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Influence of Effective Micro-Organisms (EM) and some Antioxidant on Storability and Fruit Quality of Mandarin under Cold Storage Conditions

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ABSTRACT

To assess the influence of both effective microorganisms and some antioxidants as post-harvest coating treatments on storability and fruit characteristics of Mandarin cv. Balady. Treatments applied significantly showed a reduction pattern of their fruit weight losses, decay percentage, and total losses. The combined treatments of both effective microorganisms (EM) and antioxidants were the most effective in that reduction. Also, fruit quality parameters were still maintained their quality after 45 days of storage and delayed the senescence of fruits. A gradual increase in TSS was shown. Moreover, some physiological parameters, chilling injury, and ion leakage showed a similar pattern, that highly significant values were shown on the studied fruits. Also, the malondialdehyde (MDA) content can be applied as an indicator of lipid peroxidation (LP) to measure the storability of tested fruits. The effect of antioxidants and effective micro-organisms have to be in an increasing pattern in all applied treatments that are used after 45 days of storage; the highest concentration of H_2O_2 was observed in control ones, the lowest content was observed at combined treatment, which may reflect the critical roles in reducing reactive oxygen species and mitigation of chilling injury. In addition to total phenol content were decreased significantly, as the obtained results showed a positive relationship among ascorbic acid and loss of phenolics. After 15 days of storage higher percentage of antioxidant capacity (DPPH) was observed, then a gradual decrease was recorded in fruit treated with combined treatment. Both treatments of antioxidant and effective microorganisms significantly affected the levels of antioxidant enzymes i.e catalase activity (CAT) and ascorbate peroxidase (APX). Therefore, the combined treatment may be the best treatment that maintaining the quality of stored fruit of Balady mandarin.

Keywords: Antioxidant, Effective micro organisms, Chilling injury, Ion leakage, Catalase.

INTRODUCTION

Citrus is one of the various and important fruit trees, grown in Arab Republic of Egypt, since its acreage increased to approximately 432000 hectares producing 5884000 tons fruits according (Faostat, 2018). The importance of its agriculture and economy is demonstrated by the great potential as a valuable source in nutrition, e.g. rich in vitamins, sugars, aroma and flavour compounds.. It takes the third position among the fruit crops produced in the world after grapes and apples. Balady mandarin is recognized as one of the most important citrus species that Egyptian consumers and export market prefer it because of its flavor and easy peeling. Therefore acreage tends annually to increase due to the high demand for both local and export markets; 46869 hectares produced about 1068351 tons of fruits (Faostat, 2018) were recorded ,its Production of Mandarin fruits pointed out about 20% of the total production of citrus fruits in Egypt. Mandarins as a softer citrus fruit are spoiled more quickly within the activity of both physiological and pathological causes that increases storage weight loss, fruit decay and peel breakdown (Hadian-Delijou *et al.*, 2017) and loss of characteristic quality. Mandarins fruits can be kept in great quality for only 15–30 days under ambient storage conditions (Junmatong *et al.*, 2015). Issued studies cleared that cold storage reduced losses of fruit weight and decay %, decreased titratable acidity, continually increased TSS as well as TSS/acid ratio and slowed down enzyme activities which resulted in delaying of declining vitamin C contents (Rokaya *et al.*, 2015) on tangarins, (Junmatong *et al.*, 2015) on mango and (Obenland, *et al.*, 2011) on mandarins. Antioxidants conflict with the production of free radicals and also play a key role in inactivating them and the main enzymatic antioxidants are superoxide dismutase, catalase, glutathione peroxidase, ascorbate peroxidase and glutathione reductase, while a non-enzymatic portion composed of low molecular weight antioxidants, namely proline, thiol, ascorbic acid and glutathione (Blokhina *et al.*, 2003). Imbalance safety of the cell membrane is considered the first damage induced by (CI) chilling injury (Rui *et al.*, 2010). Although ion leakage (IL) and malondialdehyde content (MDA) are widely used by investigators in fruits and vegetables to assess the safety of the cell membrane, it is possible to accurately evaluate the effects of chilling injury(Aghdam and Bodbodak, 2013). Oxidative stress from overabundance oxygen species (ROS) as hydrogen peroxide (H_2O_2)and superoxide anion ($O^{2\bullet-}$) has been joined with the event of cooling injury in fruits (Hodges *et al.*, 2004). The poisonousness of ROS depends on its responses with numerous cell components, which cause a course of oxidative reactions and the subsequent inactivation of enzymes, lipid peroxidation, protein corruption, and DNA defilement (Scandalios,1993). Ascorbic acid (VC) is a significant antioxidant, forward with alpha-tocopherol, and many other nutrients. On the other hand (Santerre *et al.*, 1988 ; Sapers *et al.*, 1989) have presented Vitamin C as a useful measure in restrain enzymatic browning of fruits and vegetables. Several

