

Exogenous Melatonin Application Prolongs Citrus Fruits (*Citrus sinensis*) Shelf-life Quality by Enhancing Some Phytochemical Traits

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Abstract

This study investigated the impact of melatonin treatments (1 mM and 2 mM) on the post-harvest quality of orange fruit during 30 and 60 days of cold storage. Parameters such as titratable acidity (TA), total soluble solids (TSS), vitamin C, antioxidant capacity, total phenolic compounds (TPC), total flavonoids compounds (TFC), enzymatic activities (PAL, CAT), and color were evaluated. Melatonin significantly improved fruit quality by maintaining higher levels of total soluble solids, vitamin C, and antioxidant capacity. Both treatments effectively reduced weight loss and enhanced the activity of antioxidant enzymes. While 2 mM melatonin showed greater efficacy in the initial stages of storage, 1 mM demonstrated better stability in maintaining quality over extended periods. Melatonin treatments also influenced color parameters, suggesting potential improvements in visual appeal. These findings highlight the potential of melatonin as a natural preservative for enhancing the post-harvest quality and extending the shelf life of orange fruit. Further research is needed to optimize melatonin concentrations and explore its integration with other preservation techniques for sustainable and efficient fruit management.

Keywords: Antioxidant, Fruit quality, Post-harvest, PAL enzyme, Weight loss

Introduction

Extending the shelf life of fruits is essential to reducing food waste, maintaining nutritional value, and ensuring the availability of fresh produce in distant markets (Bhosale & Sundaram, 2011). Longer shelf life minimizes economic losses for producers and retailers while providing consumers with consistent access to quality fruits. Effective preservation techniques, such as refrigeration, freezing, and controlled atmosphere storage, play a critical role in maintaining fruit quality during storage

and transport. These methods significantly contribute to the sustainability of food supply chains by reducing spoilage and waste (Singh *et al.*, 2024; Tadapaneni *et al.*, 2014).

Oranges, as one of the most widely consumed citrus fruits globally, hold significant economic and nutritional importance. According to official statistics, the annual orange production in Iran is approximately 3 million tons, placing the country ninth in the world in terms of orange production (Sidana *et al.*, 2013). They are valued for their richness in vitamins, antioxidants, and dietary fiber, which



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make them a staple in many diets. However, the high perishability of oranges poses challenges in their post-harvest handling (Tütem *et al.*, 2020). Factors like weight loss, microbial spoilage, and nutrient degradation can limit their shelf life, leading to substantial losses during storage and distribution. Thus, there is a pressing need for effective strategies to enhance their post-harvest quality and extend their shelf life (Khathir *et al.*, 2019; Sicari *et al.*, 2017).

Melatonin has emerged as a promising natural compound in improving the post-harvest management of fruits (Saud *et al.*, 2023). Traditionally recognized for its role in regulating sleep and circadian rhythms in animals, melatonin is now known to be synthesized in plants as well. In plants, it performs multiple physiological functions, including stress regulation, antioxidant activity, and growth modulation (Saroj *et al.*, 2023). Its potential as a post-harvest treatment lies in its ability to mitigate oxidative stress, delay ripening, and maintain fruit quality during storage (Xue *et al.*, 2021). Melatonin enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), which help in reducing ROS levels in fruits, thereby mitigating oxidative damage (Qu *et al.*, 2022). Melatonin treatment also upregulates enzymes involved in the phenylpropanoid pathway, such as phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), and polyphenol oxidase (PPO), leading to increased accumulation of phenolic compounds, flavonoids, and lignin, which enhance disease resistance and delay senescence (Michailidis *et al.*, 2021).

Melatonin is particularly appealing because of its compatibility with sustainable agricultural practices. Unlike synthetic chemicals that may have adverse effects on health and the environment, melatonin is a naturally occurring, non-toxic compound (Sharma *et al.*, 2024). It aligns with growing consumer demand for organic and environmentally friendly produce. Additionally, melatonin can be integrated with other preservation methods, such as cold storage or edible coatings, to create

a multifaceted approach for fruit preservation.

