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Reduction of chilling injury of 'Washington' navel orange fruits by melatonin treatments during cold storage

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ABSTRACT

Cold storage is used to improve the efficiency of storage and handling of orange fruits, but the fruits are exposed to chilling injury (CI). Antioxidant enzymes are part of the antioxidant defence system against CI of 'Washington' oranges during cold storage, which controls storage/handling efficiency. In this study, melatonin (ME) was used on fruits to reduce cold damage. To assess the action of ME influences; fruits were picked from the tree at the commercial maturity stage and divided into two groups that were uniform in size and colour. Then, they were immersed in ME solution at 0 µmol, 10 µmol, 100 µmol or 1,000 µmol for 20 min at 20 ± 1 °C. The treated fruits were stored at 4 ± 1 °C and 95 ± 1% RH for 4 weeks during 2019 and 2020 seasons. The physical and chemical characteristics of the stored fruits were measured every week up to the end of the storage period. Results indicated that immersing fruits in 1,000 µM ME minimised the CI-index and the water loss%, while preserving the orange peel colour (h°) during cold storage. On the other hand, the same treatment caused slight changes in soluble solid content (SSC%), maintained ascorbic acid (AA) content and the stability of total acidity (TA%), enhanced the antioxidant enzymes activities (AEAs) such as ascorbate peroxidase (APX), catalase (CAT), and superoxidase dismutase (SOD) and also reduced the rate of malondialdehyde (MDA) and ion leakage (IL) during cold storage. Moreover, it minimised hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet-}$) production and caused pronounced results to be exhibited in antioxidant capacity. Overall, the 1,000 µM ME treatment for orange fruits afforded more tolerance against cold storage stress.

KEYWORDS: antioxidant enzymes activities, ascorbic acid, catalase, ion leakage, peel colour, superoxide anion, water loss

ABBREVIATIONS: AA, ascorbic acid; AEAs, antioxidant enzymes activities; APX, ascorbate peroxidase; CAT, catalase; CI, chilling injury; H_2O_2, hydrogen peroxide; IL, ion leakage; MDA, malondialdehyde; ME, melatonin; $O_2^{\bullet-}$, superoxide anion; SOD, superoxidase dismutase; TA, total acidity; SSC, soluble solid content.

INTRODUCTION

Fruit species including oranges have gained more popularity recently. They include high content of non-nutritive, nutritive and bioactive compounds such as flavonoids, phenolics, anthocyanins and phenolic acids, as well as nutritive compounds such as sugars, essential oils, carotenoids, vitamins and minerals. They have distinct flavour and taste, excellent medicinal value and health care functions as well (Gundogdu et al., 2014; Ersoy et al., 2018; Engin and Mert, 2020; Kaskoniene et al., 2020). Citrus fruits are the most commercial fruit crop, intended for regional consumption and export. 'Washington' navel orange (*Citrus sinensis*, L. Osbeck) has the most significant importance among other fruit crops in Egypt, where the cultivated area reaches

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127,200 hectares and produces 3,197,046 tons of fruits annually; Egypt is considered the largest citrus exporter and ranked as the sixth-largest producer in the world (FAOSTATE, 2019). The cold storage strategy is broadly applied to extend the post-harvest period of the fruits together with ensuring quality (Koyuncu 2020; Strano et al., 2021). However, low-temperature stress passages cause numerous physiological processes throughout the storage period such as chilling injury (CI) (Jannatizadeh, 2019). Cold storage <10 °C increases CI rates on orange fruit, which appear as brownish pit-like holes in the flavedo, the outer coloured part of the peel (Sun et al., 2019). Chilling stress increases the membrane phase transmission from a fluid liquid-crystalline into a rigid solid-gel directly, and thus induces a significant electrolyte leakage, which results in shortage of intracellular ATP (iATP) that is accompanied by higher activity of lipoxygenase (LOX) and phospholipase D (PLD) enzymes as well as a higher accumulation of reactive oxygen species (ROS); so, membrane integrity loss through higher accumulation of malondialdehyde (MDA) is attributable to per-oxidation of membrane unsaturated fatty acids (Aghdam et al., 2018). Plant cells are preserved towards the effects of ROS by a complicated antioxidant system. This includes lipid-soluble antioxidants, water-soluble reductants and enzymes (Racchi 2013; Hasanuzzaman et al., 2019; Meitha et al., 2020). Therefore, numerous studies were conducted on the CI phenomena by applying different treatments on fruits such as salicylic acid (Sayyari et al., 2009), arginine (Babalar et al., 2018) and polyvinyl alcohol (PVA) mixed with ascorbic acid (AA) (Lo'ay et al., 2019). Also, polyamines, as positively charged compounds, supply an extra stress resistance to plants (Mirdehghan et al., 2007). They can increase the scavenging capacity of ROS because polyamines bond to negatively charged cellular compounds inclusive of proteins membrane phospholipids (Mathaba et al., 2013), and thus enhance the strength and functions of cell wall (Gill and Tuteja, 2010). N-acetyl-5-methoxytryptamine (Melatonin [ME]) was found in all vegetation (Dubbels et al., 1995). ME exhibits an exceptional multiplicity of actions in plants as it is considered to be a beneficial biogenic amine and safe not only as an endogenous and signalling compound for the stress alleviation of biotic and abiotic but also a potent free radical scavenger and antioxidant (Tan et al., 2013; Shi et al., 2015; Lee and Back, 2016). Compared to the classic antioxidants like AA, α-tocopherol and glutathione, ME exhibits a more powerful antioxidant activity (Kükner et al., 2004; Tan et al., 2013). Exogenous ME is involved in the reduction of stress, delaying the senescence of fruit, and preserving the nutritional value of some horticultural crops in cold storage (Aghdam and Fard, 2017; Cao et al., 2018; Liu et al., 2019). The effectiveness of any experimental treatments depends on their capability to enhance the activities of the antioxidant enzymes throughout the storage period (Rivera et al., 2004). So, the aim of this study is assessment the impact of ME treatments on the behaviour of 'Washington' navel orange fruits throughout cold storage for 4 weeks, and also estimation of the quality of the fruits to ascertain their tolerance during the transport process.

