

EFFECT OF EXOGENOUS MELATONIN AND CHITOSAN TREATMENTS ON QUALITY AND BIOCHEMICAL CHANGES OF 'BALADY BANZAHIR' LIMES DURING SHELF LIFE

A. D. Al-Qurashi^{1*} and M. A. Awad^{1,2}

¹Department of Arid land Agriculture, Faculty of Meteorology, Environment and Arid land Agriculture, King Abdulaziz University, P.O.Box. 80208, Jeddah, Saudi Arabia.

²Pomology Department, Faculty of Agriculture, Mansoura University, El-Mansoura, Egypt.

*Correspondence authors' e-mail: aalqurashi@kau.edu.sa

ABSTRACT

Limes undergo metabolic changes during shelf life resulting in rind disorders and green color fading that decrease fruit marketing. To retain fruit quality, some alternatives have been tested in attempts to retain quality and delay senescence during shelf life. In a completely randomized design experiment, the effect of 0.5 mM melatonin (MT) and 1% chitosan (CT) postharvest dipping either alone or in combination on quality of mature-green 'Balady Banzahir' limes during 20 days of shelf life at 23± 1°C and 60–70% RH were evaluated. MT or CT treatments retained fruit quality during shelf life measured as higher green peel color (lower a* values) and titratable acidity (TA) and lower weight loss, total soluble solids (TSS)/TA ratio compared to that of untreated ones. Vitamin C content increased until 8 days in all treatments followed by a dramatic decrease thereafter and was higher in treated fruit than the control. Total flavonoid content (TFC) in peel increased with fluctuation until 16 days but sharply decreased thereafter while, in pulp it decreased with fluctuation in all treatments. In both peel and pulp, treated fruit retained higher TFC content than the control. Antioxidant activity increased with fluctuations in both peel and pulp and was higher in treated fruit than the control. MT or CT treatment showed higher peroxidase (POD) and lower PPO activities during shelf life than the control. However, MT and CT combination treatment provided no further positive effects on most quality parameters. Overall, postharvest dipping in 0.5 mM MT or 1% CT could be an effective treatment to maintain quality of 'Balady Banzahir' limes during 16 days of shelf life.

Keywords: *Citrus aurantifolia* Swingle, Melatonin, Chitosan, Shelf life, Quality

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INTRODUCTION

Limes (*Citrus aurantifolia* Swingle) are one of the most commercially important citrus fruit being consumed almost daily as a drinking juice or a flavoring agent of many cultural cuisines in Saudi Arabia (SA). The green fragrant peel and the pulp with high antioxidants including vitamin C, phenolics, carotenoids and organic acids are important quality parameters that determine fruit freshness and marketability (Rodrigo et al. 2013; Tavallali 2019). Limes are non-climacteric fruit that are harvested and marketed at full mature-green stage with fairly good shelf life. However, limes undergo metabolic changes during shelf life resulting in rind disorders and green color fading that decrease fruit quality and market demand (Rodrigo et al., 2013; Tavallali, 2019). Melatonin (MT) known as N-acetyl-5-methoxytryptamine that was identified in plants in 1995 (Dubbels et al., 1995). It is considered as a phytohormone involved in several processes including enzymes

activities, ripening and senescence of fruit or leaves as well as tolerance to various biotic and abiotic stresses (Arnao and Hernández-Ruiz, 2015 and 2018; Xu et al., 2019). Postharvest dipping in 0.1 mM L⁻¹ of MT effectively delayed ripening, senescence and retained quality of peaches stored at ambient conditions (Gao et al., 2016) and reduced chilling injuries by enhancing antioxidant systems during cold storage (Gao et al., 2018a; Cao et al., 2016). In many fruit, postharvest MT dipping delayed ripening, enhanced antioxidant systems, retained quality of strawberries (Aghdam and Fard, 2017), bananas (Hu et al., 2017), pears (Zhai et al., 2018), cherries (Wang et al., 2019a), pomegranates (Jannatizadeh, 2019), mangoes both at cold storage (Rastegar et al., 2020) and ambient conditions (Liu et al., 2020). In citrus fruit, 1.0 g/l MT dipping retained freshness, vitamin C and TSS and reduced respiration rate and ethanol accumulation in jelly oranges 'Aiyuan 38' during 60 days of cold storage (Wang et al., 2019b). Chitosan, a polysaccharide cationic semipermeable edible coat is widely applied to reduce decay and rates of both

respiration and transpiration, and consequently retain fruit quality and freshness (Kerch, 2015). It has been applied to retain quality of various citrus such as mandarins (Contreras-Oliva *et al.*, 2012; El Guilli *et al.*, 2016; Gao *et al.*, 2018b), tangerines (Chien *et al.*, 2007; Plácido, 2016), oranges (Taghinezhad and Sharabiani, 2018), and limes (El-Mohamedy *et al.*, 2015). However, there is no available studies on the effects of MT either alone or in combination with chitosan on limes quality during shelf life at ambient conditions. The current study aim to evaluate the impact of 0.5 mM MT and 1.0% chitosan as postharvest dipping treatments either alone or in combination on 'Balady Banzahir' limes as an attempt to maintain quality during shelf life at ambient conditions.