Oxidative stress, caused by the accumulation of reactive oxygen species (ROS), is a major factor contributing to post-harvest quality loss in fruits (Neog & Saikia, 2010). ROS accelerate cellular damage, leading to faster ripening, senescence, and spoilage (Hailu *et al.*, 2008). Melatonin acts as a potent antioxidant by scavenging ROS and enhancing the activity of antioxidant enzymes, thereby reducing oxidative damage. Furthermore, it can regulate the production of ethylene -a hormone central to fruit ripening- by inhibiting its biosynthesis, slowing down ripening processes, and prolonging the storage life of fruits (Berra & Rizzo, 2009).

Studies have shown that melatonin application can effectively maintain the quality of various fruits during storage. For instance, melatonin treatment has been found to delay weight loss, reduce microbial decay, and retain firmness in fruits like strawberries, bananas, and tomatoes (Arshad & Haghshenas, 2025; El-Mogy *et al.*, 2019; Zang *et al.*, 2022). Melatonin has demonstrated the ability to slow respiration rates, inhibit fungal growth, and preserve vital nutrients, such as ascorbic acid, during extended storage. These findings highlight its potential as a natural and eco-friendly alternative to synthetic preservatives (El-Mogy *et al.*, 2019).

Despite its promise, challenges remain in the large-scale adoption of melatonin in post-harvest management. Key limitations include the need for standardized application protocols and the lack of extensive field-level research under practical conditions. Moreover, economic feasibility and scalability must be addressed to make melatonin treatments viable for widespread commercial use.

In conclusion, melatonin represents an innovative and sustainable solution to the challenges of post-harvest fruit management. Its ability to delay ripening, maintain quality, and extend shelf life offers significant benefits for reducing waste and improving the availability of high-quality fruits. As research continues to optimize its application, melatonin could play a pivotal role in creating more

efficient and sustainable post-harvest systems for oranges and other perishable fruits.

Materials and Methods

Plant Materials and Experimental Design

Orange fruits (*Citrus sinensis* or *Citrus × sinensis*) were harvested at the mature green stage. To ensure consistency, the fruits were selected based on uniformity in shape, color, and size, with a preference for medium-size specimens. The selected fruits were initially weighed, and only those of medium size were selected for further analysis. Fruits displaying any signs of blemishes or disease were excluded from the study. Fruits were selected and separated, then subjected to immersion treatment in melatonin solutions at concentrations of 1 and 2 mM, as well as a control treatment, for one minute. After the treatment, the fruits were exposed to open air to allow their surfaces to dry completely. Fruits were stored in a cold room for 2 months at $0 \pm 1^\circ\text{C}$ and a humidity of 90-95%. Measuring the desired parameters was carried out on days 30, and 60 after applying the treatments.

Qualitative Analysis of Fruit

To assess the qualitative changes in oranges during storage, sampling was conducted on the 14th day of cold storage. The qualitative analyses included evaluations of color, weight loss measurement, total soluble solids (TSS), titratable acidity (TA), vitamin C content, and various antioxidant properties. These properties encompassed total and enzymatic antioxidant activities, including catalase activity and phenylalanine ammonia-lyase activity, as well as total phenol and total flavonoid contents.

Color Parameters Measurements

The color of orange fruits was non-destructively measured using a colorimeter (CR-400, Konica Minolta Inc., Tokyo, Japan) following the method described by Giglio *et al.* (2023). The color parameters, including color difference index (ΔE), chroma (C), and hue angle (H), were calculated based on the *L* value (lightness), *a* value (green to red), and *b* value (blue to yellow).

Weight Loss Measurement

The weight of the fruits was measured during each experimental period using a precision scale.

Total Soluble Solids (TSS) Measurement

The total soluble solids (TSS) content was measured using a handheld refractometer (ATAGO model). A few drops of orange juice were placed on the refractometer, and the corresponding value was read from the graduated scale. Before starting the measurements, the refractometer was calibrated. After each reading, the device was rinsed with distilled water and dried for the next measurement (Marandi *et al.*, 2010).

