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The role of melatonin and pomegranate seed oil in maintaining the quality of Mexican limes during storage

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ABSTRACT

Mexican limes have a limited shelf life due to color changes and reduced freshness. This study evaluated the effects of melatonin (ML) and pomegranate seed oil (PSO) on maintaining postharvest quality at 20 ± 2°C and 50-60% relative humidity. The results showed that melatonin at 100 µM+PSO and 200 µM+PSO exhibited the highest overall fruit acceptability. The control group displayed the most weight loss (20.8%), while PSO demonstrated the least (12.5%). With the exception of the PSO treatment, all other treatments exhibited significantly elevated levels of phenols, flavonoids, and antioxidants. The PSO and control treatments demonstrated the lowest catalase (69 U/g FW) and peroxidase (53.5 U/g FW) activities, while the highest polyphenol oxidase activity (99.6 U/g FW) was also observed in these groups. The control and PSO treatments also exhibited the highest total soluble solids (TSS) content (8.2%) and the lowest acidity (8.5%). Overall, most traits exhibited significant differences between the treatment groups and the control, excluding the PSO treatment.

Keywords Citrus, Postharvest quality, Shelf life, Antioxidant.

INTRODUCTION

Citrus aurantifolia, is a citrus cultivar extensively farmed in tropical zones (Romero-Romero*et al.*, 2020). Citrus are fruits valued for their color, taste, and nutrients – including sugars, acids, vitamins, fiber, polyphenols, and flavonoids. Storage alters lime quality, causing peel discoloration, weight loss, lowering fruit quality and market value (Chen *et al.*, 2019). This process negatively impacts fruit quality, leading to significant losses in nutritional value, taste, and overall sensory appeal. This ultimately results in a substantial amount of agricultural produce being discarded (Pott *et al*., 2020). Despite the inevitability of fruit overripening, the development of sustainable and innovative postharvest technologies is crucial to delay these changes across the entire supply chain for various fruits.

Melatonin, is a tryptophan-derived substituted indolamine, being found in almost all living organisms. Melatonin, a bioactive compound, has recently become a focus of interest in

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agriculture for its role in regulating plant physiological processes (Arabia *et al.,* 2022). Melatonin plays a crucial role in preserving plant proteins, chlorophyll levels, and photosynthetic efficiency. This ultimately leads to improved postharvest quality in various horticultural products (Madebo *et al.,* 2022). Postharvest treatments are typically performed by immersing the fruit in melatonin prior to storage. In numerous fruits, postharvest melatonin dipping has been shown to delay ripening, enhance antioxidant systems, and maintain the quality of cherries (Wang *et al.,* 2019a) and mangoes during both cold storage (Rastegar*et al.,* 2020) and ambient conditions (Liu *et al.,* 2020). In citrus fruits, dipping in 1.0 g/l melatonin solution preserved freshness, ascorbic acid, and total soluble solids (Wang *et al.,* 2019b).

Pomegranate seed oil (PSO) is a recycled product obtained from the extraction of pomegranate seeds and its production is currently the preferred method for valorizing the non-edible part of pomegranate (Kaseke *et al.,* 2020, Gumus *et al.,* 2020). Furthermore, pomegranate seed oil (PSO), which is rich in conjugated linoleic acid (CLA) including punicic acid, exhibits potent antioxidant activity. Its application as a natural food additive offers the potential for extending fruit shelf life and valorizing agricultural waste (Cortez-Trejo *et al.,* 2021). the presence of minor secondary metabolites with beneficial effects on human health has encouraged the utilization of PSO as a pharmaceutical ingredient in food supplement formulations (Paul and Radhakrishnan 2020). (Teodosio *et al.,* 2018b) examined the impact of *Chlorella sp.* and pomegranate seed oil (PSO) coatings on extending the storage life of *Spondias tuberosa* fruits under refrigeration, finding that these treatments effectively delayed ripening, maintained fruit firmness, and preserved a vibrant green color compared to untreated controls.

Extending the shelf life of lime fruit is crucial for minimizing postharvest losses. This research explores a novel approach by investigating the potential of melatonin to improve the postharvest quality and longevity of lime fruit. The purpose of this study is to investigate how melatonin and pomegranate oil, both individually and in combination, affect the quality and shelf life of Mexican lime fruits. These treatments may provide promising potential in developing sustainable methods for improving fruit quality and prolonging shelf life. This research will advance our understanding of fruit preservation and provide valuable solutions for the agricultural sector.

MATERIALS AND METHODS

Mexican lime samples were obtained from a commercial orchard in Rodan City, Hormozgan Province, Iran, at approximately 57°E and 27°N, at 185 meters. The fruits, collected at the mature green stage, were selected for uniformity and health, then washed for one minute before testing.

Treatment of fruits

This study employed six treatment groups, as detailed in Fig. 1. The fruits were flooded in their designated treatment solutions at ambient temperature for 10 min. Following a 2-hour drying period, the fruits were individually placed in disposable plastic containers and stored. Each treatment comprised 3 replicates, each containing 9 fruits in separate, sealed containers. The containers were maintained at a consistent temperature of 20 ± 2 °C and relative humidity of 50-60% for 24 days.

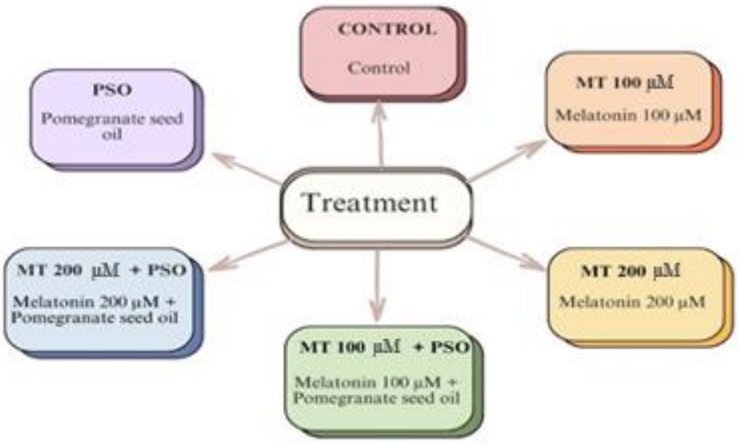


Fig 1. Experimental treatments and corresponding abbreviations.

Overall visual acceptability (OVA)

The study utilized a standardized subjective approach to evaluate the overall visual acceptability (OVA) of the fruits, employing a detailed four-point scoring system derived from established criteria (Mohammadi*et al.*, 2024b) to comprehensively assess visual attributes and overall fruit conditions as detailed in Table 1.

Table 1. Visual acceptability scoring criteria.

Score	Quality description
4 (Excellent)	Fruits exhibit exceptional freshness, firmness, and overall quality. The peel is glossy and free of dehydration, shriveling, or discoloration.
3 (Good)	Fruits are marketable and meet acceptable quality standards despite minimal shriveling and softening.
2 (Not Saleable but Edible)	Fruits display moderate symptoms of shriveling, dryness, color loss (from green to orange), and ripening. Fruits remain edible but lack optimal aroma, taste, and color.
1 (Poor Quality)	Fruits exhibit severe shriveling, darkened peel color, and signs of decay.