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kinds of molds (Fungi) in different nuts that are already roasted can be removed by application of Vitamin C solution along with water (Clark, 2015). Vitamin C is a good antioxidant that holds fruit from darkening and enhances the destruction of bacteria during the drying process. Ascorbate has been exhibited in numerous qualitative examinations to possess significant antioxidant activity (Smirnoff, 2005, Arrigoni and De Tullio, 2000). Vitamin C is one of the most frequently studied antioxidants in extracellular fluids. It can neutralize ROS in the aqueous phase before lipid peroxidation is initiated, and it can also regenerate vitamin E. (Percival, 1998). Tocopherol (V.E) is a gathering of mixes made uniquely by photosynthetic microorganisms and are required in the extinguishing and scavenging of responsive oxygen (O_2) (Neely *et al*., 1988) and function as profoundly viable recyclable string response eliminators for the evacuation of polyunsaturated polyunsaturated fatty acid (PUFA) radical species created during Lipid peroxidation. Tocopherol (V.E) is recognized as a superior antioxidant in bio-membranes, where they play both antioxidant and non-antioxidant functions. It is also considered as a general antioxidant to strengthen the stability of the membrane, containing a quenching or scavenging of ROS like H_2O_2. Tocopherol (V.E) centralized of the chloroplast thylacoid membrane. Of the four isomers of tocopherols (α -, β-, γ-, d-) found in plants, tocopherol is the most important antioxidative activity due to the appearance and behavior of 3 methyl groups in its molecular formation (Kamal-Eldin and Appelqvist, 1996 ; Orabi *et al*., 2017). Effective Microorganisms (EM) becomes put into implementation since 1980 (Higa, 2005). It is a composite of three essential microorganisms; photosynthetic bacteria, lactic acid bacteria and Yeasts used to increase the biological activity of plant and its fruits although replace the application of artificial fertilizers and modifying microbial diversity and intercommunication in soils and plants (Primavesi and Kinjo, 1997). Studies hold that this compound can influence soil quality, plant development, yield, outcome quality and improve fruit storability to be useful and other developments will be significantly improved performance (Imai and Higa, 1994). Therefore, the purpose of this study intended to recognize the impact of using effective microorganisms and some antioxidants on the storability of Balady mandarin fruits and their fruit quality.

MATERIALS AND METHODS

This examination was completed during the two progressive seasons 2016-17 2017-18: on 10-year-old Balady Mandarin (*Citrus reticulata*, Blanco) trees budded onto sour orange (*Citrus aurantium*, Linn) rootstock to study the effect of both effective microorganisms and some antioxidant on storability of Balady mandarin fruits and fruits quality during different cold storage periods. The trees are grown on clay loam soil in the Mansoura University's experimental orchard.

1. Experimental trails

Regular fruits of Balady Mandarin free from physical injury and infections were harvested at maturation stage of SSC 9.0 ± 0.1% from the experimental orchard of Mansoura University. The harvested fruits were graded, cleaned with tap water, immersed in warm chloride water for 3 minutes., cleaned with cotton cloth and air dried and then divided into Five similar groups (200 fruits/group). Each group was dipped for 7 min in solution of one of these treatments: control = tap water (T1), Effective Microorganisms (EM) at 10ml/l (T2), EM at 10ml/l+ α-tocopherol (V.E) at 1000ppm (T3), EM at 10ml/l+ Vitamin C (V.C) at 1000ppm(T4) and mix of EM+V.E+V.C at 10ml/l, 1000ppm and 1000ppm, respectively (T5). Thereafter, the fruits were left to dry at ambient air. Fruits of each treatment divided into two groups, the first one to determine the chemical characteristics and the second one to measure physical characteristics, were packed in carton boxes, and each treatment replicated three times for either physical or chemical properties and stored for 45 days under cold storage at 4c°±1 and 85-90% (R.H.). The fruit samples from tested treatments were periodically examined at 15 days intervals and were taken for physical and chemical analysis and the results obtained were represented as the mean of the two trial years.

2. Physical properties of the fruits

Weight Loss percentage

Initial weight was individually registered before storage and re-weighted at each sampling date. Fruit weight loss percentage was determined according to the following formula: [(Wi – Ws)/Wi] X 100

Where; Wi = initial fruit weight before storage and Ws = fruit weight at the end of sampling date.

Decay percentage

It was calculated by weighting each sample of decayed fruit at 15 days intervals during cold storage using that equation:

Decay (%) = (Weight of decayed fruits/Initial weight of stored fruits) × 100

Chilling injury symptoms

Chilling injury were assessed by estimating the visible surface symptoms and browning area of the peel and internal breakdown, classified as 5 levels A–E (Promyou *et al*., 2008*)*. A: No injury; B: slight injury (1–20%); C: moderate injury (21–50%);D: severe injury (51–80%); E: very serious injury (81–100%) of the affected fruit. The : CI index was determined using the following formula:

CI index = £ (CIL) × (NFL) / TNF

Where; CIL = Chilling injury level ,NFL =Number of fruit at that level and TNF =Total number of fruit

3. Chemical properties of the fruits

Total soluble solids (S.S.C)

It was determined by using Carlzeiss hand refracto-meter and calculated as a percentage according to (A.O.A.C. ,1980)

Titratable acidity (TA)

5 ml of clear sample were titrated against 0.1 N sodium hydroxide solution utilizing phenolphthalein as a pointer. (TA) was declared as 1g citric acid in 100 ml of juice, according to (Ranganna, 1979).

SSC/acid ratio

This ratio was calculated from the obtained results of fruit juice SSC and the percentage of acidity.

Ascorbic acid content (Vitamin C)

Vitamin C was determined by the titrimetric method using 2,6-dichlorophenol indophenol and 6% C2H2O4 oxalic acid. It was calculated as mg/100ml juice as indicated by (A.O.A.C. ,1980).

Total phenols content

In fruit juice extract total phenols content was determined by the colorimetric method of folin–Denis as described by(Danil and George, 1972) and the results were expressed as mg gallic acid/ g fw.

Ion leakage percentage (IL %)

Exactly 5 g of peel were weighted and cut to disks. Disks were cleaned 3 times in distilled water and put into 20 ml mannitol for 3 hours(Hakim *et al*., 1999). EC of the aqueous condition was estimated using a conductivity meter, after which the tissue samples were killed by heating to 100 °C. in a water bath for 20 minutes. The cooking process makes it possible to measure the conductivity again and to calculate the IL% from the uncooked peeling samples as follows:

IL (%) = EC (after 3 Hr.)/ EC (after boiling) × 100

Malondialdehyde (MDA)

MDA is commonly used as an index of lipid peroxidation. determine the MDA content, and exactly weighed sample of 2.5 g of peel was crushed in a blender and blended with 25 ml of 5% metaphosphoric acid (w/v), 500 µl of 2% butylated hydroxytoluene in ethanol (w / v) and subsequently homogenized by a blender. The component was separated and centrifuged at 15,000 rpm for twenty minutes. Then, mix 1 ml of the supernatant solution, 100 µl of 2% (w / v) butylated hydroxytoluene, 0.50 ml of 1% (w / v) thiobarbituric acid formed in 50 mM NaOH and 0.50 ml of 25% HCl (v / v)to mix chromogen and that the response mixture was incubated at 95 ° C for thirty minutes and the resulting color was measured with a spectrophotometer at a wavelength of 532 nm (Dhindsa *et al*., 1982).