MATERIALS AND METHODS

Experimental setup

In order to assess the influences of different concentrations of ME on 'Washington' navel orange fruits, at maturity stage fruits were harvested from a commercial orchard existing in the Dakahlia Governorate, Egypt (30.04°N, 31.25°E). On the same day of harvesting, 1 h later, 600 fruits were selected to be identical in colour, shape and size as well as without any peel defects. They were divided into two main groups to assess the physical and chemical characteristics; each one contains 300 fruits divided into four treatments. Each treatment consists of 75 fruits with three replicates (25 fruits/replicate). Initially, the fruits were washed by distilled H_2O to remove the microbial and dust content loaded from the field. At laboratory temperature, the exogenous ME treatments were applied using four different concentrations (0 mM, 10 mM, 100 mM, and 1,000 mM) by immersing for 20 min (Jannatizadeh, 2019). Treated fruits and control were stored at 4 ± 1°C and 95 ± 1% RH for 4 weeks. All chemical reagents and ME were procured from Sigma, USA.

Physical measurements

CI-index, water loss% and orange peel colour (hue angle, h°)

The CI-symptoms in orange fruits appear as brownish wrinkled areas/spots, where their number and size increase with the duration of cold storage. Based on the orange peel necrosis and the intensity of the browning colour, the CI-index was measured visually in a range of 0 (no CI-symptoms) to 5 (very severe CI-symptoms). CI-index has been determined using the formula following the method of Sayyari et al. (2009); CI (%) = Σ (value of scale) × (number of fruit with the corresponding scale number)/(total number of fruit × 4) × 100. The water loss was determined at the end of each storage utilising the equation following the method of Sun et al. (2019) and it was expressed as a percentage based on the initial fruit weight prior to the cold storage at harvest time. Water loss (%) = [(initial weight – final weight)/initial weight] × 100. The orange fruit peel colour (hue angle, h°) was evaluated by overall storage duration intervals following the method mentioned by Schirra (1992).

Chemical measurements

Soluble solid content (SSC%), total acidity (TA%) and SSC/TA-ratio

At laboratory temperature, SSC% of orange juice was determined utilising the refractometer, a hand digital, Model MASTER-PM Cat. No2393 (ATAGO, Japan).

For TA% determination, 20 ml of orange juice was used to titrate with 0.1 N NaOH. On this basis, the maturity index was assessed, according to Schirra et al. (1998), by calculating the SSC/TA-ratio.

Antioxidant enzymes activities (AEAs)

About five grams of peel tissue was blended with 50 mM phosphate buffer (pH 7.8), 0.2 mM of EDTA and 2% polyvinyl polypyrrolidone (PVPP). After centrifugation 30,000 × *g* for 20 min at 4 °C, supernatant was recovered and utilised for the activities of antioxidant enzymes as described by Aghdam and Fard (2017). For determination of catalase (CAT), 3 ml of the reaction mixture (12.5 mM H_2O_2, 0.2 ml of enzyme extract and 50 mM (pH 7.0) phosphate buffer) was used. One unit of CAT enzyme activity was identified based on a decreasing of absorbance at 240 nm per min, where the decomposition of H_2O_2 has been occurring. For determination of ascorbate peroxidase (APX), 3 ml of the reaction mixture (0.1 ml of enzyme extract, 9 mM AA, 50 mM phosphate buffer (pH 7.0) and 12.5 mM H_2O_2) has been used. Following the decrease in absorbance at 290 nm due to AA consumption, the APX activity has been assayed. Superoxide dismutase (SOD) activity has been specified based on its capability to prevent the nitro blue tetrazolium (NBT) photochemical reduction. For SOD determination, 3 ml of the reaction mixture (14 mM methionine, 50 mM phosphate buffer (pH 7.8), 1 mM (NBT), 3 mM of EDTA, 0.1 ml of enzyme extract and 60 mM riboflavin) was used. Through observing the absorbance at 560 nm, the blue formazan formation was detected. One unit of superoxide dismutase activity was identified as the enzyme amount which inhibits the reduction of NBT to the extent of 50%. For peroxidase (POD) activity, 3 ml of the reaction mixture, including of phosphate buffer (pH 6.0), enzyme extract, 0.1% guaiacol and 2% H_2O_2, was used after incubation for 5 min. The absorbance of mixture was recorded at 460 nm, where one unit was displaying an increase of absorbance per minute (Tian et al., 2005). Determination was calculated as Unit · g^{-1} protein based on the total soluble protein in samples by the method of Bradford (1976).