Weight loss

The weight of the fruits was measured using a digital scale with an accuracy of 0.01 g and the percentage of weight loss was calculated using formula (1). (Dong and Wang 2018):

$$WL = \frac{W_0 - W_f}{W_0} \times 100 \qquad (1)$$

Where, *WL* represents is the weight loss percentage, W_0 is the initial weight and W_f is the final weight.

Total phenolic content (TPC) and total flavonoid content (TFC)

Fruit juice samples were blended with 80% methanol to extract phenolic and flavonoid compounds. After centrifugation at 4000 ×g the supernatant was collected for further analysis.

The total phenolic content (TPC) of the lime extracts was measured using a modified version of a method by (Azizi*et al.*, 2010). its absorbance at 750 nm was measured using a spectrophotometer (Cecil 2501).

A method (Chang *et al.*, 2002) was used to quantify total flavonoid content (TFC). the absorbance at 415 nm was measured using a UV-visible spectrophotometer.

Antioxidant activity

The antioxidant potential of lime extracts was evaluated using the DPPH assay, following a modified method from (Azlim Almey *et al.*, 2010). the percentage of DPPH radical scavenging activity was computed utilizing the following formula:

$$AA = \frac{A_c - A_s}{A_c}$$

Where, *AA* is the antioxidant activity percentage, A_c is the control and A_s is the sample.

Activities of antioxidant enzymes

Lime peel was homogenized in 20 mL of sodium phosphate buffer containing EDTA, Triton X-100, and PVPP, then filtered and centrifuged. The supernatant was collected as the crude extract for antioxidant enzyme assays.

Catalase (CAT) enzyme activity was measured based on the decrease in hydrogen peroxide absorption at a wavelength of 240 nm.

Peroxidase (POD) activity was measured by the conversion of guaiacol to tetraguaiacol. The reaction mixture included sodium phosphate buffer, guaiacol, H_2O_2, and crude enzyme extract, with absorbance at 470 nm recorded every 30 seconds for 2 min (WANG and HAN 2009).

Polyphenol oxidase (PPO) was extracted from 0.5 g of sample homogenized in phosphate buffer and centrifuged. The reaction mixture, including catechol and phosphate buffer, was incubated, and 60 μL of PPO was added. Absorbance at 420 nm was measured.

Total soluble solids (TSS) and titratable acidity (TA)

The TSS concentration in fruit juice was quantified by employing a digital refractometer (DBR 95) and is expressed as a percentage. TA was assessed through titration of samples using a 0.1 M sodium hydroxide solution until a pH of 8.2 was reached. The outcomes are expressed in terms of citric acid equivalents (Kumar *et al.,* 2021).

Fruit color

The peel color of the fruits was objectively assessed using a Konica Chroma meter CR-400, measuring color characteristics such as hue angle (H°), chromaticity (C), and luminosity (L*), based on the CIE Lab system. While a* and b* values vary from negative to positive and negative to positive, respectively, L* values indicate brightness (Fawole *et al.*, 2020).

Statistical analysis

The study utilized a completely randomized design (CRD) within a factorial experimental framework. Statistical analysis was performed using least significant difference (LSD) tests at $p \leq 0.05$ with SAS software. It should be noted that the nonparametric Kruskal-Wallis test was used for the overall visual acceptance trait. Principal component analysis (PCA) was conducted using the XLSTAT program. For Pearson correlation analysis, the tool available at https://www.bioinformatics.com.cn/en was employed.

RESULTS

OVA

Figs 2a and b illustrate that both control and PSO-treated fruits experienced color changes and quality decline after 24 days. In contrast, fruits treated with a combination of melatonin (ML) and PSO retained better quality and freshness, with minimal color changes. Among the treatments, the combination of different melatonin concentrations with PSO achieved the highest marketability.

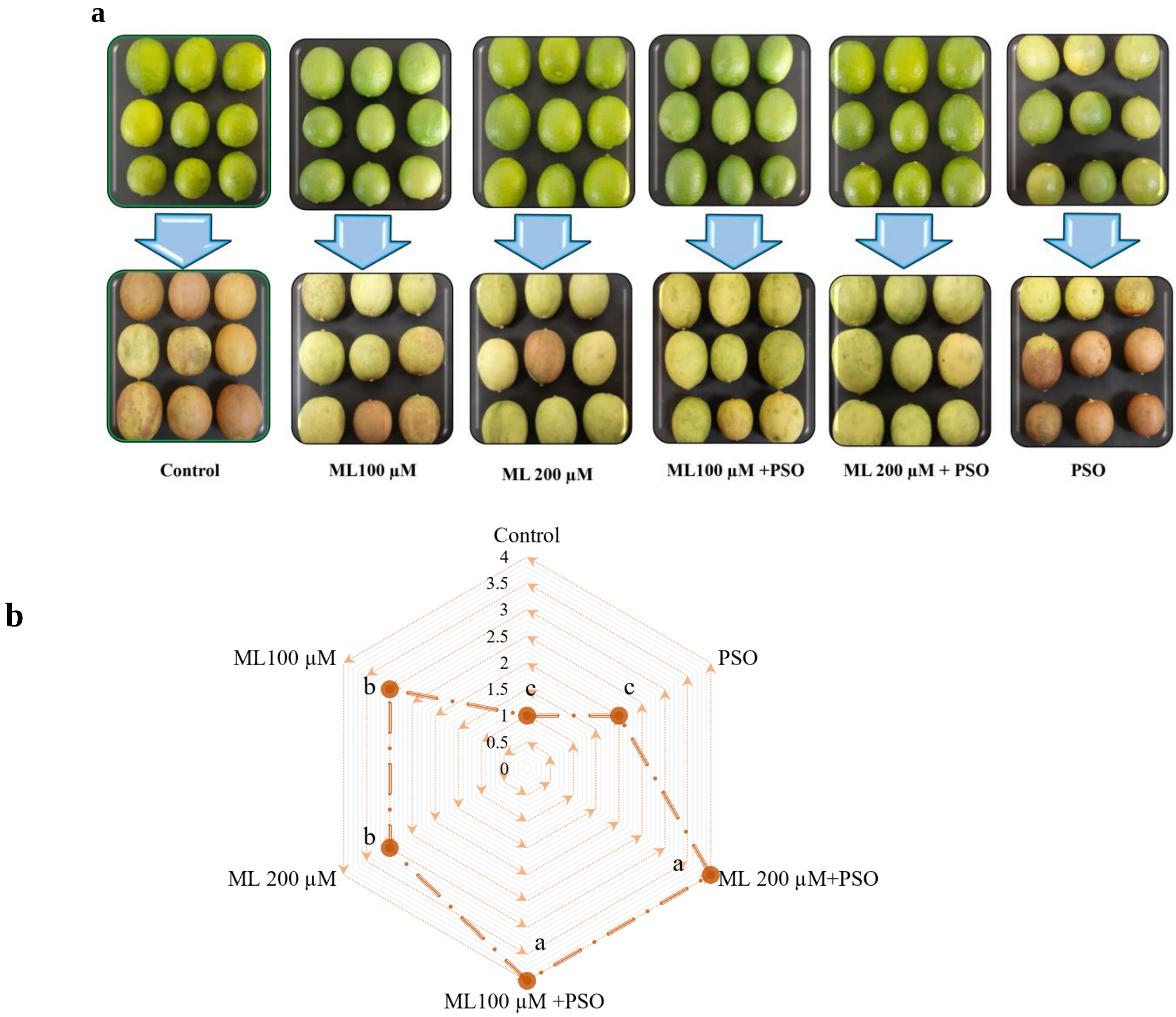


Figure 2. The effect of treatments (distilled water, 100 µM Ml: 100 µM Melatonin, 200 µM Ml: 200 µM Melatonin, 100 µM Ml+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM Ml+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil) on overall visual acceptability of Mexican lime fruit stored for 24 days at 20±2 °C and 50-60% RH.