Antioxidant activity

Antioxidant The antioxidant activity (AA) was discovered in fruit peels by the DPPH method (2,2-diphenyl-1-picrylhydrazyl) according to (Brand-Williams *et al*., 1995). 1 g of peel was extracted with 10 ml of methanol (85%). Then 1 ml of the extract was mixed with 1.0 ml DPPH (0.1 mmol / l) and 1.0 ml Tris-HCl. The mixtures were vibrated vigorously and left to stand for 30 minutes (under dark). By a spectrophotometer at 517 nm, the absorbance was measured. The antioxidant activity was revealed in terms of the percentage of a free radical scavenger.

Hydrogen peroxide concentration

One gram of fruit peel was cut into small pieces and homogenized with10 ml of 0.1% (w/v) trichloroacetic acid (TCA) on an ice bath. Then mixture was centrifuged at 12.000 g for 15 min. An aliquot of 0.5 ml of the resulting supernatant was transferred to test tube and 0.5ml of supernatant and 0.5 ml of 10mM potassium phosphate buffer (pH7.0) and 1 ml potassium iodide (1M). The absorbance of the mixed solution was measured at 390 nm by spectrophotometer. The content of H_2O_2 was expressed in m mole per liter (mM/l) by using a standard curve according to(Velikova *et al*., 2000).

Antioxidant enzyme activity

Five grams of iced peel tissue was homogenized in a pre-cooled mortar in the presence of 10 ml of 50 mM potassium phosphate buffer (PH7) along with 1% (w / v) insoluble polyvinylpyrolidone (PVP) and 0.1 mM EDTA. The extraction methods were replicated twice and the supernatants were blended and raised to a certain volume, which was the crude enzyme extract. All procedures were performed at -4 ° C and the resulting supernatant was collected and dialyzed prior to the enzyme test. Ascorbate Peroxidase activity(APX) was determined according to (Nakano and Asada, 1981). One unit of Ascorbate Peroxidase was defined as the quantity of enzyme that breaks down 1.0 µM of ascorbate per min. Catalase Enzyme activity (CAT) was evaluated by using a method of (Aebi ,1983). The activity of CAT was calculated by the reduction of absorbance at 240 nm for 1.0 min as a result of H2O2 consuming.

4. Statistical analysis

The obtained data were analyzed as completely randomized blocks design using the software package of Co-Stat, Ver. 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93940, USA). The differences among treatment means (three replicates for each) were compared with Duncan's multi-range test at 5% level according to(Duncan, 1955).

RESULTS AND DISCUSSION

1. Fruit weight loss, Decay and Total loss Percentages

Results presented in Fig (1) showed that the tested treatments significantly reduced percentage of weight loss, decay and total loss during different cold storage periods compared to the control. The highest losses were recognized in control fruits that recorded 15.83 %, 9.01% and 24.29% for fruit weight loss, decay and total loss, respectively after 45 days of cold storage. , Meanwhile, treating fruits with combination of antioxidants (V.C+V.E) and effective microorganisms (EM) was the most effective application that minimized weight loss, decay and total loss to 5.14%, 2.40%, 7.54 %, respectively at 45 days of the cold storage. It appears that the fruit weight loss % was significantly increased continuously with prolonging the cold storage period up to 45 days. The fruit weight loss% is mostly due to water loss caused by evaporation and transpiration and the value of dry matter was missed by respiration. The loss of water from fruit after harvest is a serious problem caused shrinkage and weight loss (Sun *et al.*, 2010). Our results are in agreement with those of (Lin *et al*., 2007) who indicated that using ascorbic acid at 1000ppm in grapes caused a lower progressive physiological loss in weight. Similar results are reported : by(Lin *et al*., 2007) in pear and (Siddiqui and Gupta ,1995) with ber fruits. Ascorbic acid protected various fruits against harmful effects of oxidative processes and biotic stresses due to improved levels of antioxidants that might prevent much softening and rotting of fruits (Paliyath and Subramanian, 2008). application of ascorbic acid at 100 ppm conferred the least decay occurrence rate in guava. However, low concentration of V.C significant in preventing decay incidence (Gill *et al*., 2014).

2. TSS, TA and TSS/TA ratio

In general, data in Fig (2) showed gradual increase in TSS during cold storage periods of different treatments as well as control. Significant variance was observed among the cold storage periods of 0, 15, 30 and 45 days and the tested treatments. Where, control treatment recorded the highest TSS values (11.25 and 11.31 %) for 30 and 45 days and followed in descending order EM, EM+V.E, EM+V.C and mixture of EM +V.E+V.C Meanwhile, after 45 days of cold storage, mixture of EM +V.E+V.C gave the lowest TSS comparing with other treatments and control and recorded 10.17%. In addition, the control (water only) fruits were significantly higher than other treatments. The increased values in soluble solids could be due to fruit weight loss and subsequently fruit juice concentration (Khademi and Ershadi, 2013). Where, the decrease in sucrose synthesis in treated fruits may result in lowered enzyme activity that reduced ethylene production (Martínez-Esplá *et al.*, 2017). TSS of guava fruits was at its lowest with Ascorbic acid 1000 ppm stored under cold conditions (Rajput *et al*., 2015). α-

tocopherol treatment improved chemical properties (total sugar, TSS, Vit C, total anthocyanin and TSS/acid value) of pomegranate fruits (Fayed, 2010). Moreover, α- tocopherol – induced increase in total soluble sugars was observed in citrus (Kostopoulou *et al.*, 2015).