MDA accumulation, ion leakage (IL%) and AA

MDA content was assessed following the procedure depicted by Iturbe-Ormaetxe et al., (1998) using thiobarbituric acid method. About 2.5 g of grinded orange flavedo was blended with a mixture of 500 µl of butylated hydroxytoluene (2%, w/v) as well as 25 ml of metaphosphoric acid (5%, w/v) well in ethyl alcohol. Through determining the 1,1,3,3-tetraethyoxypropane in the range from 0 mM to 2 mM of TBARS that has been equal to MDA in the range from 0 Mm to 1 Mm, the calibration curves have been obtained. Stoichiometrically, tetraethyoxypropane is transformed into MDA through the acid-heating step of the experiment. Regarding the IL% determination; 5 g of orange sample (peel) was cut into discs and washed using demineralised water (three times) and placed in 20 ml of 0.4 M mannitol for 3 h at 24 °C; then the sample was measured as the initial electrical conductivity of the solution (EC1). After that, the sample was cooked at 100 °C for 30 min in a water bath to measure the final leakage after leaving the sample at room temperature (EC2). The IL was calculated based on IL (%) = (EC1/EC2) × 100 (Hakim et al., 1999). AA has been estimated by a titration process employing 6% oxalic acid and 2,6-dichlorophenolindophenol reagent (AOAC, 1995).

H_2O_2, $O_2^{\bullet-}$ production rate, and antioxidant capacity (DPPH%)

The H_2O_2 content was determined following the procedure depicted by Xu et al. (2012). One gram of peel tissue has been added to 5 ml acetone. After centrifugation 6,000 × *g* for 15 min at 4 °C, the clear extraction has been packed. We added 1 ml of the latter clear extraction to 0.2 ml ammonia and 0.1 ml titanium sulphate (5%), and then centrifuged 6,000 × *g* for 10 min at 4 °C. The pellets (titanium-peroxide complex) that formed have been dissolved in 3 ml of sulphuric acid 10% (v/v) and centrifuged 5,000 × *g* for 10 min at 4 °C. The absorbance of the resulting supernatant was determined at 410 nm. Using H_2O_2 as a standard curve, the H_2O_2 content was calculated and then expressed as mmol · min^{-1} · g^{-1} fresh weight (FW). $O_2^{\bullet-}$ production rate was determined through the formation of nitrite from NH_2OH in the presence of O_2 according to the procedure depicted by Yang et al. (2011). At 530 nm, the absorbance has been recorded. To determine the production rate of $O_2^{\bullet-}$ from the reaction equation of NH_2OH with O_2, a standard curve with NO_2 was used. The production rate of $O_2^{\bullet-}$ has been identified as mmol · min^{-1} · g^{-1} FW. Regarding the antioxidant capacity, 3 g peel tissue was added to 30 ml methyl alcohol and then centrifuged at 10,000 × *g* for 15 min. The resulting clear extraction (1 ml) was added to 3 ml of 0.1 mM 2,2- DPPH that dissolved in methyl alcohol. At room temperature, the reaction mixture was incubated in dark for 20 min. Using a spectrophotometer, the absorbance has been determined at 517 nm and consequently the DPPH radical scavenging impact has been recorded. The DPPH% of the sample has been determined (Cao et al., 2018) as follows: [(Abs control − Abs sample)/Abs control] × 100.

Statistical analysis

The average data of two growth seasons (2019–2020) for the present study has been examined statistically. The Co-Stat software package, Ver. 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93940, USA) was used. Data have been analysed as a completely randomised design (CRD) with three replications. The means of all examined treatments results have been contrasted utilising Duncan's Multiple Range Test at $p \leq 0.05$.

RESULTS AND DISCUSSION

CI-index, water loss%, and orange peel colour (hue angle, h°)

All ME treatments showed a higher keeping of fruit peel colour (h°) and a significant decrease in the CI-index and water loss% compared to the untreated fruits (control) during cold storage for 4 weeks. Results of CI-index, water loss% and orange peel colour (h°) as physical properties of the 'Washington' navel orange fruits at cold storage intervals up to 4 weeks are shown in Figure 1. The current results indicated that the 1,000 mM ME treatment caused the lowest value of CI-index compared to the other treatments. Such treatment recorded (1.02) at the 3rd week, and then developed slightly to (1.03) at the end of storage time, while the control treatment presented the highest value (4.09) at the end of storage period. With respect to the water loss% and the orange peel colour (h°), the same treatment resulted in a significant decrease of the water loss% (3.84%) and a slight decrease in the peel colour of the fruits (h° = 69.67) compared to the