Titratable Acidity (TA) Measurement

The titration was performed using 10 mL of orange juice with 0.1 N sodium hydroxide (NaOH) solution (4 g/L) until the pH reached 8.2. The amount of acid in the juice was then expressed as a percentage based on the volume of NaOH consumed during the titration, according to the method described by Selcuk and Erkan (2015). The titratable acidity was calculated in terms of citric acid equivalent (the predominant acid in strawberries) using Equation (1).

$$TA = \frac{S \times N \times F \times E}{C} \times 100$$

Total Phenol Content (TPC)

The total phenolic content was determined by combining 2 mL of a 2% sodium carbonate solution, 2.8 mL of distilled water, and 100 μL of a 50% Folin-Ciocalteu reagent with 100 μL of the fruit juice. The absorbance was recorded at 720 nm after a portion of the incubation time, using a control sample for reference. Gallic acid was utilized as the standard for constructing the calibration curve. The phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of fresh plant weight, following the procedure described by Meda *et al.* (2005).

Total Flavonoid Content (TFC)

The total flavonoid content was assessed using a colorimetric assay at 380 nm, in accordance with the procedure outlined by

Pirogov *et al.* (2016) Specifically, A 30 μL aliquot of fruit juice was mixed with 150 μL of 5% sodium nitrite, 300 μL of 10% aluminum chloride, and 1000 μL of 1 mol/L sodium hydroxide. After incubating the mixture in the dark for 30 minutes, the reaction was diluted to a final volume of 5 mL with double-distilled water, and the absorbance was measured at 380 nm. The flavonoid content was calculated using a quercetin standard curve and expressed as milligrams of quercetin equivalent per milliliter of fruit juice (mg QE/mL).

Antioxidant Activity

The antioxidant activity was measured using the DPPH free radical scavenging assay. A specified volume of methanolic extract was mixed with DPPH solution and incubated in the dark for 15 to 30 minutes. Absorbance was then measured at 517 nm using a spectrophotometer. The activity was calculated using a formula, with a control sample (80% methanol) to calibrate the spectrophotometer and measure the absorbance of the DPPH solution without the extract (Chiou *et al.*, 2007).

Vitamin C

The ascorbic acid (AsA) content was estimated using the method described by Vithana *et al.* (2018). The total ascorbic acid content in fruit pulp was determined by homogenizing the sample in an extraction solution, centrifuging the mixture, and measuring the absorbance of the supernatant after reaction with Dichloroindophenol reagent. The results were quantified using L-ascorbic acid as a standard and expressed as mg/kg of fresh weight.

Measurement of Catalase

Catalase (CAT) enzyme activity in the fruit was measured using the method described by Boominathan and Doran (2002). The reaction mixture consisted of 900 μL of 10 mM hydrogen peroxide (H_2O_2) prepared in phosphate-buffered saline (without PVP) and 100 μL of fruit juice placed in a glass cuvette. The decomposition of H_2O_2 , catalyzed by

catalase, was monitored spectrophotometrically by measuring the decrease in absorbance at 240 nm within 1 minute using a Uvi Light XS 5 SECOMAM spectrophotometer. The catalase activity was then calculated based on the rate of H_2O_2 decomposition.

$$\text{Units } \left(\frac{\text{mM}}{\text{min}} \right) = \frac{\Delta\text{OD} / \text{min}(\text{slope}) \times \text{Vol. of assay (0.0003)}}{\text{Extinction Coefficient (43.6)}}$$

Phenylalanine Ammonia-Lyase (PAL) Activity Assay

Phenylalanine ammonia-lyase (PAL) enzyme activity was measured following the procedure outlined by D'Cunha (2005). PAL enzyme activity was measured by incubating a reaction mixture of potassium phosphate buffer, phenylalanine, distilled water, and fruit juice. After incubation, the reaction was stopped, and absorbance was measured. The activity was calculated using a cinnamic acid standard curve and expressed in mg of cinnamic acid per 100 g of fresh weight.

Experimental Design

The experiment was conducted using a completely randomized design with four replications. The treatments applied included 1 and 2 millimolar melatonin. Data were organized in tables and graphs were generated using Excel software. Statistical analysis of the data was performed using SAS 9.2 software, and mean comparisons were conducted using Duncan's multiple range test at the 5% probability levels.

Result and Discussion

The analysis of variance (ANOVA) (Table 1) shows that melatonin concentration (C) had a significant effect on most of the measured traits, including antioxidant activity, phenolic content, and weight loss, indicating its strong influence on postharvest fruit quality. Storage time (T) also significantly affected key parameters such as vitamin C and antioxidant percentage. The interaction between concentration and time (C \times T) was significant

for several traits, suggesting that the effect of melatonin varies depending on the duration of storage.