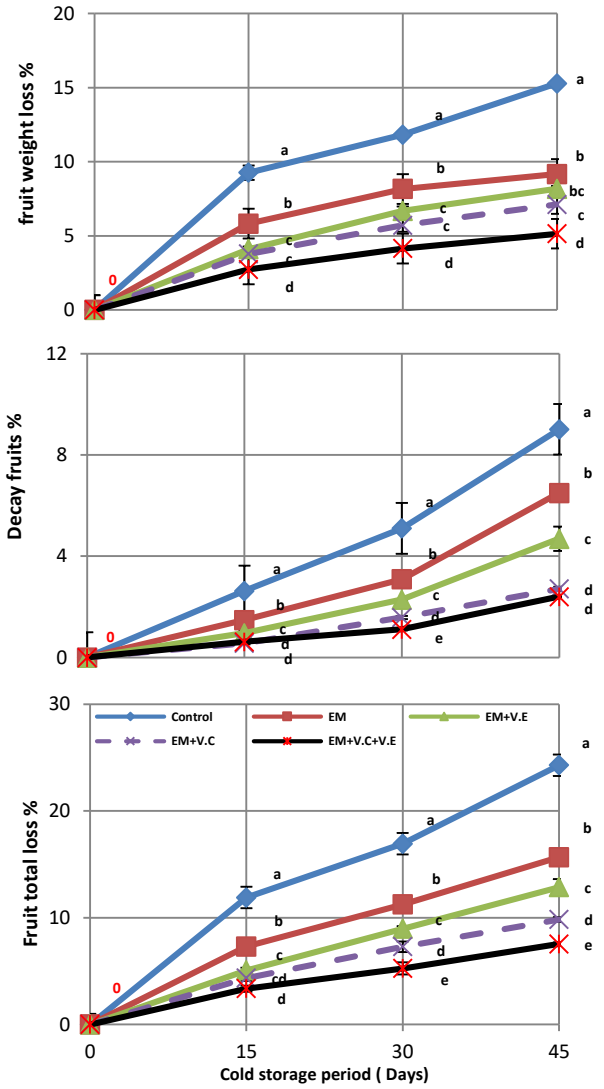

Fig. 1. Effect of some antioxidants and effective microorganisms on weight loss %, decay % and total loss% Balady mandarin fruits during 45 days cold storage period .The vertical bars indicate for standard error (SE, n=3). Data in each storage period followed by the same symbol are not significant different according to Duncan's multiple range test. EM= effective microorganisms, V.E= α-tocopherol, and V.C= Vitamin C.

Total acidity (TA) ratio was high in zero time and recorded 1.27 %, slowly decreased during 15, 30 and 45 days of cold storage. But treatments of the effective microorganisms and antioxidants maintained TA values significantly higher than the control. Although TA was slightly declined during different experimental periods up to 45 days of the cold storage, mixture of EM+VC+VE treatment had significant keeping influence on TA in the tested fruits followed by EM+VC treatment, they recorded 1.05% , 0.98 % sequentially at 45 days of cold storage. These results are consistent with the results of Echeverria and Valich (1989) who stated that low acid content in fruits with increased storage time could be attributed to consumption of the organic acids accumulated in cellular respiration and change the ratio of acids to total sugars. As for TSS/TA ratio data in Figure 2 revealed that there was a gradual increase in TSS/TA ratio with significant variance between the tested treatments and also cold storage periods. The decline in total acid contents led to an increase in TSS /acid ratio over the same time period. After 15, 30 and 45 days of cold storage It was observed the same trend in fruits treated with EM+ V.C+ V.E followed by EM+ V.C treatment to arrange the second in this respect. In addition, the treatment of EM+ V.C+ V.E reflected lower TSS/acid values as compared with all treatments. This indicates that EM+ V.C+ V.E treatments maintained high fruit quality. Our results agree with those of Hussein and El-Greatly, (2007) who reported : the enhancing effect of antioxidant on fruit characteristics.

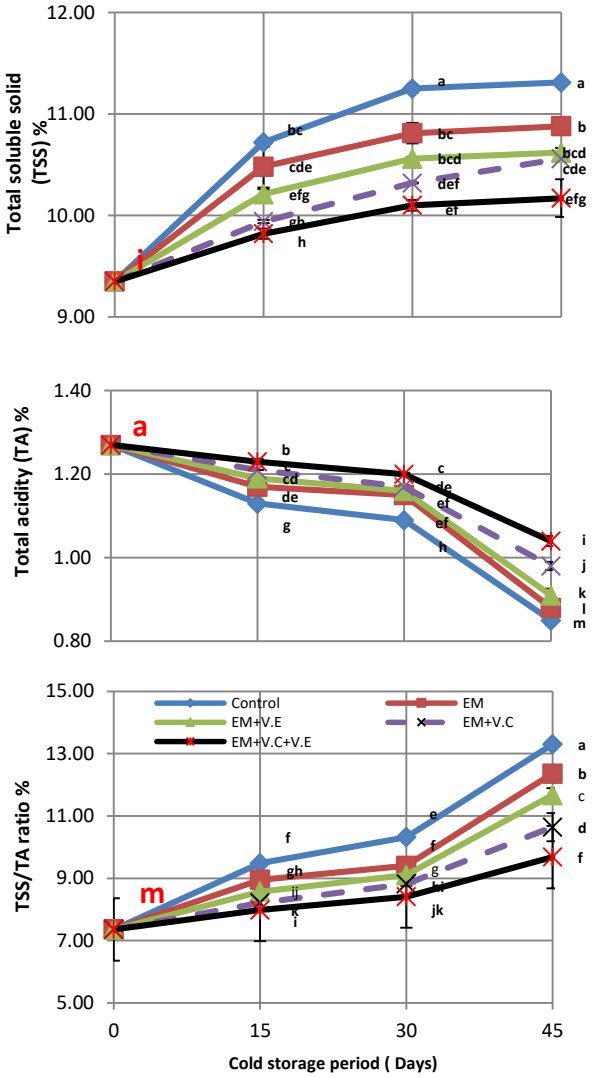

Fig. 2. Effect of some antioxidants and effective microorganisms on TSS %, TA % and TSS/TA ratio% in Balady mandarin fruits during 45 days cold storage period. The vertical bars indicate for standard error (SE, n=3). Data in each storage period followed by the same symbol are not significant different according to Duncan's multiple range test. EM= effective microorganisms, V.E= α-tocopherol, and V.C= Vitamin C.