MATERIALS AND METHODS

Fresh lime fruit samples and postharvest dipping treatments: In May, 2020 growing season, uniform samples of 'Balady Banzahir' lime fruits were harvested at mature-green stage from a commercial orchard in Najran Province, SA and rapidly transported to the postharvest laboratory at KAU, Jeddah. In the laboratory, again, sound fruit that are similar in shape, size and peel color were selected. A completely randomized experimental design with three replicates (70 fruit of each) were setup for each treatment. Fruit of each treatment/replicate were drenched in either water (control), 0.5 mM MT or 1% chitosan solutions either alone or in combination for 15 min. The wetting agent Tween 20 was included in all treatments formulations at 1ml/l. All treatments/replicates were air dried for 1 h, weighed and stored at 23 ± 1 °C and 85–90% (RH) in perforated cardboard (each of 70 fruit) for up to about 20 days. A separate three replicates (30 fruits of each) for each treatment were stored at the same conditions and periodically weighed at 0, 4, 8, 12, 16, and 20 days for weight loss calculation and expressed in percentage. At the beginning of shelf life (0 day) and after 4, 8, 12, 16, and 20 days, random samples (10 fruit for each) per replicate were collected for quality parameters measurements. Following peel color measurement, samples of both pulp and peel were collected, sliced and stored at -80 °C for enzymes, TFC and antioxidant activity determinations. Additional portion of fruit pulp were directly used for juice extraction for TA, TSS and vitamin C measurements.

Peel color, TSS, TA and vitamin C measurements: Ten fruit (from each replicate) at each time 0, 4, 8, 12, 16, and 20 days of shelf life were randomly selected for the estimation of peel color using a Minolta Chroma Meter CR-410 (Minolta Camera Co. Ltd., Osaka, Japan). The values of L^* , a^* and b^* were measured in the middle of each of the ten fruit/replicate. $\text{Chroma} = (a^{*2} + b^{*2})^{1/2}$

represented the hypotenuse of a right triangle with values ranging from 0 = least intense to 60 = most intense. The chroma values indicate the saturation of the color. TSS and TA contents were quantified in composite juice samples collected from the ten fruit. Vitamin C content was quantified by the method of Ranganna (2000) and results expressed as mg/100 ml⁻¹ juice.

Total flavonoid content: The methanolic extract preparation for both peel (consists of flavedo and albedo) and pulp of the fruit samples were carried out following the procedure described by Awad *et al.* (2017). The filtrated methanol extract was used for TFC and antioxidant activity measurements. TFC was also calorimetrically estimated in the methanol extract as detailed in Awad *et al.* (2017).

Antioxidant activity assay: DPPH radical scavenging of methanol extract for both peel and pulp of fruit was estimated by applying the method of 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Ao *et al.*, 2008) as previously declared (Awad *et al.*, 2017).

PPO and POD activities assays: The crude extract for enzymes measurements in both peel and pulp was prepared as described by Awad *et al.* (2017). PPO (EC 1.14.18.1) activity was spectrophotometrically estimated as previously declared (Jiang *et al.*, 2002) while, the method of Miranda *et al.* (1995) was applied for POD (EC 1.11.1.7) activity estimation as previously explored (Awad *et al.*, 2017).

Statistical analysis: Data statistical analysis were performed by applying a two ways ANOVA for a completely randomized design with 3 replications using SAS program (SAS Institute Inc., 2000, Cary, NC., USA). The least significant differences (LSD) at $P \leq 5\%$ was applied for significant differences comparisons among treatments means. The entire experiment was repeated twice, in order to confirm the reproducibility of the results.

RESULTS AND DISCUSSION

Peel color: There were significant interaction effects between treatments and shelf life periods on all of the measured parameters, thus we passed over the main effects and focused instead on the interactions. Chromaticity L^* (lightness or darkness) of peel increased in the control after 4 days, remain constant up to 16 days followed by a significant increase after 20 days (Fig. 1a). While, in the treated fruit, it remain fairly constant until 8 days, then increased thereafter, and was lower after 20 days than the control. Chromaticity a^* values (–greenness to + redness) of peel gradually increased during shelf life and were lower in all treated fruit than the control (Fig. 1b). After 20 days, MT and CT+MT

treated fruit showed lower a^* values than CT alone. Chromaticity b^* (– blueness to + yellowness) increased in the control up to 8 days and fluctuated thereafter. While, it increased in the treated fruit until 12 days and then decreased thereafter (Fig. 1c). After 20 days, the treated fruit showed lower b^* values than the control. The chroma values had a rather similar trend to b^* value, it increased with fluctuations during shelf life and was lower after 8 and 20 days in the treated fruit than the control (Fig. 1d). There were no significant differences in chroma values among the MT, CT and CT+MT treatments. The loss of peel green color with yellowing due to chlorophyll degradation and carotenoid synthesis reduce fruit freshness and limit marketability of limes (Bisen and Pandey, 2008; Tavallali, 2019). The increase of a^* value during ripening reflects chlorophyll degradation by the action of chlorophyllase, as well as to the accumulation of carotenoid and xanthophyll pigments which lead to decrease of greenness and/or increasing redness (Rodrigo *et al.*, 2013). Our results comply with Zhang *et al.* (2018) where MT treatment slowed the changes in L^* and a^* values and delayed senescence of litchi fruit. However, MT treatment had no effect on L^* , a^* and b^* values of mango peel during cold storage and shelf life while, it increased L^* values of strawberries during cold storage compared to the control (Liu *et al.*, 2018). Our results comply with Plácido *et al.* (2016) where 2% chitosan coating maintained green color and delayed yellowing of tangerines peel during cold storage. On the other hand, 8 g/l chitosan coating showed no effect on ‘Ortanique’ mandarins color during shelf life (El Guilli *et al.*, 2016). Chitosan coating form a semipermeable film on fruit surface and thus regulate gas exchange, reduce respiration and ethylene production rates, delay color alteration and maintain quality of various horticulture commodities such as cucumber and bell peppers (Kerch, 2015) and guava during storage (Hong *et al.*, 2012).