Weight loss

The proportion of weight loss in treated lime fruit was much lower than that of the control group, according to a comparison of weight loss in stored lime fruit (Fig. 3). The control group exhibited the maximum weight loss at 20.8%, while the minimum weight loss was observed in the PSO treatment at 12.5%.

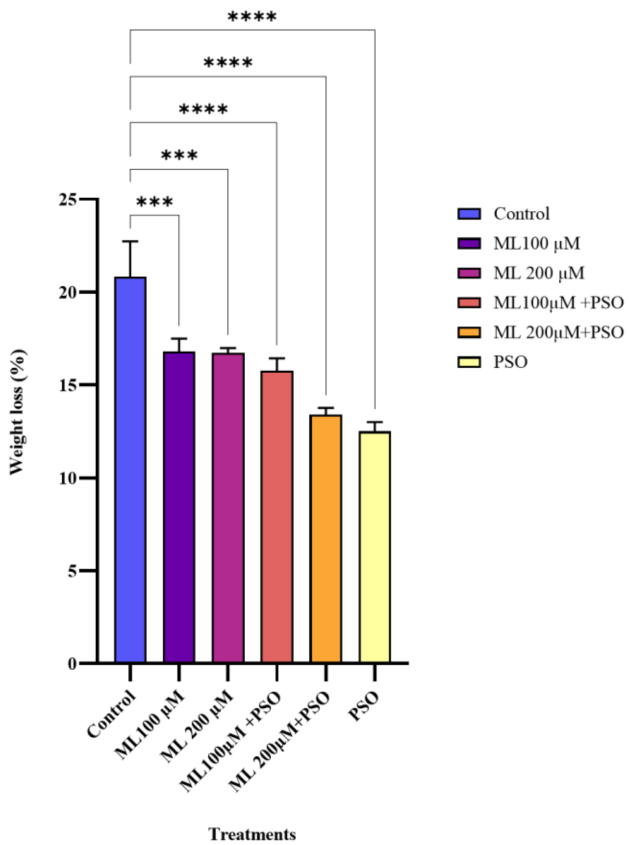


Figure 3. The effect of treatments (distilled water, 100 µM Ml: 100 µM Melatonin, 200 µM Ml: 200 µM Melatonin, 100 µM Ml+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM Ml+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil) on weight loss of Mexican lime fruit stored for 24 days at 20±2 ℃ and 50-60% RH. Data represent mean values of n = 3, and the error bars represent standard errors (SE) of the means. Statistical analysis was performed using an LSD test at $p \leq 0.05$ level.

TPC, TFC, and antioxidant capacity

A general decline in TPC was observed across most treatments during storage, indicative of phenolic degradation. The control and PSO-treated samples exhibited the most pronounced decrease in phenolic levels. In contrast, samples treated with 100 µM ML+PSO demonstrated the highest retention of phenolic content, followed by those treated with 100 µM ML (Fig. 4a).

Fig. 4b shows the total flavonoid content of the fruit after storage. fruits treated with 200 µM and 100 µM ML did not show a notable decrease in flavonoids overall. On the other hand, the total flavonoid content of the PSO-treated and control samples significantly decreased throughout storage. Notably, the control sample had the lowest flavonoid content, at 0.57 mg/g.

Analysis of the results showed that the antioxidant capacity of all samples decreased during storage. This decrease was significantly more pronounced in the control and PSO-treated groups compared to the other treatment groups. In contrast, samples treated with melatonin alone or in combination with PSO showed superior antioxidant capacity retention, with no significant difference observed between these treatments (Fig. 4c).