3. Chilling injury (CI)

Chilling injury (CI) assessment revealed significant variation among antioxidant treatments and control during chilly storage. In all treated Balay mandarin fruits CI indications appeared after thirty days of storage. Highest Chilling injury was recognized after 45 days of storage, at the same time lowest of Chilling injury was obtained in mixture of EM+V.C+V.E treatment that record 0.33 % contrasted to the control (water) 4%, and there was no significant difference between EM+V.E and EM+V.C. (Fig 3). The mixture of this compounds decreased Chilling injury symptoms by inducing antioxidant activities under low temperature, mixed treatments of AsA and chitosan showed a most significant effect in inhibiting browning (Sun *et al*., 2010). As it is known, injury degree of treated litchi fruits can be expressed as relative leakage rate (Jiang *et al*., 2001). Corresponding to the results achieved in this investigation, delay in the occurrence of CI is associated with improving catalase activities. Most of these changes were reduced H_2O_2 content in fruit treated with EM in combination with antioxidants (V.C & V.E). The decline of H2O2 content decreased oxidation of lipid, which is linked with (IL) decrease, and consequently the degree of injury decreased accordingly.

4. Ion leakage (IL)

The interaction effect of effective microorganisms with antioxidants and storage time on IL was significant. IL increased during chilled storage for both treated and un treated fruits, but it was higher in untreated fruits than treated once. Maximum IL% obtained in control (62.59 %) after 45 days of storage. At this time EM+V.C+V.E treatment reduced IL% by 25.42 % compared with control. So that, the lowest amount of IL was obtained in EM+V.C+V.E treatment and EM+V.C which decreased IL% by 25.42% and 29.50% respectively compared to the control (Fig3). Ion leakage (IL) is an indirect measurement of the integrity and stability of the cell membrane. This index has often been used as a qualitative indicator of Chilling injury (Liu *et al*., 2018). Peroxidation of lipid regularly expands the (IL) in fruit cells during chilled storage (Nukuntornprakit *et al*., 2015). Low temperature increases the formation of H_2O_2, which influences the safety of the membrane. The breakdown of H_2O2 by catalase (CAT) is one of the mechanisms for reducing damage to cell membrane under stress conditions. in this study increasing the activity of CAT and reducing the rate of H_2O_2 may maintain the cell membrane safety . In litchi fruit ascorbic acid (AA) reported a significant effect in Ion leakage (IL) level throughout storage period (Sun *et al*., 2010).

5. Malondialdeldehyde (MDA)

Data illustrated in Fig (3) showed that the quantity of malondialdeldehyde increased significantly through chilled storage. It has also been, indicated that applying antioxidants mixed with effective microorganisms reduced the amount of malondialdeldehyde significantly. The rank of MDA was noticed in EM+V.C+V.E treatment with a difference of 88% contrast to the control after 45 days. Changes in MDA level are often used as an indicator of lipid peroxidation occurring from cold stress . Results registered that during cold storage, MDA increased significantly via immutable membrane damage. Application of EM+both of antioxidants was the most effective treatment for reducing malondialdeldehyde. The structure of the cell membrane changes due to lipid peroxidation in cold storage, thus increasing the level of MDA under these conditions. Larkindale and Huang, (2004) reported that causing the reactive oxygen species (ROS) accumulation in plant cells under cold storage conditions cause lipid peroxidation. Lipid oxidation is more probable to be the result of a series reaction involving molecular oxygen. However, this process depends on starting event that is due to the attack of hydroxyl radical or another radical (including molecular oxygen)(Purvis, 2003). Such results could show that EM +, both antioxidants, could reduce MDA production and maintain membrane safety by prevention the lipid peroxidation process. Supporting our results, plums treated with ascorbic acid had less generation of ROS and MDA (Liu *et al*., 2014).