Weight loss: Regardless of treatment, weight loss of lime fruit exhibited a continuous increase during shelf life reaching 16.26% in the control after 20 days, but was lower in treated fruit after 8 to 20 days. MT-treated fruit exhibited lower weight loss than CT after 8, 16 and 20 days (Fig. 2a). The CT+MT treatment showed similar or slightly higher weight loss than each one alone. Generally, a water loss of about 10% of initial fruit weight induce wilting, accelerate browning, membrane disintegration and senescence, and ultimately limit fruit marketability (Robinson *et al.*, 1975; Kays and Paull, 2004; Lufu *et al.*, 2020). In the current experiment, weight loss was around 10% in treated fruit but about 12% in the control after 16 days. At this time, most of the control fruit exhibited peel browning and welting/shriveling symptoms in contrast to treated ones that showed such symptoms only after 20 days.

Postharvest weight loss is generally attributed to both water loss (by transpiration) and respiration (El Guilli *et al.*, 2016; Plácido *et al.*, 2016; Tavallali, 2019). Our results validated those of Rastegar *et al.* (2020) and Liu *et al.* (2020) on mangoes and Gao *et al.* (2016) on peaches in which postharvest MT treatment reduced weight loss, respiration and ethylene production rates during cold storage and ambient conditions. In addition, postharvest 1.0 g/l MT dipping retained freshness and reduced respiration rate and ethanol accumulation in jelly oranges ‘Aiyuan 38’ during 60 days of cold storage. Similar results on weight loss reduction during storage by chitosan coatings have been reported on oranges (Taghinezhad and Sharabiani, 2018), tangerines (Plácido *et al.*, 2016) and mandarins (El Guilli *et al.*, 2016). In contrast, chitosan coating at 0.6%, 1.2% and 1.8% did not affect weight loss of ‘Oronules’ mandarins during cold storage and shelf life (Contreras-Oliva *et al.*, 2012). Chitosan as a semipermeable coat is expected to reduce water loss and delay fruit wilting and shriveling as previously reported on other citrus fruit (El Guilli *et al.*, 2016; Plácido *et al.*, 2016). A low molecular weight chitosan coating at 1.5% reduced respiration rate and weight loss during storage and shelf life of Murcott tangor fruit (Chien *et al.*, 2007).

TSS, TA and TSS/TA ratio: TSS content decreased after 4 days and then slightly increased during shelf life in the treated fruit while, in the control, it remained fairly constant until 12 days and increased thereafter (Fig. 2b). There were no significant differences among the treatments and the control in TSS content at the end of shelf life. TA content dramatically decreased during the first 4 days, gradually increased until 12 days and then decreased thereafter in all treatments. After 8 and 12 days, TA content was higher in treated fruit than the control (Fig. 2c). The TSS/TA ratio dramatically increased during the first 4 days, remained constant until 8 days, then decreased after 12 days followed by a sharp increase thereafter (Fig. 2d). TSS/TA ratio was lower in all treated fruit after 4 and 8 days than the control while, after 12 and 16 days, the MT and CT treated fruit resulted in lower TSS/TA ratio than the control. As non-climacteric fruit, sugars could be synthesized from organic acids by the glycolytic enzymes leading to the increase in TSS content during storage (Rapisarda *et al.*, 2008). The decrease in TA might be due to the use of organic acids as substrate for energy production and alcoholic fermentation during storage (Rapisarda *et al.*, 2008). Exogenous MT application delayed the increase in TSS, and maintained TA of ‘Guifei’ mango fruit during ripening at ambient conditions (Liu *et al.*, 2020). On the contrary, MT application showed no significant effects on TSS and TA of another mango cultivar (Rastegar *et al.*, 2020) and strawberries during storage and shelf life (Liu *et al.*, 2018). Similar to our results, TSS content was

slightly increased in tangerines during one month of cold storage at 10 °C with no effect of chitosan coating (Plácido *et al.*, 2016). Also, chitosan treatment showed no or slight effects on TSS and TA of both ‘Ortanique’ (El Guilli *et al.*, 2016) and ‘Oronules’ mandarins (Contreras-Oliva *et al.*, 2012) during storage. Chien *et al.* (2007) reported that a low molecular weight chitosan coating maintained higher TA and lower TSS of ‘Murcott’ mandarins than the control, in contrast to a high molecular weight chitosan that had no effect. Chitosan coating delayed citric acid breakdown and maintained higher organic acids in mandarins during cold storage (Gao *et al.*, 2018b).