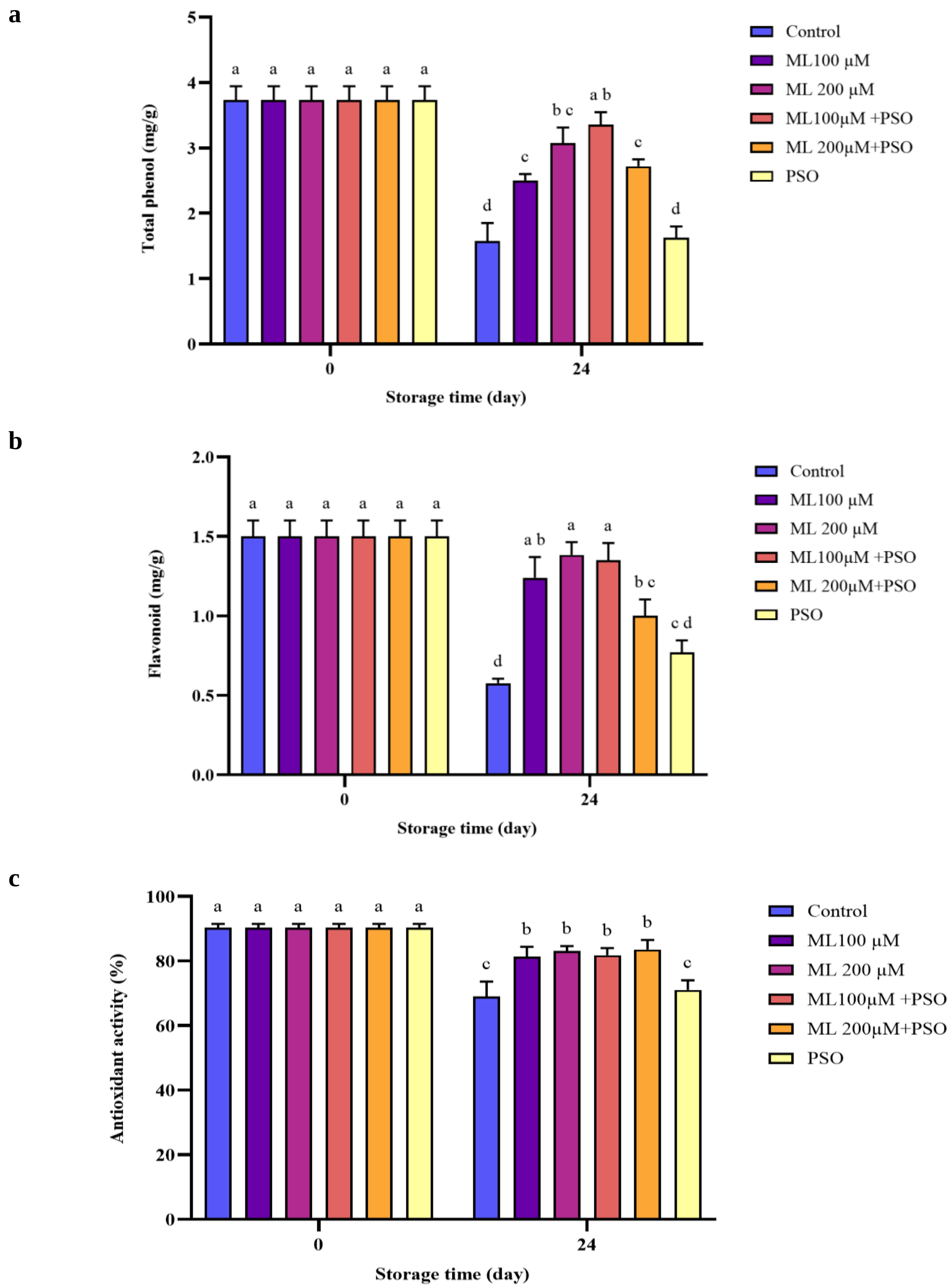


Figure 4. The effect of treatments (distilled water, 100 µM Ml: 100 µM Melatonin, 200 µM Ml: 200 µM Melatonin, 100 µM ML+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM ML+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil) on a) total phenols, b) flavonoids, and c) antioxidant activity of Mexican lime fruit stored for 24 days at 20±2 ℃ and 50-60% RH. Data represent mean values of n = 3 and the error bars represent standard errors (SE) of the means. Statistical analysis was performed using the LSD test at $p \leq 0.05$ level.

The activity of antioxidant enzymes

CAT

CAT activity remained constant in the control and PSO-treated samples throughout the storage period (Fig. 5a). Conversely, fruits treated with other combinations of ML and PSO exhibited a significant increase in CAT activity.

PPO

A significant increase in enzymatic activity was observed in both the control (99 U/mg FW) and PSO-treated samples ((98 U/mg FW)) after 24 days of storage. In contrast, no significant differences in enzymatic activity were found among the other treatment groups when compared to the initial values (Fig. 5b).

POD

After a 24-day storage period, treated fruits showed a rise in POD activity. The highest levels of enzyme activity were shown by the 100 and 200 μM ML + PSO treatments. On the other hand, the control and PSO-treated groups showed a minor drop-in POD enzyme activity in comparison to the original values.