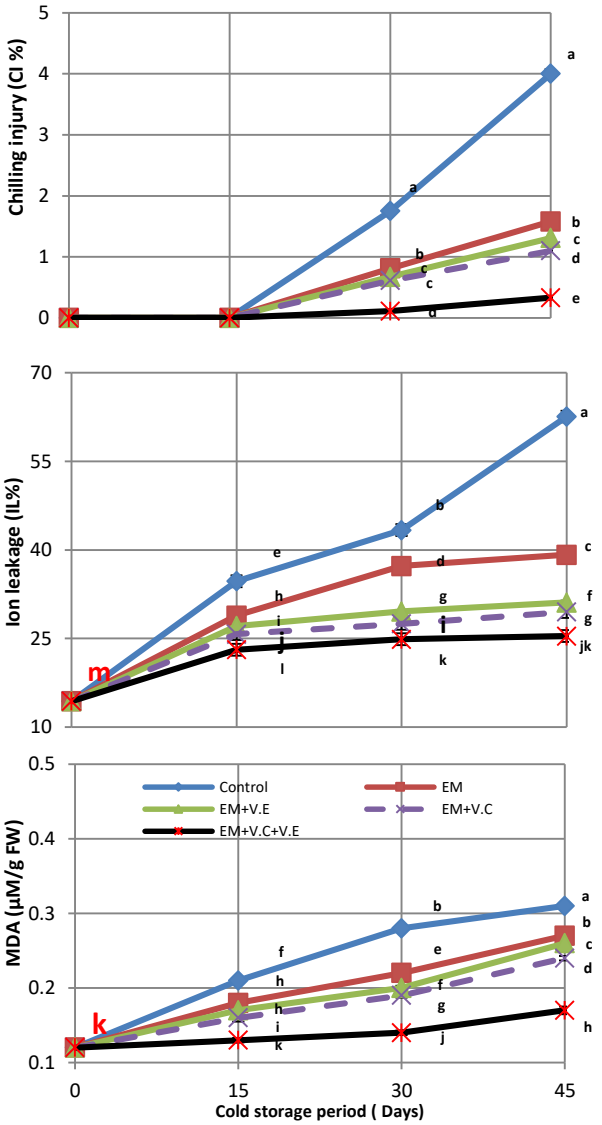

Fig. 3. Effect of some antioxidants and effective microorganisms on Chilling injury %, Ion leakage % and malondialdeldehyde (MDA) (µM/g FW) in Balady mandarin fruits during 45 days cold storage period. The vertical bars indicate for standard error (SE, n=3). Data in each storage period followed by the same symbol are not significant different according to Duncan's multiple range test. EM= effective microorganisms, V.E= α-tocopherol, and V.C= Vitamin C.

6. Total phenols

Results in Fig (4) shows the changes of total phenol (TP) content in Balady mandarin fruits during 45 days of cold storage at 4°c. Decreasing TP was observed in all treatments

after 15 days of cold storage. The lowest TP content (442mg/100g FW) was observed in control TP content (1118mg/100g FW) was obtained in fruit treated with mixture of EM+V.C+V.E. Interactions of antioxidants, effective microorganisms and cold storage period significantly differed in total phenol content. The study of Rekha *et al*., (2012) has shown a direct relation between antioxidant activity of citrus species and phenolic contents. These results agree with previous reports where antioxidant treatments as ascorbic acid reduced the loss of phenolics (González-Aguilar *et al*., 2005) because ascorbic acid reduce the quinones, product of the polyphenol oxidase (PPO) reaction with the phenolic compounds. Therefore, any factor that has restrained effects on PPO can increase the polyphenol content (Toivonen and Brummell, 2008 and Liu *et al*., 2014) defined that ascorbic acid resulted in lower PPO activity in plum during the storage time . Hussien *et al*., (2015) demonstrated that application of GSH or α-TOC increased total phenols and flavonoids in cotton plants grown under normal irrigation compared to the control. Phenolic compounds may exert their antioxidant activity in different ways. They may immediately scavenge some reactive species (ROS), including hydroxyl, peroxyl and superoxide radicals, operating as chain breaking antioxidants, they may defeat lipid peroxidation recycling other antioxidants, such as α-tocopherol (V.E).

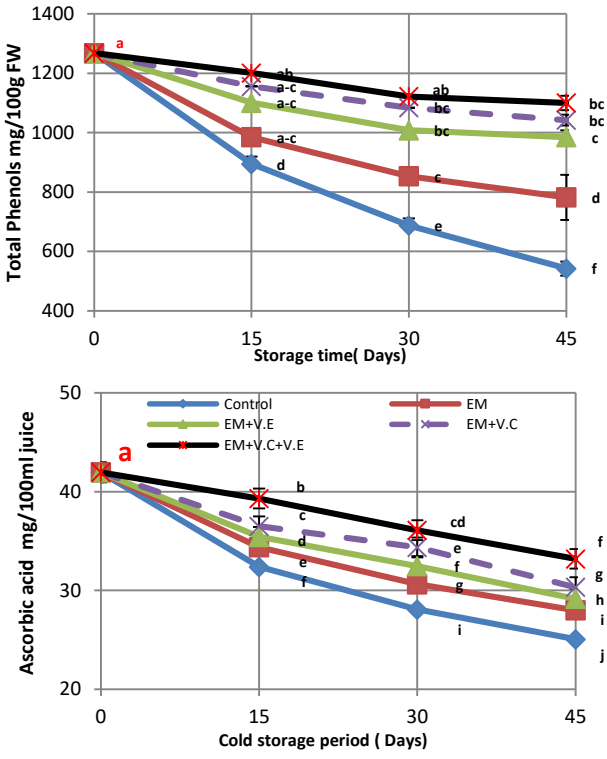

Fig. 4. Effect of some antioxidants and effective microorganisms on content of total phenols and Ascorbic acid in Balady mandarin fruits during 45 days cold storage period. The vertical bars indicate for standard error (SE, n=3). Data in each storage period followed by the same symbol are not significant different according to Duncan's multiple range test. EM= effective microorganisms, V.E= α-tocopherol, and V.C= Vitamin C.

7. Ascorbic acid

The ascorbic acid content of Balady mandarin fruits under cold storge showed a clear tendency to decrease Fig (4). The highest ascorbic acid content was observed in combination of both antioxidants and effective microorganisms with 33.20 (mg/100ml) followed by Em+ V.C with 30.33 (mg/100ml) ,the lowest content was obtained in control with 25.07 (mg/100ml). The ascorbic acid content were significantly declined with extended storage duration ,the rate of decreases were low due to use antioxidant and effective microorganisms(Gonzalez-Aguilar *et al.*, 2008)find out that the treatment of citric acid and ascorbic acid prevented the degradation of the vitamin C, suggesting that the oxidative reactions were the main cause of deterioration, and that the use of antioxidants could prevent such losses. (Mapson, 1970)reported that decrease in Ascorbic acid throughout storage could be due to alteration of Ascorbic acid to dehydroAscorbic acid or due to action of Ascorbic acid oxidase, (Rajive *et al.*, 2013) reported higher TSS, titratable acidity and ascorbic acid in litchi fruits treated with ascorbic acid after harvest.