Table 1- ANOVA analysis for the Effects of Melatonin Concentration and Storage Time on Postharvest Orange fruits

Variable	D F	T A	TS S	Vi t C	L*	a*	b*	Chro ma	Hu e	Loss Wei ght	Antioxi dant %	Flavon oids	Phen ols	PA L	CA T
Concentr ation (C)	2	11. 9*	0.1 2*	62. 3**	10. 7*	7.1 7**	13. 9*	0.95*	101. 5**	122.1* *	102.9**	15.95**	332.9**	36.1 *	7.59 *
Time (T)	1	1.5 ns	0.1 ⁿ s	71. 7**	2 ^{ns}	42*	4.6 8*	0.25 ^{ns}	20.9 **	115.9* *	18.9*	12.8**	49.2**	209. 1*	1.11 ns
C × T	2	0.1 5*	0.1 5 ^{ns}	8.4 7*	0.0 2 ^{ns}	12. 8*	0.8 6 ^{ns}	1 ^{ns}	4.97 **	3.11**	372.1**	27.41*	33.14**	0.43 **	2.93 *
Error	1 7	0.9	0.3 4	2.5	1.5	1.0 5	0.9 4	0.54	1.32	1.53	3.5	1.65	3.5	1.34	1.22
CV%		7.6	14. 3	3	2.1	3.6 1	2.9 3	5.5	4.6	7.29	3.02	4.76	2.77	7.9	21.3 1

* and ** significant at 0.05 and 0.01, ns: Not-significant

Titration Acidity (TA)

The titratable acidity (TA) of orange juice exhibited notable variations during storage among different treatment concentrations (Fig. 1). On Day 30, there were no significant differences in TA levels between the control and 2 mM treatments, while the 1 mM treatment showed a slightly higher value, though this difference was not statistically significant. By Day 60, a distinct pattern emerged, with the 2 mM treatment displaying a significant increase in TA compared to both the control and 1 mM treatments. The 2 mM treatment recorded the highest TA value (1.5 g/100 ml), which was significantly greater ($p < 0.05$) than the other treatments. In contrast, the control and 1 mM treatments maintained relatively stable TA levels over time, with no significant differences observed between them. These results indicate that higher concentrations (2 mM) may lead to increased acidity during extended storage, potentially influencing the sensory characteristics of the

juice.

Based on our findings, the application of melatonin can improve the content of fruit acidity. This effect was statistically significant at a concentration of 2 mM, whereas at 1 mM. Although the increase was not significant, but effectively prevented a decline in acidity (Fig. 1). Melatonin applications have been shown to maintain fruit quality characteristics during cold storage. This includes maintaining firmness, delaying changes in titratable acidity, and preserving organic acid concentrations (Carrión-Antolí *et al.*, 2022; Kucuker *et al.*, 2024). In a study, melatonin application on peach fruits was effective in maintaining the concentration of organic acids, including titratable acidity, although the effect varied depending on the concentration and compound (Kucuker *et al.*, 2024). A study on 'Newhall' navel oranges showed that melatonin treatment increased titratable acidity, suggesting an inhibition of fruit quality deterioration and delayed senescence (Ma *et al.*, 2021).

