatment contributes to amelioration of chilling injury and activation of antioxidant enzymes in tomato fruit. Post. Biol. and Technol., 79: 1–8.

تأثير وضعية الثمار على الشجرة وحساسيتها لأعراض البرودة ونشاط مضادات الأكسدة الانزيمية لثمار المانجو صنف 'عويس' خلال التخزين البارد

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أجريت هذه الدراسة خلال موسمى 2015 و2016 بمزرعة خاصة بمحافظة الشرقية على أشجار مانجو صنف 'عويس' عمرها 10 أعوام ومنزرعة فى تربة رملية على مسافة زراعة 8×10 متر. تعتبر ثمار المانجو من الثمار الحساسة للإصابة بأعراض البرودة خلال فترة التخزين الطويلة و بخاصة تحت 13 درجة مئوية. تحت درجات الحرارة المنخفضة يحدث العديد من التغييرات الفسيولوجية التي من شأنها اظهار الآثار الجانبية للتخزين البارد على الفواكه من أعراض البرودة مما يقلل من العائد الاقتصادى. تهدف الدراسة إلى اختبار تاثير اوضاع ثمار المانجو على االاشجار مثل الجانب المشمس (SUN ؛ فاكهة تتعرض لأشعة الشمس معظم اليوم) (وجانب الظل SHA ؛ فاكهة تنمو على الجانب المظلل من الأشجار)(وداخل المظلة INS ؛ تنمو الفاكهة داخل مظلة الشجرة)على سلوك الثمار تحت التخزين البارد (C ° 1 ± 4 و96 RH ٪) لمدة 35 يومًا خلال موسمين متتاليين (2015-2016). من الواضح أن الفاكهة التي تم حصادها من INS اظهرت قلة فى ظهور اعراض البرودة على الثمارخلال مدة 35 يومًا من التخزين. كما أظهرت الثمار التي تم حصادها من INS بشكل ملحوظ أعلى نشاط فى مضادات الاكسدة الانزيمية مثل أسكوربات بيروكسيديز (APX) ، بيروكسيديز (POD) ، الكتاليز (CAT) ، و السوبر اكسيد ديسموتاز (SOD) مقارنة بالثمار المقطوفة من مواقع الاخرى. بالإضافة إلى ذلك ، انخفض انتاج او توالد كل من $O_2^{\bullet-}$ و H_2O_2 في الثمار المقطوفة من داخل الاشجار كما اعطت قلة فى نتائج نفاذية الخلايا، وخاصة كمية منخفضة في كل من malondialdehyde (MDA) و نتائج اكسدة البروتين carbonyl (PCG)طوال مدة التخزين. أشارت هذه النتائج إلى أنه يمكن أخذ مواقع الثمار على الشجرة فى الاعتبار عند الحصاد لتصنيف الفواكه قبل التخزين البارد.