Total flavonoid, vitamin C, and antioxidant activity:

TFC in fruit peel increased with fluctuation until 16 days followed by a sharp decrease thereafter (Fig. 3a). The treated fruit retained higher TFC content in peel than control especially after 4, 16 and 20 days. While, in pulp, TFC decreased with fluctuation during shelf life reaching a lowest level (about 50% of the initial) (Fig. 3b). The treated fruit retained higher TFC content in pulp than control during shelf life especially after 8 and 16 days. Citrus fruit are good source of natural antioxidants including phenolics, flavonoid, carotenoids and vitamin C (Guimaraes *et al.*, 2010; Enejoh *et al.*, 2015; Narang and Jiraungkoorskul, 2016). Our results are in agreement with those of Tavallali (2019) where total phenolic content of both ‘Tahiti’ and ‘Persian lime’ fruit decreased during storage. In a similar study on peaches, Gao *et al.* (2018a) reported that total phenolic content decreased during cold storage but at a lower rate in 0.1 mM MT pre-storage treated fruit. They found that MT treatment enhanced glucose-6-phosphate dehydrogenase, shikimate dehydrogenase and phenylalanine ammonia lyase, activities but inhibited PPO and POD activities which would favor phenolics accumulation. MT dipping has been reported to retain higher total phenolics and TFC in mango (Rastegar *et al.*, 2020) and litchi (Zhang *et al.*, 2018) fruit during cold storage and shelf life. Our results comply with previous studies where chitosan treatment retain higher phenolics by regulating gas exchange and/or by its eliciting properties activating phenolics biosynthesis (Meng *et al.*, 2008; Kerch, 2015). However, chitosan coating at different rates showed no effects on total phenolic and flavonoid of ‘Oronules’ mandarins during cold storage and shelf life (Contreras-Oliva *et al.*, 2012). Moreover, they found that uncoated mandarins stored at 20 °C for 9 days contained higher content of hesperidine, narirutin and didymin than coated one, which could be related to the higher weight loss of uncoated fruit under this storage condition. Vitamin C content gradually increased until 8 days in all treatments followed by a dramatic decrease thereafter to a lowest level at the end of shelf life (Fig. 3c). The treated fruit significantly retained higher vitamin C content than the

control during shelf life especially after 4 to 12 days. Vitamin C represent an important contributor for the total antioxidants activity of citrus fruit (Pretel *et al.*, 2004). In a similar study, vitamin C content of Kagzi limes tended to increase until 18 days followed by a decrease trend thereafter in both coated and uncoated fruit during 24 days storage at ambient conditions (Bisen and Pandey, 2008). Our results on MT are in agreement with Wang *et al.* (2019b) where 1.0 g/l MT dipping retained vitamin C of jelly oranges ‘Aiyuan 38’ during 60 days of cold storage. In other fruits, MT-treatment retained higher vitamin C than the control during cold storage of mangoes (Rastegar *et al.*, 2020) and at ambient conditions of peaches (Gao *et al.*, 2016). Our results comply with studies on other citrus fruit where chitosan treatment maintained higher ascorbic acid of tangerines (Plácido *et al.*, 2016), Kinnow mandarin (Baswal *et al.*, 2020) and pummelo (Nie *et al.*, 2020) during storage than the control. On the contrary, chitosan coatings had no effect on ascorbic acid content of ‘Oronules’ mandarins during cold storage and shelf life (Contreras-Oliva *et al.*, 2012). During the first 4 days, antioxidant activity of peel decreased in the control, increased in the MT and CT+MT but remain constant in the CT treatment (Fig. 4a). Following that, it was increased with fluctuation during shelf life in all treatments. The treated fruit maintained higher antioxidant activity than control during shelf life. In pulp, during the first 4 days, antioxidant activity decreased in the control, but remain fairly constant in the other treatments and thereafter increased with fluctuation in all treatments (Fig. 4b). The treated fruit showed higher antioxidant activity than the control during shelf life. Phenolics, carotenoids and vitamins contents contribute to the non-enzymatic antioxidant system that protect fruit against oxidative stress (Rivera-Cabrera *et al.*, 2010). On the contrary to our results Tavallali (2019) reported that the antioxidant activity of both Tahiti’ and ‘Persian lime’ fruit decreased during cold storage plus 2 days at ambient conditions. In other fruit, our results on antioxidant activity partially contradict with those of Rastegar *et al.* (2020) in which antioxidant activity decreased in mango fruit pulp treated or not with MT during cold storage, but at the end of storage MT treated fruit retained higher antioxidant activity than control. Regarding chitosan, our results are in agreement with those of Petriccione *et al.* (2015) where 1 or 2% chitosan coated strawberries exhibited higher antioxidant activity compared to uncoated ones during 9 days of storage at 2 °C. However, chitosan coating had no effect on the total antioxidant capacity of juice of ‘Oronules’ mandarin stored for 9 days at 20 °C (Contreras-Oliva *et al.*, 2012). Moreover, they found that 0.6 and 1.2% chitosan coated-fruit exhibited lower antioxidant capacity than uncoated ones during cold storage and shelf life.