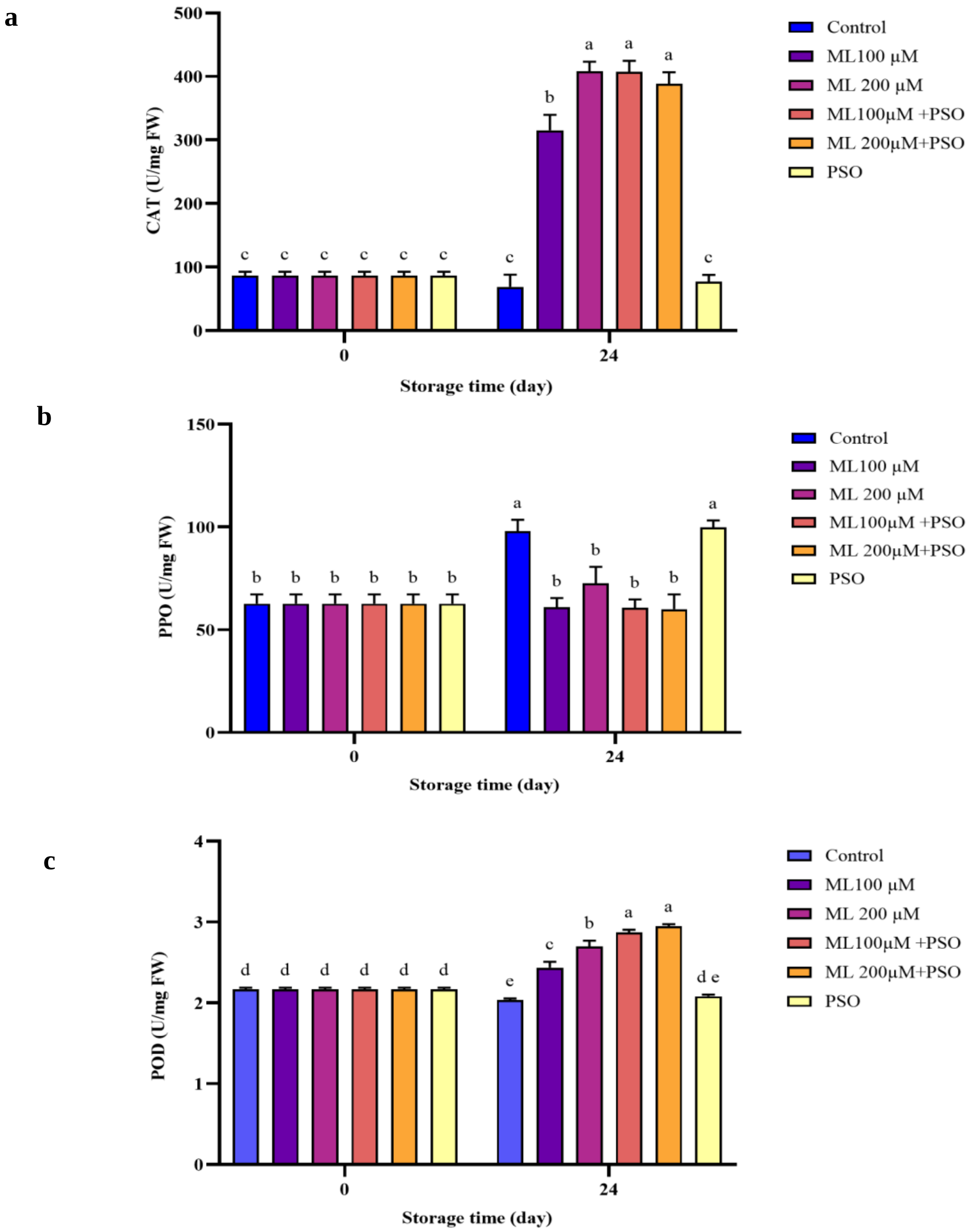


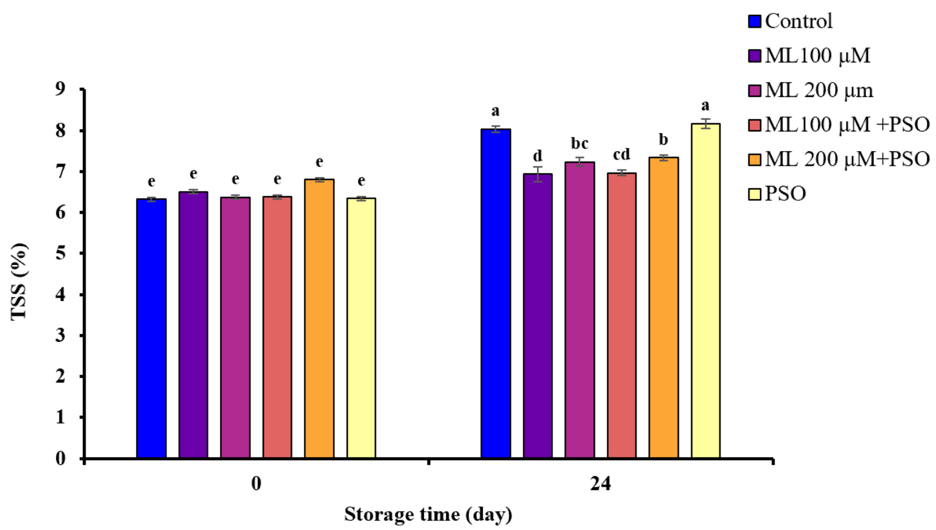
Figure 5. The effect of treatments (distilled water, 100 µM Ml: 100 µM Melatonin, 200 µM Ml: 200 µM Melatonin, 100 µM Ml+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM Ml+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil) on a) catalase, b) peroxidase, and c) polyphenol oxidase of Mexican lime fruit stored for 24 days at 20±2 ℃ and 50-60% RH. Data represent mean values of n = 3 and the error bars represent standard errors (SE) of the means. Statistical analysis was performed using the LSD test at $p \leq 0.05$ level.

TSS and TA

Fig. 6a illustrates a gradual increase in TSS content across all samples during storage. The highest TSS concentrations were observed in fruits treated with PSO (8.16%) and in the control group (8.03%). Conversely, fruits treated with 100 µM ML exhibited the lowest TSS concentration.

A general decline in TA was observed across all samples during storage, as depicted in Fig. 6b. After 24 days of storage, the lowest TA values were observed in both the PSO-treated and control groups. In contrast, fruits treated with 100 and 200 µM ML + PSO exhibited significantly higher acidity compared to the other treatments.

a



b

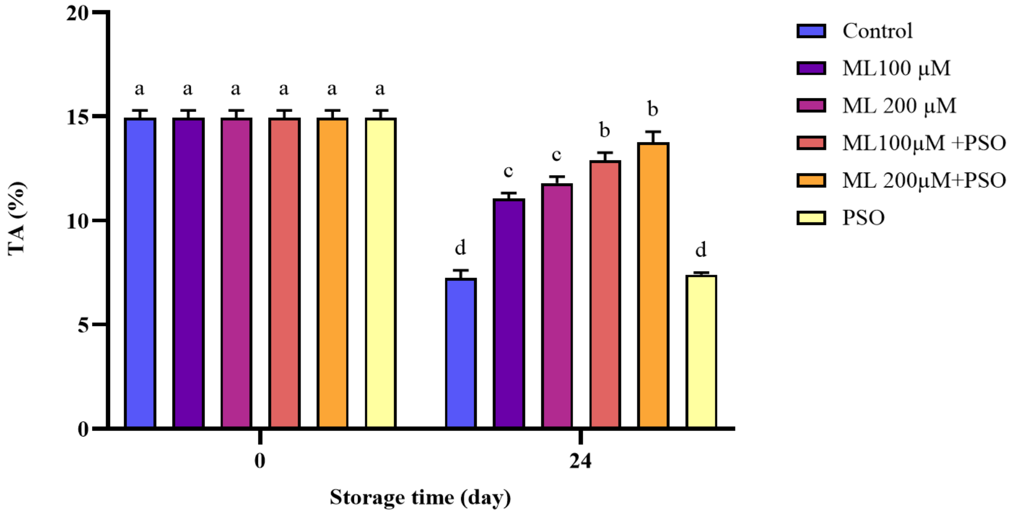


Figure 6. The effect of treatments (distilled water, 100 µM ML: 100 µM Melatonin, 200 µM ML: 200 µM Melatonin, 100 µM ML+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM ML+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil) on a) Total Soluble Solids (TSS) and b) Titratable Acidity (TA) of Mexican lime fruit stored for 24 days at 20±2 °C and 50-60% RH. Data represent mean values of n = 3 and the error bars represent standard errors (SE) of the means. Statistical analysis was performed using the LSD test at $p \leq 0.05$ level.

Color parameters

During storage, color analysis of all samples consistently showed increasing L* values, with the PSO treatment having the lowest L* at the end, while the a* index shifted from negative to positive. The highest a* was found in PSO treatment. The b* index gradually rose, with higher values in the ML 100 µM and 200 µM+ PSO treatments and the lowest in PSO alone. The Hue angle decreased uniformly across all treatments, and Chroma values slightly increased, with lower values observed in the PSO treatment. The color change index (CCI) decreased throughout the experiment, with the control group exhibiting the lowest value at the end.