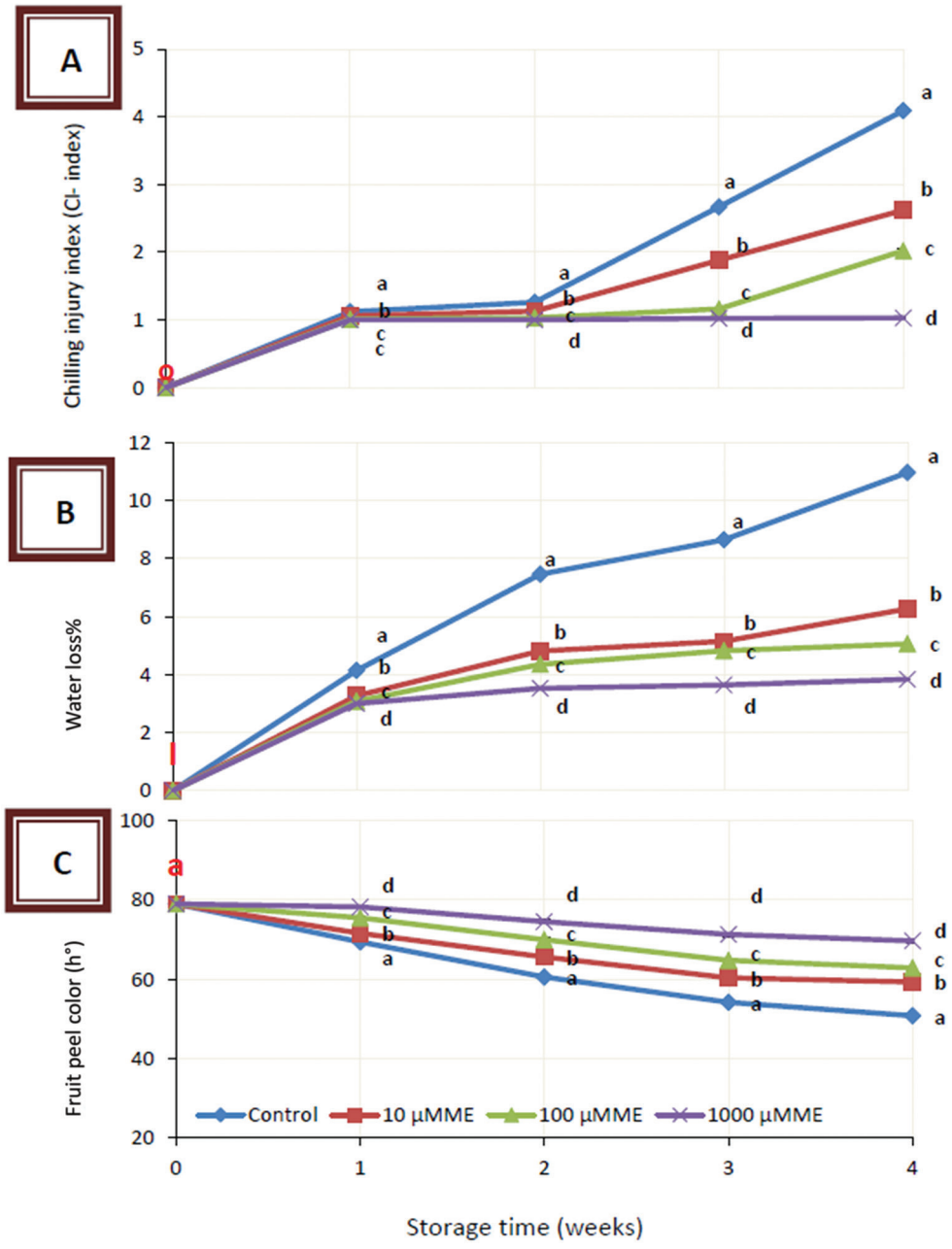


Figure 1. The effect of ME treatment at four different concentrations on (CI-index), water loss% and fruit peel colour (h°) of 'Washington' navel orange fruits during cold storage at (4 ± 1 °C and 95 ± 1% RH) for (4 weeks). The vertical bars represent standard error (±SE of $n = 3$) and the alphabetical letters pointed to the significance at $p = 0.05$ between treatments in each storage period. The average of both experimental seasons (2019 and 2020) was analysed by using Duncan's Multiple Range Test. CI, chilling injury; ME, melatonin.

control, which recorded the highest value of water loss% (10.97%) and a sharp decrease in the peel colour (h° = 50.75) at the 4th week of the cold storage period. The variation in physical parameters is due to the effect of low temperature stress, which generates ROS during long-term cold storage (Lo'ay and Doaa, 2020). The most ROS produced is the hydroxyl radical ($OH^{\bullet}$), which reacts with lipids and proteins of the plasma cell membrane to produce the MDA (Hodges et al., 2004) and protein carbonyl groups (Wang, 2006). After that, the cell membrane loses its structure and functions (Lo'ay et al., 2019), and then the cell dies (Foyer et al., 2017). More generation and accumulation of ROS is associated with more activity of membrane-degrading enzymes such as phospholipase (Aghdam et al., 2018) and LOX (Jannatizadeh, 2019). Consequently, the CI incidence symptoms appear (Lo'ay and Doaa, 2020), which depend on the equilibrium between ROS formation and antioxidant system performance under minimum temperature (Hodges et al., 2004). ME is more effective for membrane safety because it directly scavenges the ROS and indirectly improves the activity of the scavenging systems, which leads to a reduction of ROS accumulation (García et al., 2014).