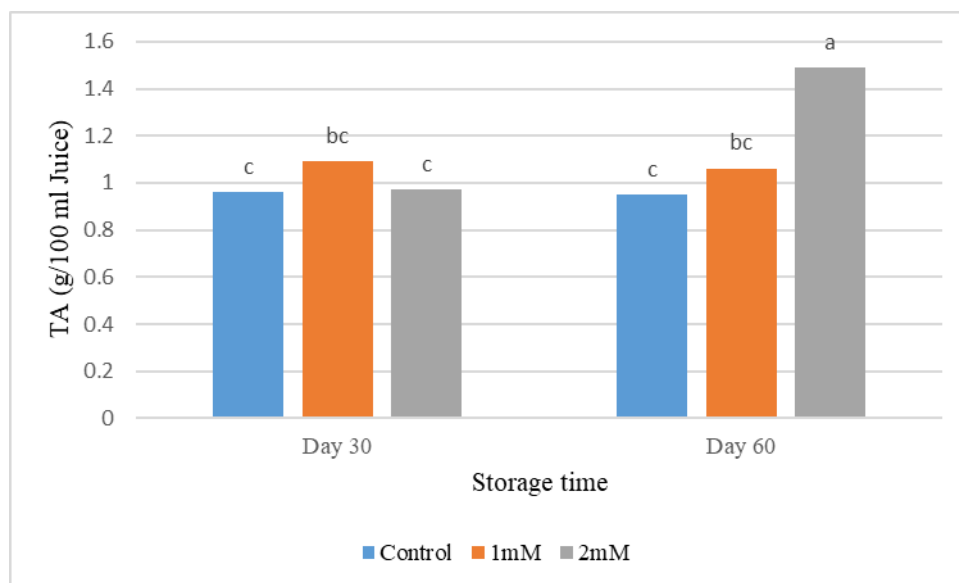


Fig. 1. Effect of different melatonin concentrations on the total acidity (TA) of orange juice over 30 and 60 days of storage

Total Soluble Solids (TSS)

The total soluble solids (TSS) content in orange juice showed significant variations among treatments and storage durations (Fig. 2). On Day 30, TSS level in the control group was significantly lower ($p < 0.05$) than those in 1 and 2 mM treatments. Among the treatments, the 2 mM group exhibited the highest TSS value, though this was not significantly different from 1 mM treatment. By Day 60, a similar trend persisted, with the 2 mM treatment maintaining the highest TSS level (approximately 14 °Brix), significantly surpassing the control and 1 mM treatments. Over time, slight reductions in TSS was observed in the control and 1 mM treatments, with the control consistently showing the lowest values at both storage intervals. These findings suggest that higher concentrations (2 mM) contribute to enhanced TSS retention during storage, potentially improving the sweetness and overall quality of the juice over extended periods.

The total soluble solids (TSS) content in oranges treated with 2 mM melatonin significantly increased compared to the control during 30 and 60 days of storage (Fig. 2). Melatonin treatments generally lead to an increase in TSS in various fruits during post-harvest storage. This is observed in pitaya,

navel oranges, nectarines, peaches, jujube fruits, pomegranates, sweet cherries, passion fruits, strawberries, and kiwi berries (Ba *et al.*, 2022; Bal, 2021; Kucuker *et al.*, 2024; Ma *et al.*, 2021; Wu *et al.*, 2023). Melatonin treatments have effectively slowed the process of senescence, reduced fruit softening, and maintained the total soluble solids content in nectarines and strawberries (Bal, 2021; Liu *et al.*, 2018). Melatonin treatments have been shown to delay the decline in fruit firmness, reduce the loss of soluble solids and titratable acids, and regulate the formation of soluble pectin by inhibiting the activities of key enzymes, including pectin methylesterase (PME), polygalacturonase (PG), cellulase (Cx), and β -glucosidase (β -Glu) (Qu *et al.*, 2022).

Vitamin C Content

The vitamin C content in orange juice varied significantly among treatments and storage durations. On Day 30, the 2 mM treatment recorded the highest vitamin C concentration (50 mg/kg), though this was not significantly different from the 1 mM treatment. In contrast, the control treatment displayed a significantly lower vitamin C level ($p < 0.05$) compared to the other treatments. By Day 60, a noticeable decline in vitamin C content was observed in all groups. However, the 1 mM treatment retained

a significantly higher concentration of vitamin C compared to the control and 2 mM treatments, with the control group showing the lowest value (30 mg/kg). These findings suggest that while both 1 mM and 2 mM treatments initially improve vitamin C retention, prolonged storage results in an

overall decline, with the 1 mM treatment exhibiting greater stability over time. This highlights the potential of moderate concentrations (1 mM) for preserving the nutritional quality of orange juice during extended storage (Fig. 3).