POD and PPO activities: POD activity in peel decreased with fluctuation until 16 days and then increased to similar levels to initial after 20 days (Fig. 5a). While, in the treated fruit, it remains fairly constant until 16 days followed by a dramatic increase after 20 days. The treated fruit showed higher POD activity than control especially after 12, 16 and 20 days. While, in pulp POD activity dramatically decreased during the first 4 days to lower level that fluctuated during shelf life in all treatments (Fig. 5b). The treated fruit maintained higher POD activity than the control especially after 12, 16 and 20 days. Antioxidant enzymes contribute to the total antioxidant system that protect cell membranes against ROS, which accumulate because of abiotic and/or biotic stresses during postharvest phase. Our results comply with Rastegar *et al.* (2020) in which MT-treated mangoes showed higher POD activity than the control during cold storage. It has been reported that 0.1 MT gave contradicting results on POD activity in peach fruit during storage where MT decreased POD activity at 1°C, in contrast to storage at ambient conditions (25–28 °C) (Gao *et al.*, 2016 and 2018). However, MT treatment showed no effects on POD activity in pear fruit during ripening at 26 °C (Zhai *et al.*, 2018). Our results comply with other studies where chitosan treatment increased

antioxidant enzymes activities including POD in tomato (Liu *et al.*, 2007) and in green asparagus (Wu and Yang, 2016) during storage. PPO activity in peel decreased during the first 4 days, then fluctuated until 12 days followed by a dramatic increase during 16 and 20 days in all treatments (Fig. 5c). The treated fruit exhibited lower PPO activity than the control after 16 and 20 days. While, in pulp, PPO activity decreased during the first 4 days, then fluctuated until 8 days followed by a dramatic increase in all treatments reaching a maximum level at the end of shelf life (Fig. 5d). The treated fruit exhibited lower PPO activity than the control after 12, 16 and 20 days. PPO is generally associated with phenolics metabolism and tissue browning of fresh horticultural commodities via oxidizing phenolic compounds into quinones during ripening and senescence (Jiang *et al.*, 2002). In confirmation to our results, PPO increased during storage and was lower in MT-treated fruit than the control in mangoes (Rastegar *et al.*, 2020) and in peaches (Gao *et al.*, 2018). The results on chitosan treatment confirm those of Meng *et al.* (2008) on table grapes and Petriccione *et al.* (2015) on strawberries where chitosan coatings retarded the increase in PPO activity, which was associated with maintaining fruit color.

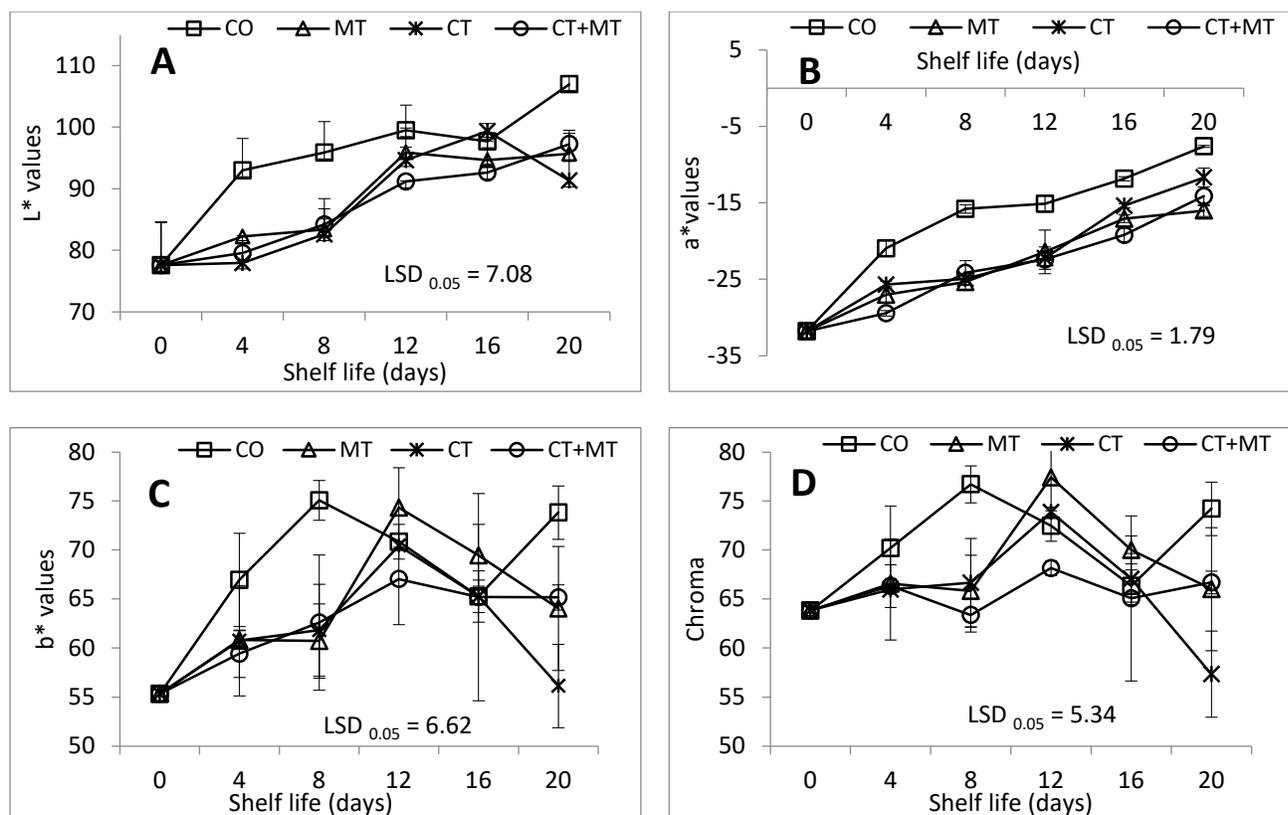


Fig. 1. Peel color parameters L* (A), a* (B), b* (C) and chroma (D) of ‘Balady Banzahir’ limes treated with 0.5 mM melatonin (MT) and/or 1% chitosan (CT) and stored at ambient (23±1 °C and 60–70% RH). Values are means ± SD from three replicates (n = 3). Means with differences within the LSD value are not significantly different $P \leq 0.05$.