SSC%, TA% and SSC/TA-ratio

The differences in chemical quality characteristics of 'Washington' navel orange fruits affected by ME treatments and cold storage period up to 4 weeks are represented in Figure 2. It was observed that the untreated fruits (control) gave the highest values of SSC% and SSC/TA-ratio, and the lowest value of TA% during 4 weeks of the cold storage compared to the ME treatments. Also, the 1,000 µM ME treatment

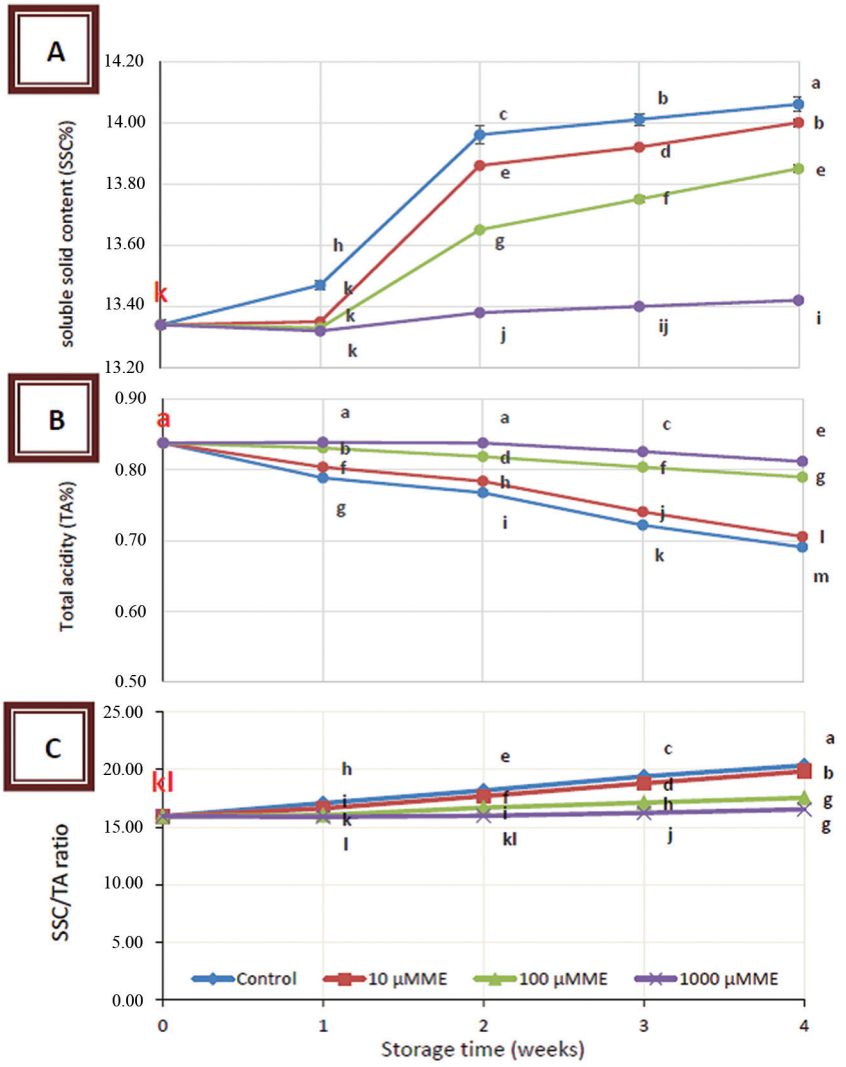


Figure 2. The effect of ME treatment at four different concentrations on (SSC%), (TA%), and SSC/TA-ratio of 'Washington' navel orange fruits during cold storage at (4 ± 1°C and 95 ± 1% RH) for (4 weeks). The vertical bars represent standard error (±SE of $n = 3$) and the alphabetical letters pointed to the significance at $p \leq 0.05$ between treatments in each storage period. The average of both experimental seasons (2019 and 2020) was analysed by using Duncan's Multiple Range Test. ME, melatonin; SSC%, soluble solid content; TA%, total acidity.

provided the lowest changes in SSC% and SSC/TA-ratio over the length of chilly storage compared to other treatments. It recorded 13.42% for the SSC% and 16.53 for the SSC/TA-ratio, while maintaining the stability of TA% at 0.812% after 4 weeks of the cold storage. The variances in SSC% and TA% measurements of stressed orange fruits through cold storage may occur because of the conversion of organic acid into sugar (Baldwin et al., 1995). Also, an increase in SSC/TA-ratio during the storage period may occur because of decreasing AA amount and acidity (Wang et al., 2014) and increasing starch enzyme activity (Brizzolara et al., 2020) under prolong storage duration. The same results have already been observed in lime (Verma and Dashora, 2000) when the fruits are stored at chilled temperatures.