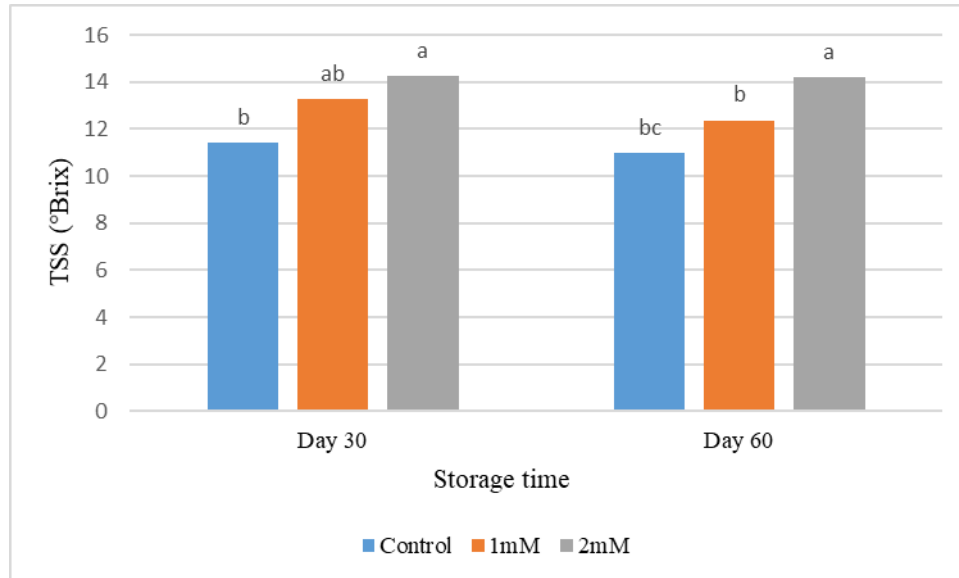


Fig. 2. Effect of different melatonin concentrations on the TSS of orange juice over 30 and 60 days of storage

In this study, vitamin C was influenced by melatonin treatment, with the application of 1 mM and 2 mM melatonin significantly enhancing its content compared to the control during both 30- and 60-day storage periods. Melatonin treatments significantly maintained higher contents of vitamin C in fresh-cut pitaya fruits during storage compared to control treatments. This suggests that melatonin can help preserve vitamin C levels in fruits post-harvest (Ba *et al.*, 2022). Also, Melatonin enhances the activity of antioxidant enzymes such as, catalase (CAT), which helps in reducing oxidative stress and preserving vitamin C (Ma *et al.*, 2021; Wang *et al.*, 2022). Furthermore, by decreasing the accumulation of reactive oxygen species (ROS) and enhancing the antioxidant defense system, melatonin helps in maintaining the nutritional quality of fruits, including vitamin C content (Song *et al.*, 2022).

Weight Loss

The graph illustrates the percentage of weight loss in samples over two storage periods (30 and 60 days) under three treatment conditions: control (untreated), 1 mM, and 2 mM. On Day 30, the control group exhibited the highest weight loss (15.25%), while the 2 mM treatment group demonstrated the lowest weight loss (5.28%). The 1 mM treatment resulted in intermediate weight loss (12.98%), highlighting the efficacy of higher concentrations in minimizing weight loss during short-term storage. By Day 60, weight loss increased among all groups, with the control group again recording the highest loss (19.05%) and the 2 mM treatment maintaining the lowest loss (11.91%). The 1 mM treatment group showed moderate weight loss (17.74%). Statistically significant differences between treatments, as indicated by the letters in the graph, underscore the effectiveness of higher treatment concentrations in reducing weight loss over extended storage periods (Fig. 4).

Melatonin treatments have consistently demonstrated a reduction in weight loss among different fruit types, including pitaya, citrus, okra, peaches, sweet cherries, and strawberries (Ba *et al.*, 2022; Ma *et al.*, 2021). Melatonin helped maintain fruit firmness and reduced weight loss by promoting stomatal closure, which minimizes water loss (Shi *et al.*, 2024).

On the other hand, Melatonin is effective in reducing weight loss in post-harvest fruits by enhancing antioxidant defenses, promoting stomatal closure, and influencing gene expression related to cell wall integrity. These effects collectively contribute to maintaining fruit quality and extending shelf life during storage (Shi *et al.*, 2024).