8. Antioxidant activity %

Data illustrated in Fig (5) appears the changes in the antioxidant activity (AA) of Balady mandarin fruits which were treated with antioxidants and effective microorganisms through cold storage. A higher level of antioxidant activity (AA) was noted in fruit treated with both Antioxidant and effective microorganisms with significant difference after 15 days of cold storage. Thereafter, a gradual decrease in antioxidant activity (AA) was observed until 45 days. The maximum of antioxidant activity content was recorded in fruit treated with both Antioxidant and effective microorganisms after15, 30, 45 days of storage .At the end of storage period treatment with EM+V.C+V.E record 51.45 % compared to control that record 39.16 %. This higher of AA may be my be attributed to higher total phenol content (TPC).Antioxidant application such as V.C,VE had a positive effect on antioxidant activity (Sun *et al*.,2010, Ehteshami *et al*., 2019)

9. Catalase activity and Ascorbate peroxidase

CAT and APX were significantly influenced by antioxidative and effective microorganisms, storage period, and their combine. The alteration in CAT, APX was demonstrated in Fig (5) , which shows that CAT, APX increased immediately in the first 15 days of storage. The higher CAT and APX were obtained in fruits treated with a combination of EM + VC + VE with ((13.65 µmol / g f.wt) and (5.63 µmol / g f.wt), respectively after 45 days higher than the control. CAT and APX play an essential role in detoxifying of H_2O_2 at minimum temperatures (Mittler, 2002) and converting free radicals species such as H_2O_2 to O_2 and H_2O (Zhang and Tian, 2009). Results showed that combination of EM+V.C+V.E increased CAT, APX at chilly storage. likewise,(Sun *et al*.,2010) reported Ascorbic acid ,CH increased CAT,APX ; (Liu *et al* ., 2014) reported that Plums treated with ascorbic acid showed significantly excessive CAT activity during the entire storage period .Also (Sadiq *et al*., 2017) reported that externally applied 200 and 300 mg L - 1 α-Toc significantly increased the activities of antioxidative enzymes (SOD, POD, and CAT). The formation and function of membranes are influenced and some oxidation products such as malondialdehyde (MDA) are presented that react with biomolecules and exert cytotoxic and genotoxic effects. Endogenous antioxidants such as vitamin C, vitamin E, etc. act as primary defense systems. However, in diseased conditions, there is an additional need for antioxidants from exogenous sources. Cells have various protective mechanisms to inhibit the adverse effects of ROS, including antioxidant enzymes (such as

superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and small molecules such as vitamins C and E.

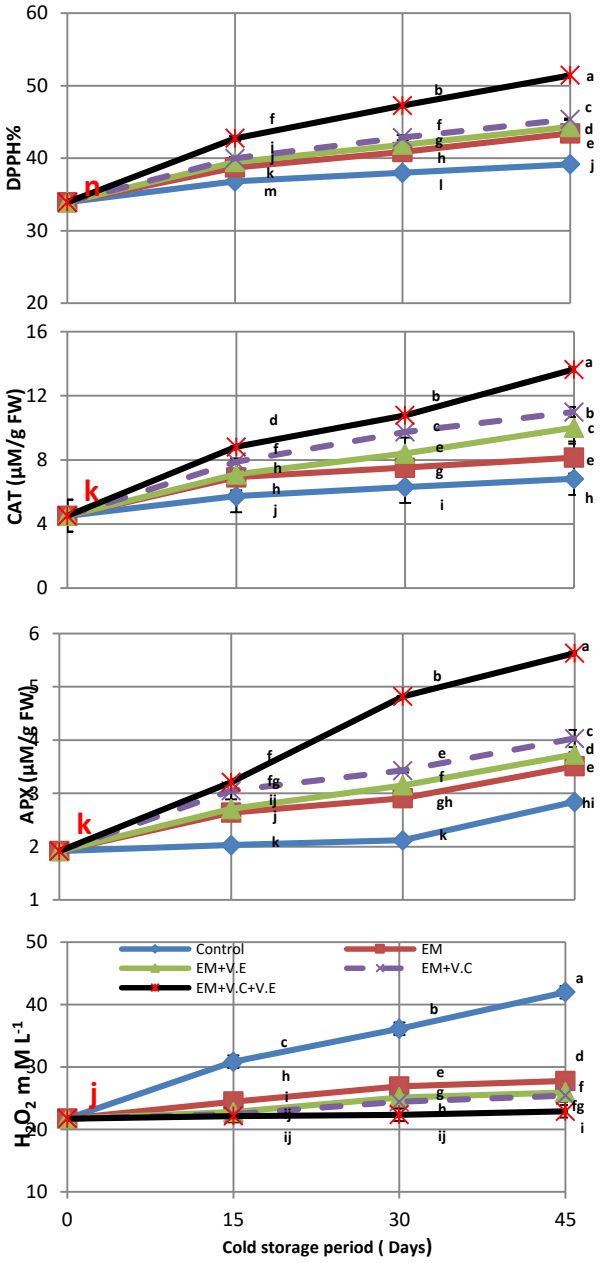

Fig. 5. Effect of some antioxidants and effective microorganisms on Antioxidant activity %, Catalase (CAT µM/g FW), Ascorbate peroxidase (APX µM/g FW) and Hydrogen peroxide (H_2O_2 mM/L)in Balady mandarin fruits during 45 days cold storage period. The vertical bars indicate for standard error (SE, n=3). Data in each storage period followed by the same symbol are not significant different according to Duncan's multiple range test. EM= effective microorganisms, V.E= α-tocopherol, and V.C= Vitamin C.