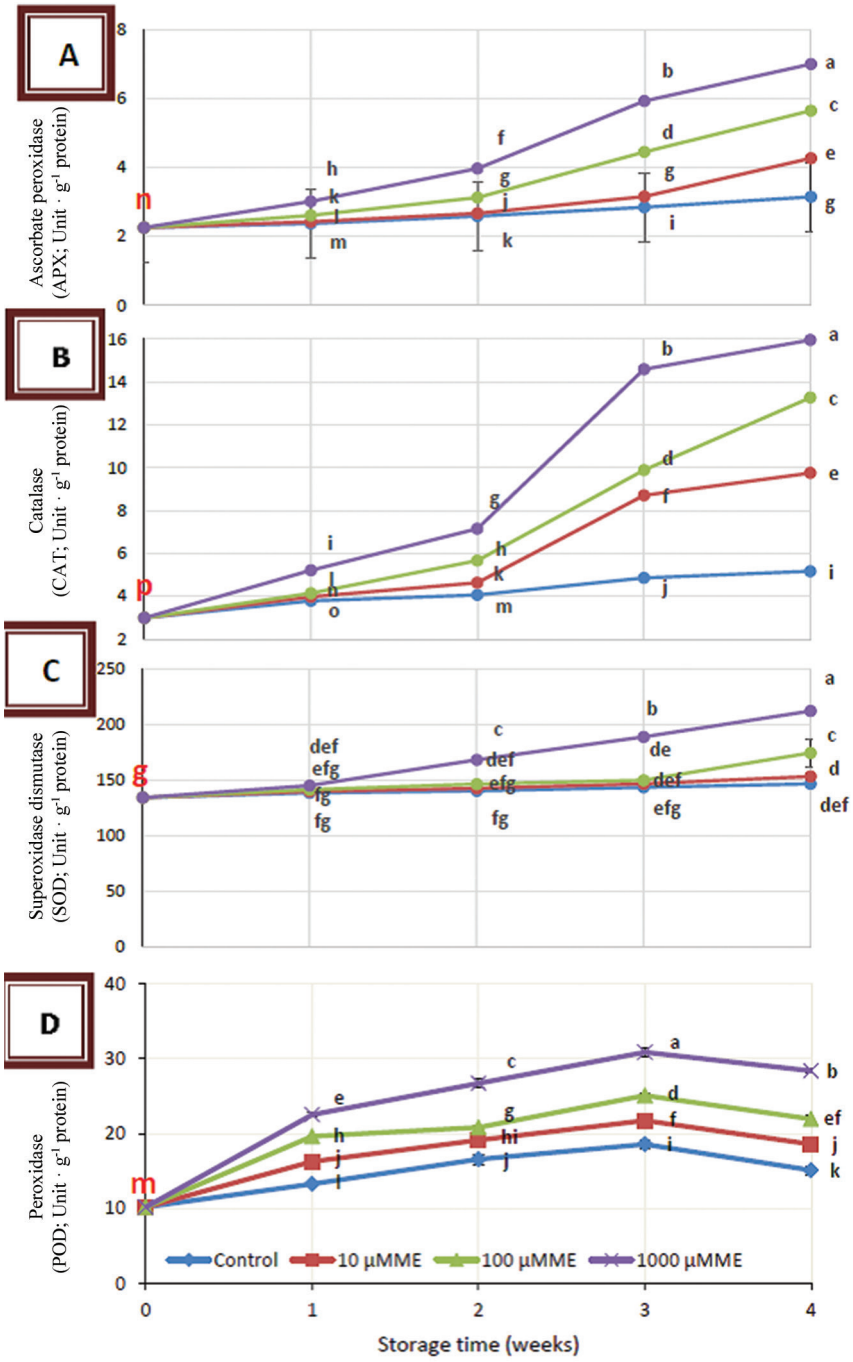


Figure 3. The effect of ME treatment at four different concentrations on AEAs of 'Washington' navel orange during cold storage at (4 ± 1°C and 95 ± 1% RH) for (4 weeks). The vertical bars represent standard error (±SE of $n = 3$) and the alphabetical letters pointed to the significance at $p \leq 0.05$ between treatments in each storage period. The values are the average of both tested seasons (2019 and 2020) was analysed by using Duncan's Multiple Range Test. AEAs, antioxidant enzymes activities; ME, melatonin.

AEAs performance

The change in AEAs in all treatments increased slightly at the 1st week, compared to the initial values, and then became differential from the 2nd week up to the 4th week of cold storage period based on ME concentrations. The activities of antioxidant enzymes, i.e. APX, CAT, SOD and POD in 'Washington' navel orange fruits affected by ME treatments and cold storage period for 4 weeks at p £ 0.05 are shown in Figure 3. The results clearly revealed that the 1,000 mM ME treatment recorded the highest significant values at the 4th week for APX (7.0 Unit · g^{-1} protein), CAT (15.96 Unit · g^{-1} protein) and SOD (212.33 Unit · g^{-1} protein), compared to the untreated fruits, which presented the lowest activities at 3.15 Unit · g^{-1}, 5.17 Unit · g^{-1} and 146.66 Unit · g^{-1} protein, respectively, while the activity of POD gradually increased until the 3rd week (30.86 Unit · g^{-1} protein) and then decreased to 28.37 Unit · g^{-1} protein at the 4th week of cold storage. Our results are in agreement with a previous study which showed that ME is an enhancer of certain antioxidant enzymes (Chao et al., 2012). The antioxidant enzymes are part of the antioxidant defence system against CI of fruits during cold storage, thus improving storage/handling (Foyer et al., 2017). The ME treatments enhanced the activities of the antioxidant enzymes during cold storage by quenching ROS generation (Ma et al., 2016).