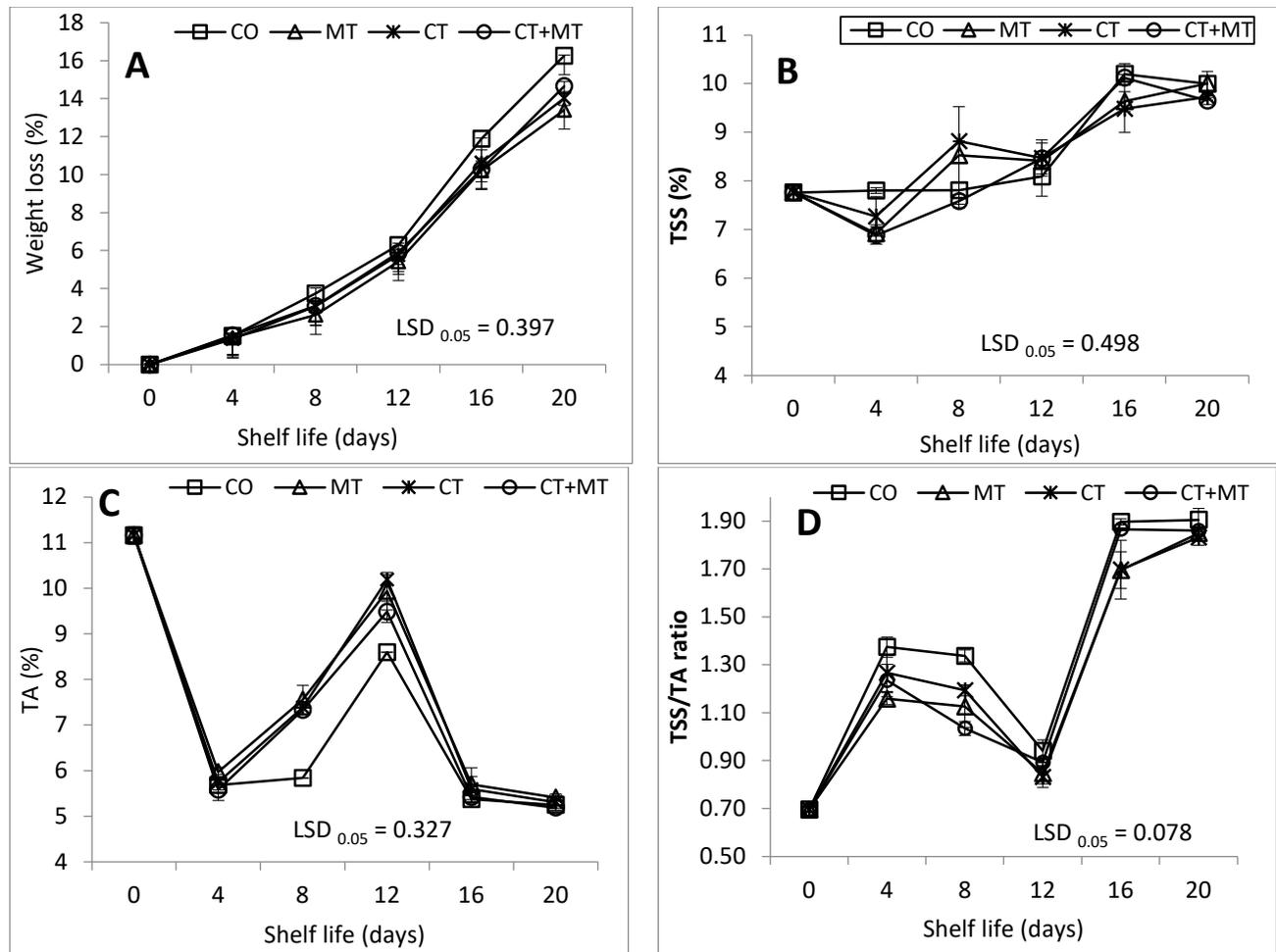
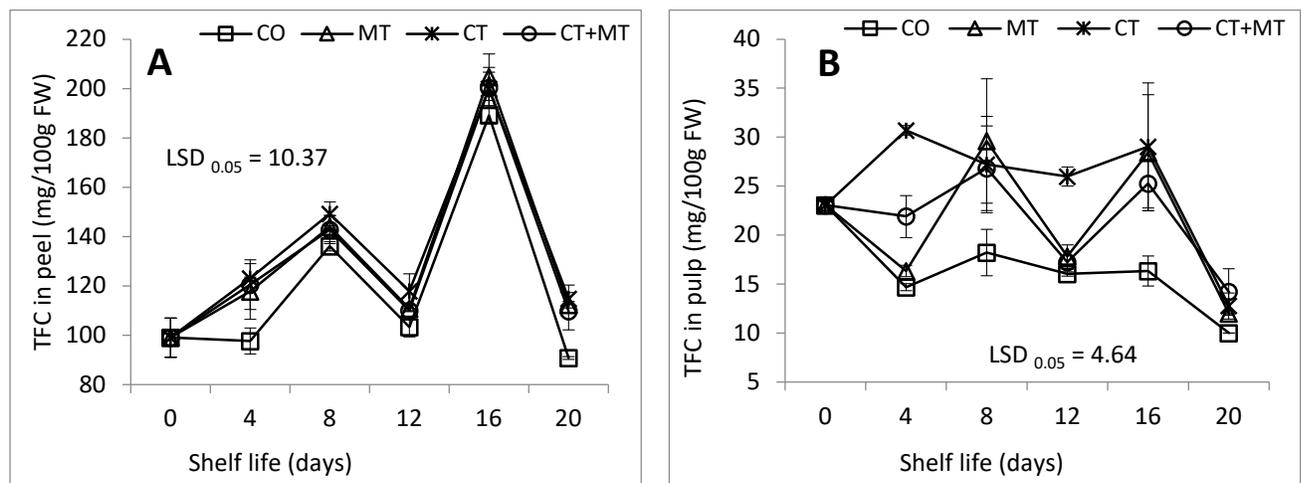