10. Hydrogen peroxide (H_2O_2)

Data shown in Fig(5) ,storage period ,treatments and their interaction affected on Hydrogen peroxide (H_2O_2) level significantly. It is observed ,the accumulation of (H_2O_2) increased in all treatments after 15 days of storage time .higher concentration of Hydrogen peroxide (H_2O_2) was recognized in the control treatment that record after 45 days (41.99 mmolL-1). Simultaneously ,the lowest content of (H_2O_2) was obtained in EM + V.C + V.E treatment that record after 45 days (22.87mmolL-1).The results of this experimental inducted significant effect of effective microorganisms and antioxidant on (H_2O_2) content during cold storage and showed positive effect on the reduction of Hydrogen peroxide (H_2O_2) production . Antioxidant enzymes such as catalase activity (CAT) are expected to increase, which play a crucial role in detoxifying reactive oxygen species and mitigating chilling injury (Mittler, 2002). Furthermore, limited(H_2O_2) production by ascorbic acid application in banana (Lo'ay and El-Khateeb, 2018). Consequently exposure of plants to unfavorable environmental pollution such as extreme temperatures, heavy metals, drought, air pollutants, lack of nutrients or salinity stress can affect the production of ROS, e.g., O_2, H_2O_2 and OH. and OH, increase. To protect against these toxic oxygen intermediates, plant cells and their organelles such as chloroplasts, mitochondria and Peroxi-Somes use antioxidant defense systems. Numerous studies have shown that the triggering of the cellular antioxidant machinery is important for protection against various stresses (Singh *et al*., 2008). The biosynthesis and accumulation of (H_2O_2) may participate in the expansion of chilling injury at low temperatures (Huang *et al*., 2016). Application of α-TOC prevented lipid peroxidation, also H_2O_2 content was decreased in wheat seedlings when α-TOC was applied(Kumar *et al*., 2013). α-TOC can also serve as a capping agent to scavenge intracellular ROS formed(Soltani *et al*., 2012).

CONCLUSION

It is apparent from the obtained results that combined treatment EM10ml/l + V.C 1000 ppm + V.E 1000ppm reduced IL, MDA and H_2O_2 that biochemical markers during cold storage ,on other hand increase antioxidant activity AA and antioxidant enzymes such as CAT and APX and TPC .In general , EM10ml/l mixture with V.C 1000 ppm + V.E 1000ppm are recommended as the best treatment to Prolong Storage period up to 45 days and maintaining quality of Balady mandarin fruits under cold storage

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تأثير الكائنات الحية الدقيقة وبعض مضادات الاكسدة على القدرة التخزينية وجودة ثمار اليوسفى تحت ظروف التخزين البارد

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أجريت هذه الدراسة خلال موسمين متتاليين 2016-2017،17-18 لدراسة تأثير كل من الكائنات الحية الدقيقة النشطة وبعض مضادات الأكسدة كمعاملات ما بعد الحصاد على تخزين وجودة ثمار اليوسفى البلدى . حيث تم تخزين الثمار لمدة 45 يوم على درجة ± 1 درجة مئوية والرطوبة النسبية 85-90٪ وتم استخدام 5 معاملات : المعاملة الأولى :الحاكمة (مياه الصنبور) ، المعاملة الثانية : الكائنات الحية الدقيقة (10 ملى / اللتر) ، المعاملة الثالثة : الكائنات الحية الدقيقة + فيتامين اى (10 ملى / اللتر + 1000 جزء فى المليون) ، المعاملة الرابعة : الكائنات الحية الدقيقة + فيتامين سي (10 ملى / اللتر + 1000 جزء فى المليون) و المعاملة الخامسة : خليط الكائنات الحية الدقيقة + فيتامين اى+ فيتامين سي(10 ملى / اللتر + 1000 جزء فى المليون + 1000 جزء فى المليون) .وتم أخذ عينات من كل معاملة بشكل عشوائي كل 15 يومًا. أظهرت المعاملات التى تم تطبيقها بشكل ملحوظ تأثيرا ايجابيا على الخصائص الفزيائية والكيميائية للثمار ومنها فقدان الوزن ونسبة التحلل والفقد الكلي مقارنةً بالكنترول ، وكانت المعاملات المشتركة للكائنات الدقيقة النشطة (EM) ومضادات الأكسدة هي الأكثر فاعلية في تقليل الفقد الكلى خلال فترة التخزين. إلى 5.14٪ و 7.5٪ على التوالي. أثبتت المعاملة المختلطة من EM و VE و VC الحفاظ على الخصائص الفيزيائية لثمار اليوسفي. علاوة على ذلك ، أظهرت نتائج اضرار البرودة و التسرب الايونى نفس التغييرات ، حيث كان لأستخدام الكائنات الحية الدقيقة منفردة او مختلطة تأثيرا ايجابيا على الثمار المخزنة. كما تم تقدير (MDA) كمؤشر على أكسدة الدهون لقياس قدرة الثمار على التخزين ، حيث اظهرت النتائج انخفاض تركيز MDA وكذلك IL مقارنتا بالكنترول ؛ وأوضحت النتائج أيضا انخفاض تركيز H_2O_2 بعد 45 يومًا مقارنتا بالكنترول ، والذي قد يعكس الدور الفعال في الحد من أنواع الأكسجين التفاعلي وتخفيف إصابة التبريد ، بالإضافة إلى انخفاض محتوى الفينول الكلي بشكل ملحوظ بعد 45 يومًا من التخزين البارد ، حيث أظهرت النتائج التي تم الحصول عليها علاقة إيجابية بين حمض الأسكوربيك وفقدان الفينولات. كما يتضح من النتائج أن حمض الأسكوربيك قد يمنع تدهور حمض الأسكوربيك الداخلي من التدهور. وكانت هناك زيادة ملحوظة فى نشاط مضادات الأكسدة (DPPH) ، وقد أثرت المعاملات بشكل كبير على مستويات الإنزيمات المضادة للأكسدة الكتاليز (CAT)و أسكوربات البيروكسيديز (APX) . من خلال النتائج المتحصل عليها يتضح ان معاملة ثمار اليوسفى البلدى بأستخدام EM+ V.C + V.E كان لها تاثير فعال فى إيطالة الفترة التخزينية وزيادة القدرة التخزينية للثمار مع الحفاظ على خصائص جودة الثمار لمدة 45 يوم تحت ظروف التخزين البارد .