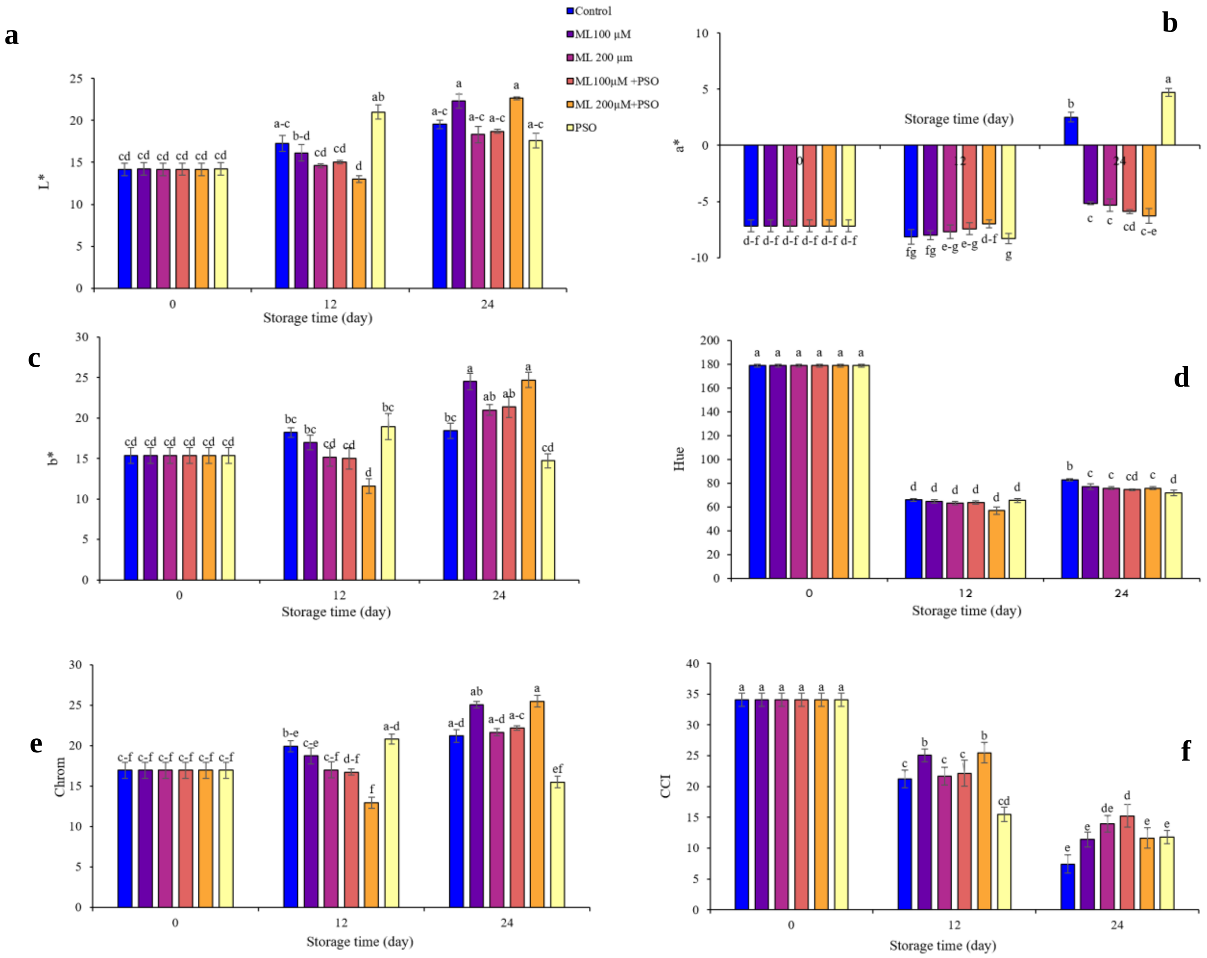
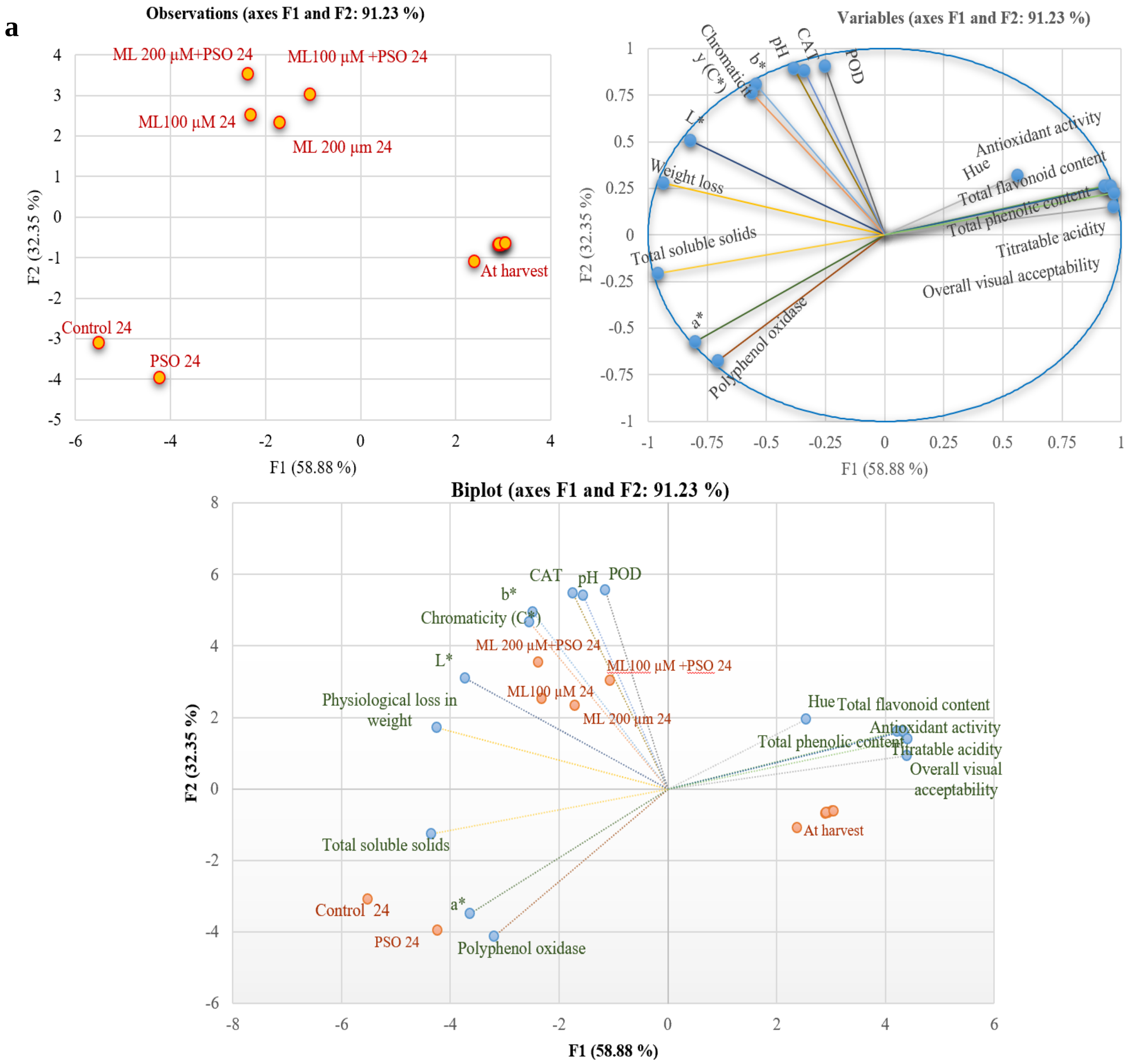


Figure 7. The effect of treatments (distilled water, 100 µM ML: 100 µM Melatonin, 200 µM ML: 200 µM Melatonin, 100 µM ML+PSO: 100 µM Melatonin with pomegranate seed oil, 200 µM ML+PSO: 200 µM Melatonin with pomegranate seed oil, PSO: pomegranate seed oil). on a) L*, b) a*, c) b*, d) Hue, e) chroma, and f) CCI of Mexican lime fruit stored for 24 days at 20±2 ℃ and 50-60% RH. Data represent mean values of n = 3 and the error bars represent standard errors (SE) of the means. Statistical analysis was performed using the LSD test at $p \leq 0.05$ level.

PCA and Pearson correlation

The study employed principal component analysis (PCA) to examine the relationships between various parameters and treatments. F1 explained 58.88% of the variance, showing positive associations with total phenol, flavonoid, titratable acidity (TA), overall visual color (OVC), and antioxidant activity, and negative correlations with weight loss, total soluble solids (TSS), and L* value. F2 accounted for 32.35% of the variance, with positive loadings for catalase (CAT), peroxidase (POD), and pH, and negative loadings for polyphenol oxidase

(PPO) and a* value. The PCA biplot revealed a strong association of PPO with PSO24 and control treatments, while the ML (100 µM) + PSO treatment was closely related to POD. Pearson correlation analysis showed a significant positive correlation between overall visual acceptability and phenol, flavonoid, antioxidant capacity, and TA, with a negative correlation to TSS and PPO.