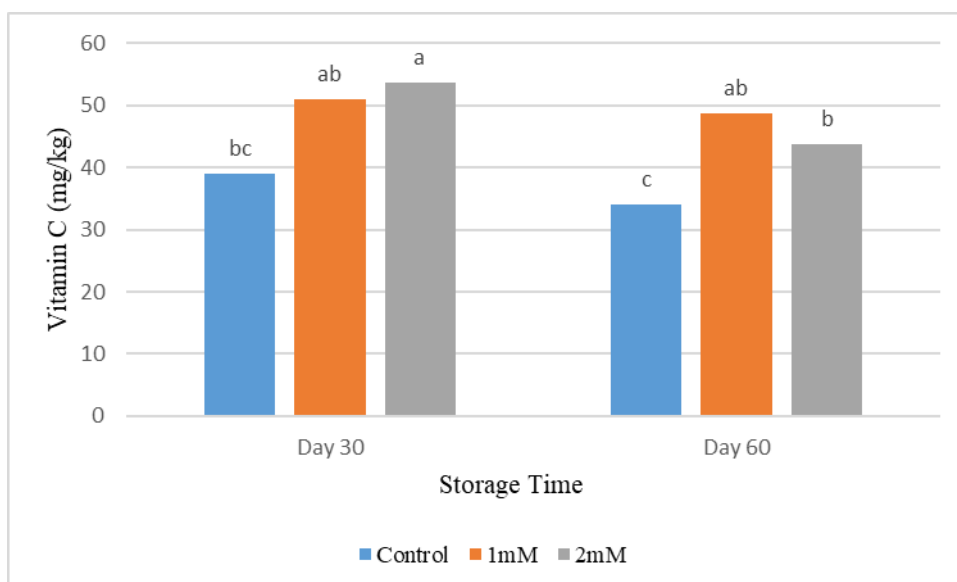


Fig. 3. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

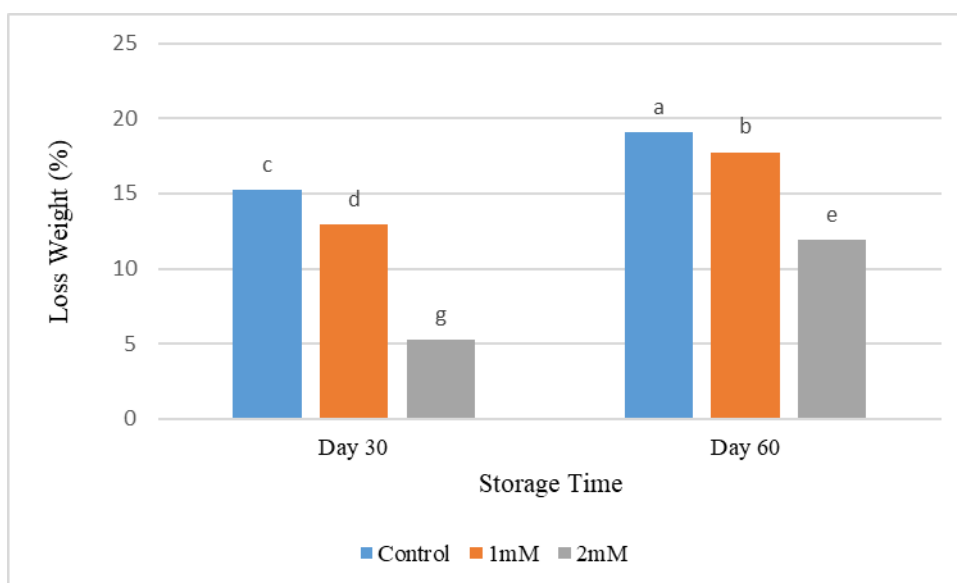


Fig. 4. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Antioxidant Capacity

The antioxidant capacity (%) exhibited significant variations among treatments and storage periods. On Day 30, the 2 mM treatment showed the highest antioxidant capacity (63.38%), which was significantly greater than that of the control (56%) and 1 mM (53.19%) treatments. These results highlight the superior effectiveness of the 2 mM treatment in preserving antioxidant levels during the early stages of storage. By Day 60, a notable shift in antioxidant capacity was observed among the treatments. The 1 mM treatment demonstrated the highest antioxidant capacity (72.3%), significantly surpassing the control (45%) and 2 mM (53%) treatments ($p < 0.05$). These findings suggest that while the 2 mM treatment is more effective in maintaining antioxidant capacity in the short term, the 1 mM treatment provides better retention over extended storage periods. This indicates the potential advantage of moderate concentrations (1 mM) for preserving antioxidant capacity, thereby

enhancing product quality and nutritional value during long-term storage (Fig. 5).