Fig. 2. Weight loss (A), total soluble solids (TSS, B), titratable acidity (TA, C) and TSS/acid ratio (D) of ‘Balady Banzahir’ limes treated with 0.5 mM melatonin (MT) and/or 1% chitosan (CT) and stored at ambient (23±1 °C and 60–70% RH). Values are means ± SD from three replicates (n = 3). Means with differences within the LSD value are not significantly different $P \leq 0.05$.



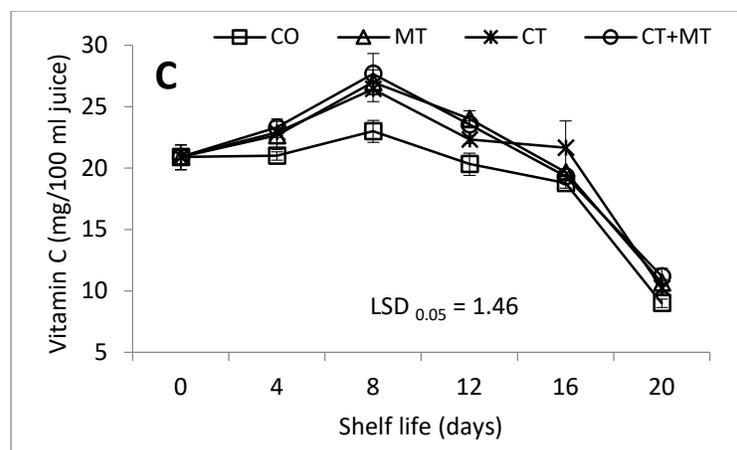


Fig. 3. Total flavonoid content (TFC) in peel (A), TFC in pulp (B) and vitamin C in pulp (C) of ‘Balady Banzahir’ limes treated with 0.5 mM melatonin (MT) and/or 1% chitosan (CT) and stored at ambient (23±1 °C and 60–70% RH). Values are means ± SD from three replicates (n = 3). Means with differences within the LSD value are not significantly different $P \leq 0.05$.