Changes of MDA accumulation, IL%, and AA

The level of MDA and IL% increased significantly at p £ 0.05, while AA level decreased dramatically, depending on the cold storage period and ME treatments,

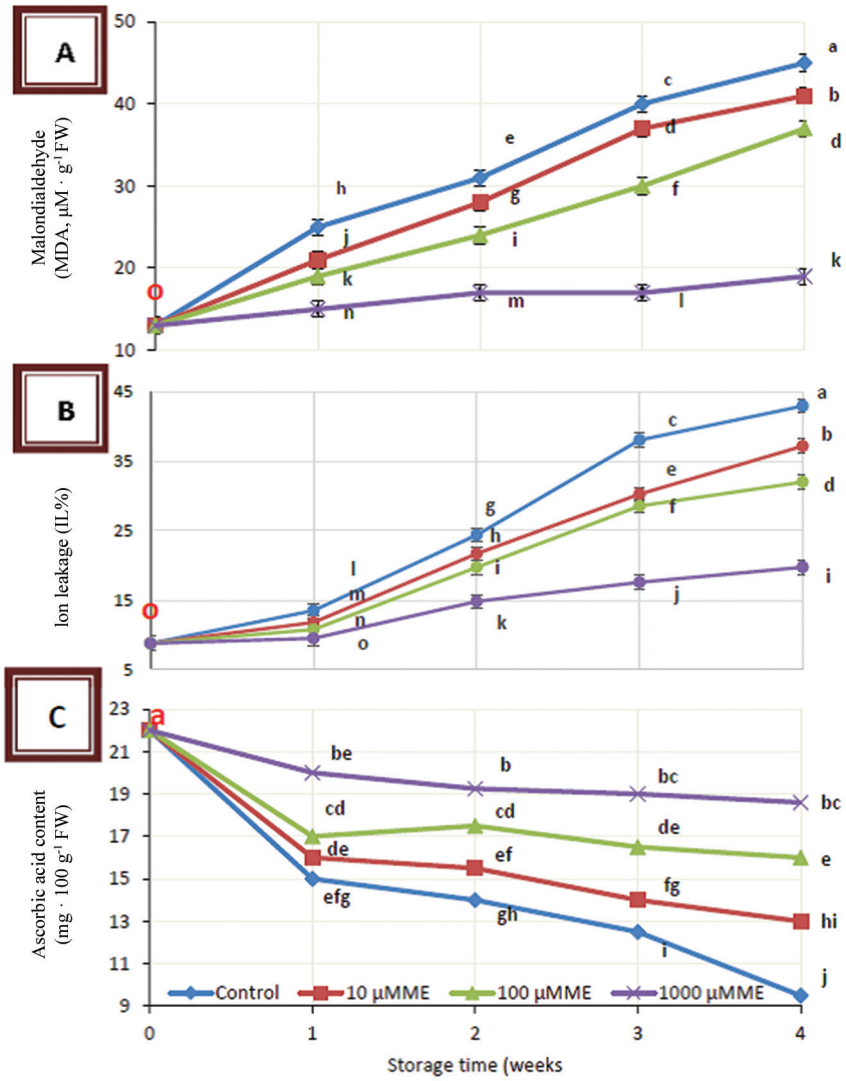


Figure 4. The effect of ME treatment at four different concentrations on the MDA accumulation (µM · g^{-1} FW), (IL%), AA content (mg · 100 g^{-1} FW) of 'Washington' navel orange during cold storage at (4 ± 1°C and 95 ± 1% RH) for (4 weeks). The vertical bars represent the standard error (±SE of n = 3) and the alphabetical letters pointed out the significance at $p \leq 0.05$ between treatments in each storage period. The values are the average of both tested seasons (2019 and 2020) was analysed by using Duncan's Multiple Range Test. MDA, malondialdehyde; ME, melatonin.

compared to the initial values (Figure 4). Moreover, the differences between ME treatments were observed in 1st week and became more obvious within the end of the cold storage duration. The obtained results indicated that the lowest values of MDA (19 mM · g^{-1} FW) and IL% (19.73%) and the highest value of AA (18.6 mg · 100 g^{-1} FW) were more pronounced with the 1,000 µmol ME treatment compared to the control, which showed a reverse trend, including the highest bulk of MDA (45 µM · g^{-1} FW) and IL% (42.90%) and the lowest value of AA (9.5 mg · 100 g^{-1} FW) at the 4th week of cold storage. Generation of ROS during long-term cold storage leads to producing MDA and increasing IL from the cell membrane (Hodges et al., 2004). The results could be associated with an improvement in scavenging of ROS by ME (García et al., 2014), and the consequent reduction in the oxidative response (Purvis, 2004) and cell death (Linster and Clarke, 2008). On the other hand, Gitto et al., (2001) reported that ME can recycle the reduced form of AA and thus increase its antioxidant efficacy. Additionally, tomato fruits treated with ME displayed a significantly high concentration of AA (Liu et al., 2016).

H_2O_2, $O_2^{\bullet-}$ production rate, and antioxidant capacity (DPPH%)

The changes in generation rates of H_2O_2 and $O_2^{\bullet-}$ as well as antioxidant capacity (using DPPH%) affected by ME treatments and the cold storage period up to 4 weeks are shown in Figure 5. The generation rates of H_2O_2 and $O_2^{\bullet-}$ increased perceptibly in all treatments except 1,000 µM ME treatment that increased slightly generation rates of H_2O_2 and $O_2^{\bullet-}$ starting from the 2nd week until the end of the storage period, while recording the lowest rates of H_2O_2 (0.11 mmol · min^{-1} · g^{-1} FW) and $O_2^{\bullet-}$ (0.31 mmol · min^{-1} · g^{-1} FW) in the 4th week compared to other ME treatments and control. The antioxidant capacity of 'Washington' navel orange fruits affected by all treatments and storage period (4 weeks) was registered as the free radical scavenging impact using DPPH reduction% method. Observing