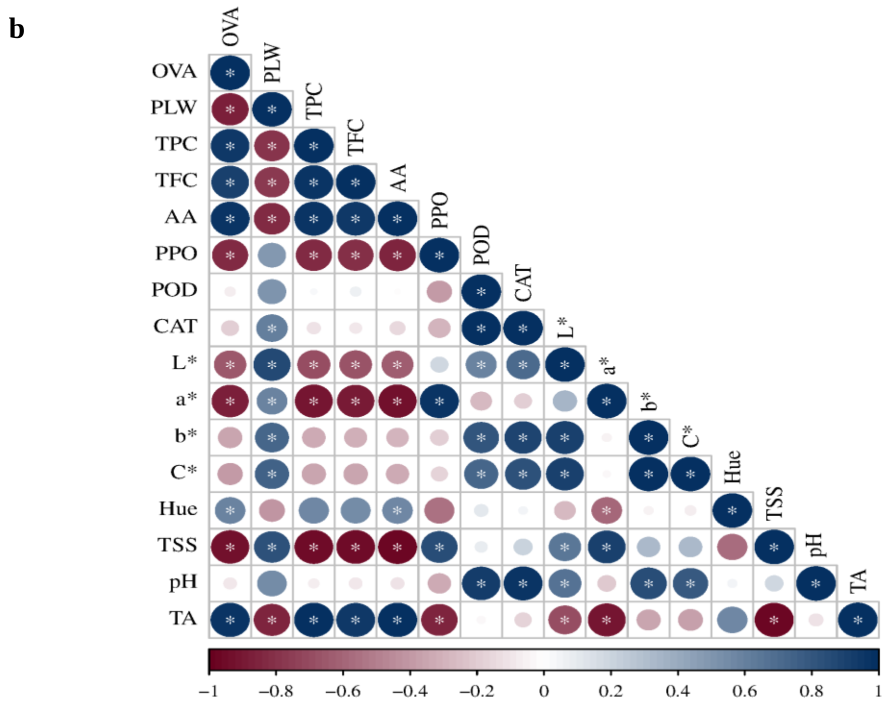


Figure 8. Principal component analysis (PCA) of treatments and variable trait relationships in Mexican lime fruit, including PCA loading plots of the examined variable traits (a). Pearson's correlation coefficients among physio-biochemical traits in Mexican lime under cold stress of 0 °C (a) and -6 °C (b).

Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) computation

TOPSIS, a prominent Multi-Criteria Decision-Making (MCDM) approach, offers a more comprehensive assessment by simultaneously evaluating and ranking treatments based on their performance across all relevant factors. TOPSIS effectively integrates multiple criteria into a single ranking system by considering both the advantages and disadvantages of each treatment. This method determines the distance of each treatment from ideal solutions, allowing for an evaluation of how closely it approaches optimal performance. This comprehensive analysis considers all relevant parameters, providing a more holistic understanding of the treatment options (Heidari *et al.*, 2022). As shown in Table 2. The study employed the TOPSIS technique to evaluate and rank various treatments based on multiple criteria. After 24 days of storage, the highest-ranked treatment for Mexican lime fruit was ML (100 µM) + PSO, with a score of 0.85, followed by ML (200 µM) + PSO, with a score of 0.83**.**

Table 2. The values of indices and their rankings for each treatment.

Score	Rank	Treatment
0.19	6	Control
0.77	4	ML 100
0.80	3	ML 200
0.85	1	ML 100+ PSO
0.83	2	ML 200 +PSO
0.21	5	PSO

DISCUSSION

Visual appeal is key for consumer preference in food, especially limes. Aroma, flavor, and color strongly influence this. Fruit quality assessments often involve evaluating physicochemical parameters and their changes during storage (Carrión-Antolí *et al.*, 2022). Excessive water loss (over 5%) in fruits can significantly diminish their visual appeal, leading to wilting and skin drying. This accelerates aging, reduces marketability, and results in economic losses (Lufu *et al.*, 2020, Liu *et al.*, 2020). Melatonin may preserve fruit quality by acting as an antioxidant, maintaining color, texture, and flavor. Studies show it improves kiwi fruit's appearance by increasing chlorophyll and carotenoid levels (Zhang *et al.*, 2023). By creating an effective barrier against evaporation and oxygen penetration, pomegranate seed oil reduces oxidation and maintains the freshness of fruits, and can be used as a useful ingredient in the formulation of edible coatings to increase the shelf life of fruits (Teodosio *et al.*, 2018a).

Weight loss in fresh produce, primarily due to water loss through respiration and transpiration, indicates quality deterioration. This loss of weight in fresh fruits corresponds to the reduction of water through metabolic processes and product degradation during storage (Ferreira*et al.*, 2020). The mechanism behind this weight loss reduction might be related to the maintenance and improvement of membrane function, as suggested by (Wang*et al.*, 2019a). Melatonin reinforces cell membranes and reduces water loss to help preserve fruit quality (Rastgoo *et al.*, 2024). Furthermore, our findings support the notion that ML treatment can significantly inhibit weight loss in fruits, as observed in kiwi berries (Zhang *et al.*, 2023). In line with our results, (Shahsavari and Rastegar 2024) also found that pomegranate seed oil coating significantly limited and minimized post-harvest weight loss in guava.

Citrus fruits are packed with flavonoids and antioxidant phenolics. These compounds fight oxidative stress by scavenging ROS, boosting the antioxidant defenses and protecting against damage (Promyou *et al.*, 2023). Studies show that melatonin effectively reduces ROS accumulation in fruits by activating both enzymatic and non-enzymatic antioxidants, thus delaying fruit aging (Liu *et al.*, 2025). Similarly, strawberries treated with melatonin exhibit increased levels of total phenolics and flavonoids, leading to delayed ripening (Liu*et al.*, 2018).

Melatonin treatment in peaches enhances antioxidant activity, minimizes oxidative stress, protects cell membranes, and delays aging after harvest (Gao *et al.*, 2016). Finally, studies on jujube have shown that melatonin application increases phenolic compounds, delaying senescence and enhancing overall fruit quality (Wang *et al.*, 2021). Melatonin increases the activity of key enzymes such as phenylalanine ammonia lyase by stimulating the phenylpropanoid pathway. This increased enzyme activity leads to the production of more phenolic compounds (Sharafi *et al.*, 2021). Pomegranate seed oil creates a barrier layer on the surface of the fruit, limiting oxygen penetration, thereby helping to preserve phenolic compounds and reduce oxidative processes. This feature plays an effective role in preserving the quality of fruits after harvest (Chavan*et al.*, 2023)

Our findings are consistent with those of (Tavallali 2018), who observed a decrease in TPC in lime fruits during storage. Similar results have been reported for other fruits, such as mangoes and litchi, where ML dipping preserved higher TPC levels for longer shelf durations. However, (Xu *et al.*, 2022) found a trend toward lower TPC increases in 'Friar' plum flesh following melatonin treatment. (Ma *et al.*, 2021) suggested that the accumulation of phenolic compounds in ML-treated fruits contributes to maintaining postharvest quality and mitigating physiological senescence. Edible coatings containing pomegranate seed oil, by retaining 70% of phenolic compounds, played an effective role in preserving the quality and bioactive compounds of strawberries during storage, which was consistent with the results of our study (Melikoğlu *et al.*, 2022). Research on *Spondias tuberosa* fruits revealed that coatings integrating *Chlorella* sp. with pomegranate seed oil generated a modified atmosphere that significantly slowed the ripening process; specifically, the formulation containing 2.0% *Chlorella* sp. combined with PSO offered the most effective preservation of ascorbic acid and phenolic compounds (Teodosio et al., 2020).