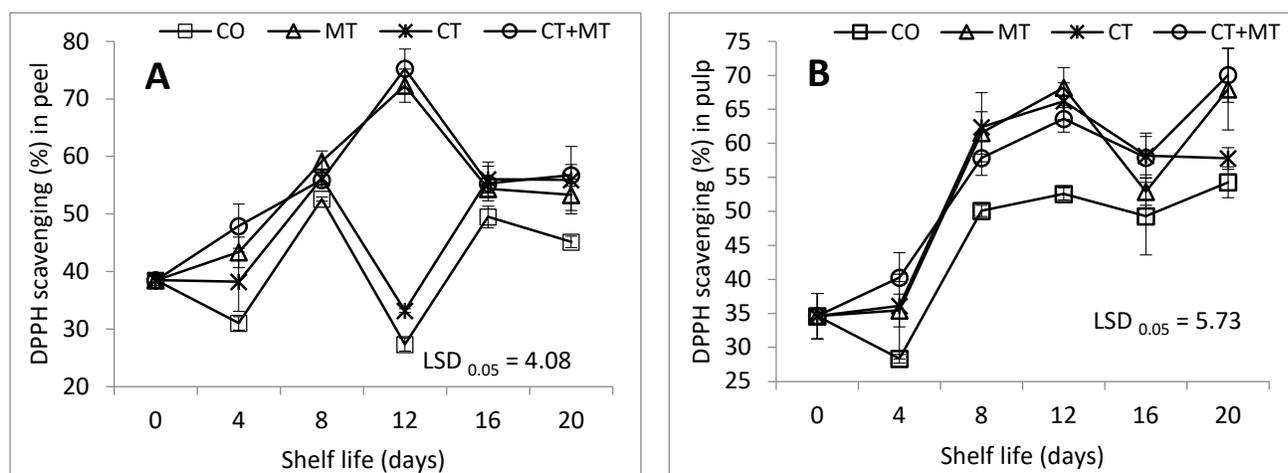
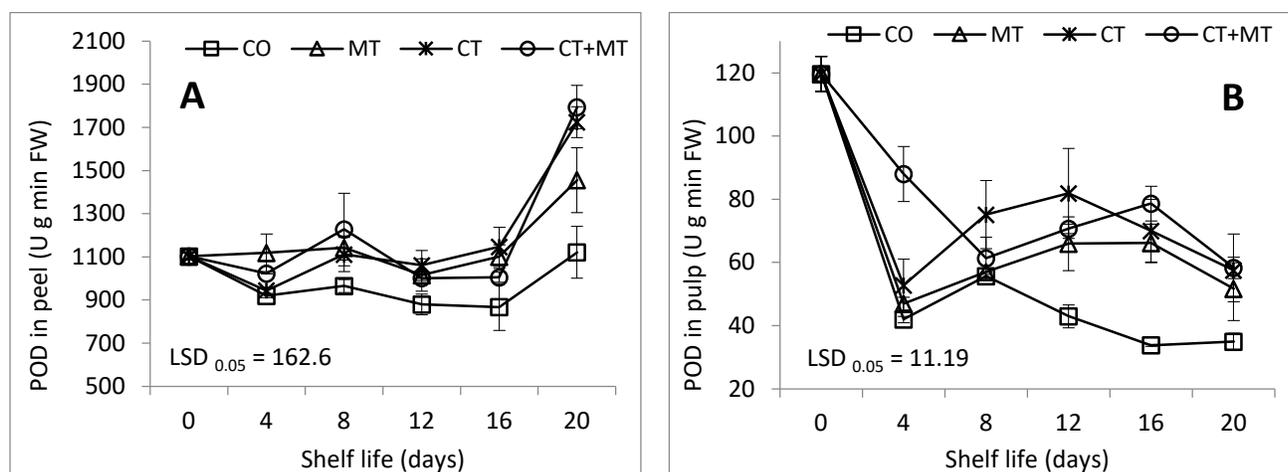


Fig. 4. DPPH scavenging (%) in peel (A) and pulp (B) of ‘Balady Banzahir’ limes treated with 0.5 mM melatonin (MT) and/or 1% chitosan (CT) and stored at ambient (23±1 °C and 60–70% RH). Values are means ± SD from three replicates (n = 3). Means with differences within the LSD value are not significantly different $P \leq 0.05$.



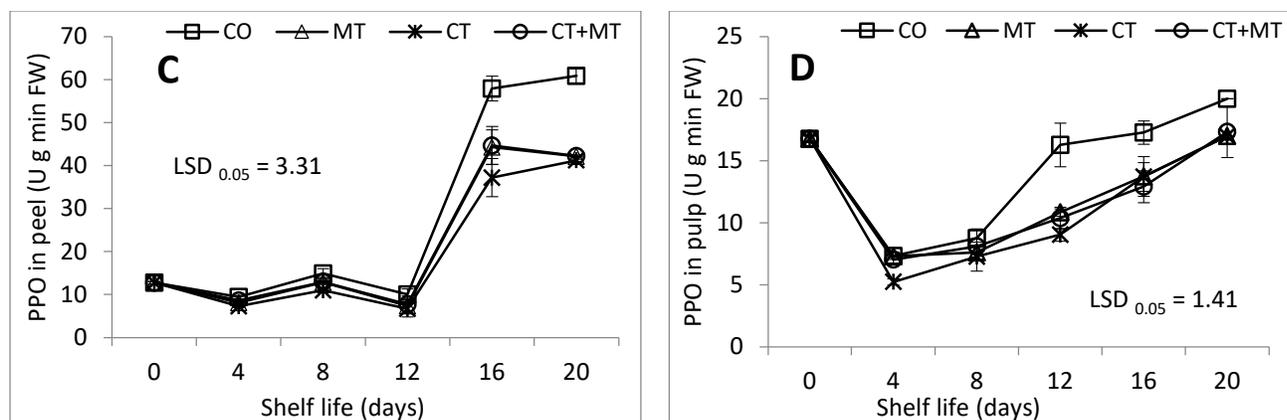


Fig. 5. Peroxidase (POD) in peel (A) and pulp (B), and polyphenoloxidase (PPO) in peel (C) and pulp (D) of 'Balady Banzahir' limes treated with 0.5 mM melatonin (MT) and/or 1% chitosan (CT) and stored at ambient (23 ± 1 °C and 60–70% RH). Values are means \pm SD from three replicates ($n = 3$). Means with differences within the LSD value are not significantly different $P \leq 0.05$.

Conclusion: Our study revealed that postharvest dipping in 0.5 mM melatonin or 1% chitosan could be an effective treatment to retain postharvest quality of 'Balady Banzahir' limes during 16 days of shelf life at ambient conditions via enhancing antioxidant system of fruit. In this context, MT or CT treatments retained green peel color, resulted in higher TA and lower weight loss, TSS and TSS/TA ratio that ultimately enhance fruit marketability compared to the control. In addition, these treatments improved the health benefits from limes by retaining higher vitamin C, and TFC contents and higher antioxidant activity than the control. However, MT and CT combination treatment provided no additional positive effects on most quality parameters of fruit, suggests a need for further study

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Conflict of Interest: The authors declare that they have no conflict of interest.

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