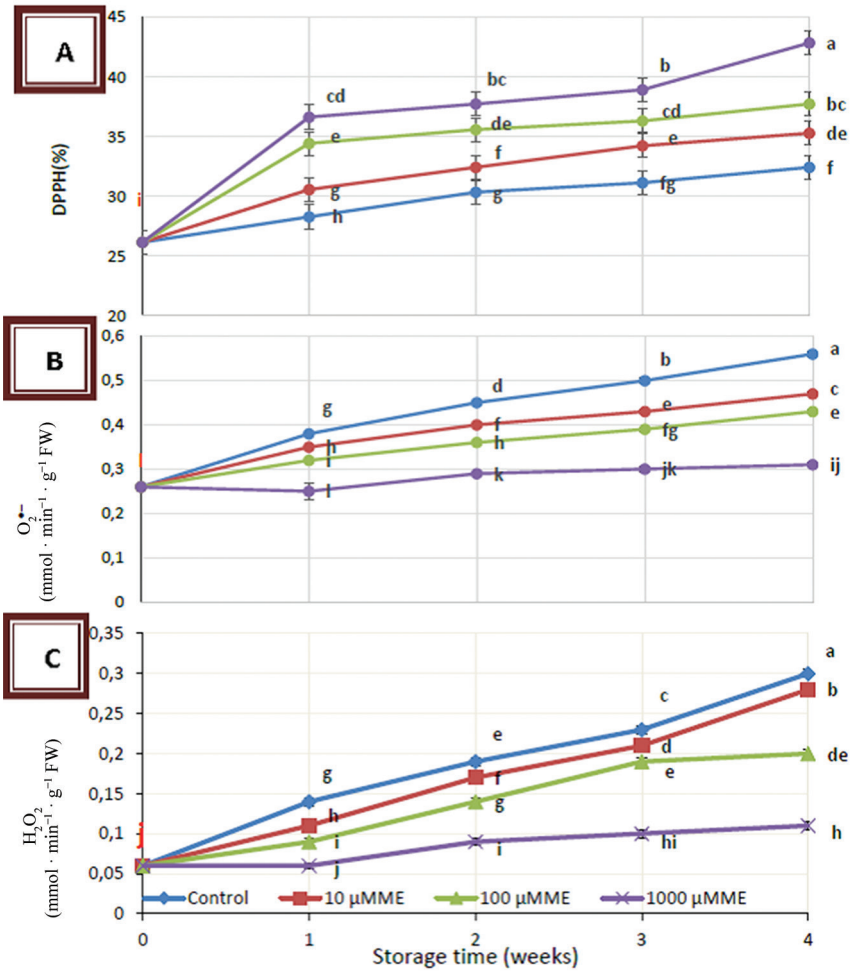


Figure 5. Shows DPPH reduction%, $O_2^{\bullet-}$ (mmol · min^{-1} · g^{-1} FW), and H_2O_2 (mmol · min^{-1} · g^{-1} FW) of 'Washington' navel orange during cold storage at (4 ± 1 °C and 95 ± 1% RH) for (4 weeks). The vertical bars represent the standard error (±SE of n = 3) and the alphabetical letters pointed out the significance at $p \leq 0.05$ between treatments in each storage period. The values are the average of both tested seasons (2019 and 2020) was analysed by using Duncan's Multiple Range Test. DPPH, diphenyl-1-picrylhydrazyl; FW, fresh weight.

the antioxidant capacity, the treated fruits exhibited varying and strong degrees of antioxidant profile compared to untreated fruit (control), which showed the lowest activity with inhibition% at 32.44% in the 4th week. The highest antioxidant activity was revealed by 1,000 µM ME treatment, which exhibited inhibition% at 42.86% in the 4th week and was more effective than the other treatments. The current results are in agreement with Ma et al. (2016) and Rapisarda et al. (2008), who reported that ME may be able to quench the generation of H_2O_2 and $O_2^{\bullet-}$ directly, or through enhancing the activities of antioxidant enzymes during the cold storage. Additionally, the relationship between CAT and APX alongside other antioxidants could be significantly linked to quenching of H_2O_2 and $O_2^{\bullet-}$ (Foyer et al., 2017 and Yang et al., 2011). On the other hand, Aghdam and Fard (2017) stated that strawberry fruits affected by ME treatments demonstrate significantly high DPPH scavenging activity during cold storage.

CONCLUSIONS

The use of 1,000 µmol ME treatment exhibited remarkable tolerance against the cold storage stress, that is represented in minimising both CI-index and the water loss and preserving the orange peel colour (h°) during cold storage duration. Furthermore, the same treatment enhanced the activities of antioxidant enzymes, recorded the lowest values of MDA and IL%, minimised the H_2O_2 and $O_2^{\bullet-}$ production and presented higher antioxidant capacity. Finally, applying ME treatment at 1,000 µmol could be recommended as the best treatment to improve the quality and storability of 'Washington' navel orange fruits during cold storage.

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AUTHOR CONTRIBUTIONS

M. S. A. and L. A. A. conceived the idea for the study and designed the corresponding experiments. M. S. A. and L. A. A. performed statistical analysis and drafted the design chart. M. S. A. and A. M. conducted the field experiments. M. S. A., L. A. A. and A. M. collected and analysed the data. M. S. A. and L. A. A. wrote the first draft of manuscript. M. S. A., L. A. A. and A. M. reviewed and prepared the final draft of manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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