Limes, rich in flavonoids with antioxidant properties, may benefit from ML, which modulates total phenolic content and mitigates physiological senescence (Khatam *et al.*, 2024). Similar to its effect in oranges, where it led to the accumulation of phenolics and maintained postharvest quality, our findings suggest a potential role for melatonin in mitigating physiological senescence in limes. Melatonin's antioxidant function extends beyond direct free radical scavenging, also enhancing the concentration of other antioxidants and antioxidant enzyme activity, ultimately increasing the postharvest life of fruits and vegetables (Ahammed *et al.*, 2020). Melatonin's amphiphilic properties enable it to penetrate cell membranes and neutralize reactive species within cells. Exogenous melatonin regulates ROS levels, preserving cell integrity and reducing oxidative damage (Sharafi *et al.*, 2021). Pomegranate seed oil acts as an antioxidant and prevents nutrient oxidation, moisture loss, and color changes, thus helping to maintain the quality and shelf life of the fruit (Drinić *et al.*, 2020). Studies have also confirmed that PSO can substantially improve the oxidative stability of plant-based oils by enhancing antioxidant potential and slowing down the degradation of beneficial compounds during storage periods (Siraj et al., 2019).

Melatonin, being amphiphilic, easily penetrates cells, neutralizes reactive species, regulates ROS levels, protects cell integrity, and minimizes oxidative damage (Onik *et al.*, 2021). Melatonin treatment in peaches boosts antioxidant enzyme activity, reduces ROS levels, and delays deterioration, highlighting its potential for enhancing postharvest fruit quality (Gao *et al.*, 2016). Melatonin is a powerful antioxidant that can neutralize up to 10 ROS molecules per molecule, significantly surpassing other antioxidants like vitamin C, vitamin E, and glutathione (Galano and Reiter 2018). PPO, which accelerates tissue browning by converting phenolics to quinones, increases with storage but is reduced in melatonin-treated fruits. Studies, including those by (Rastegar*et al.*, 2020) and (Gao *et al.*, 2018), found that melatonin decreases PPO activity in mangoes and peaches. Our results support Marak et al.'s (2023) findings, showing that melatonin administration successfully suppresses PPO and POD activity in litchi fruits, postponing browning and maintaining antioxidant levels throughout storage. This demonstrates how melatonin may improve the quality and shelf life of fruit(Marak *et al.*, 2023).

TSS and TA are key maturity indices for citrus juice quality. During storage, TSS increases due to factors such as water loss, starch degradation, and the conversion of polysaccharides and pectin to sugars (Hussain *et al.*, 2022). TSS rises with fruit maturation and storage-induced dehydration, while TA decreases due to organic acid consumption. Melatonin has been shown to delay TSS increase and preserve TA in fruits like mangoes and strawberries (Promyou *et al.*, 2023, Ma *et al.*, 2021). Our results confirm melatonin's effectiveness in regulating TSS and TA in limes, with changes linked to metabolic processes such as organic acid consumption during respiration (Bhardwaj *et al.*, 2022b). Our findings align with previous observations, suggesting melatonin's potential to regulate lime quality. The observed decrease in both TSS and TA in both treatment groups is likely due to metabolic processes within the fruit, including the consumption of organic acids during respiration (Bhardwaj *et al.*, 2022a). These results collectively indicate that ML application could be a viable approach for preserving the postharvest quality of fruits by modulating the TSS and TA.

Peel color affects consumer preferences by signaling internal quality. During storage, decreased L* indicates browning and reduced hue angle shows chlorophyll loss, allowing carotenoids to turn fruit color from green to yellow as it ripens (Liu*et al.*, 2021). In limes, as maturity progresses, chlorophyll degradation allows for the accumulation and expression of carotenoids in the flavedo (outermost layer of the peel), resulting in the fruit's color changing from green to yellow (Sun*et al.*, 2019). Melatonin use significantly reduces chlorophyll degradation and increases carotenoid production at metabolic levels in various vegetables, including broccoli. (Yan *et al.*, 2024). In ripening limes, a* increases due to chlorophyll loss and carotenoid gain, shifting color towards red. Melatonin treatment lowers a* and b*, delaying ripening. However, b* increases during storage due to chlorophyll decline and carotenoid rise. Melatonin preserves chloroplast integrity, delaying color changes and enhancing lime sensory quality. Melatonin treatment maintains chloroplast integrity by inhibiting genes and enzymes involved in chlorophyll degradation, thereby enhancing the sensory quality of fruit. Our results are consistent with those of (Promyou *et al.,* 2023) who showed that melatonin treatment effectively maintains brightness and delays color changes in strawberries. Similarly, the observed delay in fruit color development in our melatonin-treated lemons is consistent with observations in various other fruits, including Papaya (Borthakur *et al.*, 2024) and mango (Liu*et al.,* 2020). Furthermore, our findings are consistent with those of (Zhang *et al.*, 2018). who reported that melatonin application slows down the changes in L* and a* parameters and delays the onset of senescence in litchi fruit. Furthermore, melatonin treatment has been shown to preserve the color and appearance of lychee fruit, reduce skin browning, and improve overall fruit quality (Xie *et al.*, 2022). In study by (Mohammadi *et al.*, 2024a), the use of pomegranate seed oil (PSO) in combination with xanthan gum showed a significant effect on preserving the color of the fruit peel. These results indicate that pomegranate seed oil is effective in preserving the natural color and increasing the apparent shelf life of the fruit by reducing chlorophyll decomposition and delaying the synthesis of carotenoids. In addition, studies demonstrated that the application of a coating composed of 3% tamarind starch and 0.24 mL/mL pomegranate seed oil resulted in greater efficiency in maintaining fruit luminosity (L*) and delaying color development, as indicated by the C* values (Onias et al., 2019).

CONCLUSIONS

Our study investigated the efficacy of enriching melatonin using pomegranate seed oil (PSO) for the postharvest preservation of *Citrus aurantifolia*. The applied treatments demonstrated a promising ability to mitigate PPO activity, thereby reducing browning, a major quality concern in limes. Additionally, the treatments significantly improved the function of antioxidant enzymes, notably POD and CAT, indicating a strengthened antioxidant defense system within the fruit. These combined effects highlight the effectiveness of the treatments, excluding PSO alone, in enhancing the overall quality and prolonging the storage duration of Mexican lime. Our findings thus underscore the potential of melatonin as a valuable postharvest strategy for preserving the quality and marketability of limes.

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