Melatonin has been demonstrated to significantly enhance antioxidant activity in a variety of post-harvest fruits, thereby improving quality and extending shelf life (Fig. 5). Treatments with melatonin increase the activities of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), in fruits such as pitaya, peaches, and blueberries (Shang *et al.*, 2021). Melatonin also reduced the levels of reactive oxygen species (ROS) and lipid peroxidation, while promoting the activity of antioxidant enzymes such as ascorbate peroxidase (APX), glutathione S-transferase (GST), and phenylalanine ammonia-lyase (PAL) (Wu *et al.*, 2023). Melatonin treatments increased the levels of bioactive compounds, including total phenolics and anthocyanins, while preserving higher antioxidant activity throughout the storage period (Lorente-Mento *et al.*, 2021).

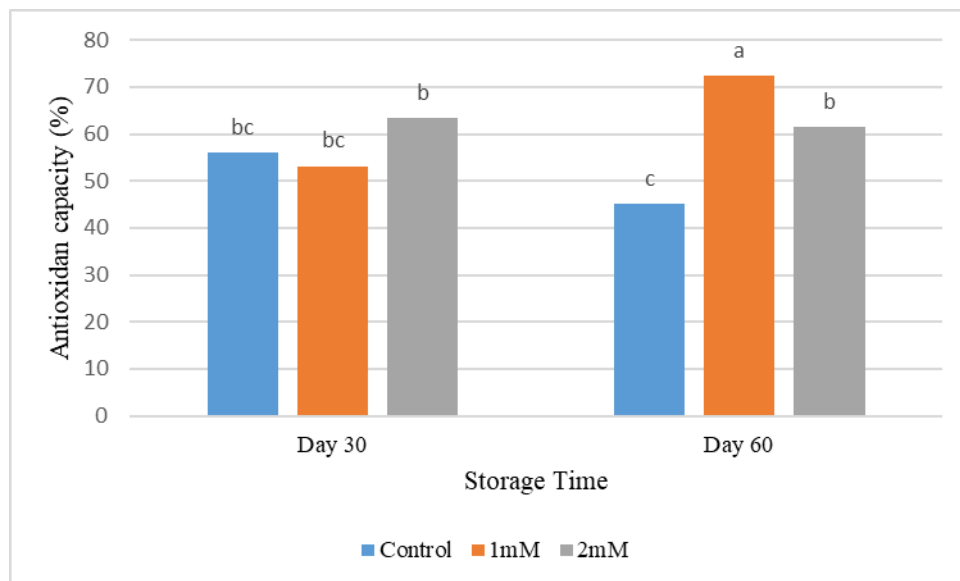


Fig. 5. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Total Flavonoid Content (TFC)

The total flavonoid content (TFC), expressed in mg QE/ml juice, showed significant variations among treatments and

storage periods. On Day 30, the 1 mM treatment recorded the highest TFC (21.82 mg QE/ml), which was significantly higher than the control (15.83 mg QE/ml) but not significantly different from the 2 mM treatment (18.82 mg

QE/ml). The control group had the lowest TFC value, highlighting the beneficial effect of treatments in enhancing flavonoid levels during short-term storage. By Day 60, notable change in TFC level was observed among all groups. The 2 mM treatment displayed the highest TFC (28.47 mg QE/ml), significantly exceeding the 1 mM treatment (24.8 mg QE/ml) and the control (19.26 mg QE/ml). These findings underscore the superior efficacy of the 2 mM treatment in maintaining flavonoid content over extended storage periods. Overall, the results suggest that higher concentrations, such as 2 mM, are more effective in preserving TFC during prolonged storage, thereby contributing to the improved nutritional and functional

quality of the product (Fig. 6).

Melatonin treatment significantly increased the total flavonoid content in orange fruits during 60 day of shelf life (Fig. 6). In a study, Melatonin treatment delayed the decrease in flavonoid content in blueberries during storage (Cao *et al.*, 2024). Also, Melatonin treatment at 0.5 mM effectively preserved the flavonoid content in litchi fruits, reducing oxidative browning and maintaining overall fruit quality during storage (Marak *et al.*, 2023). Exogenous melatonin application can increase non-enzymatic antioxidants, including flavonoids, thereby improving the fruit's antioxidant capacity and maintaining quality during storage (Zhang *et al.*, 2024).

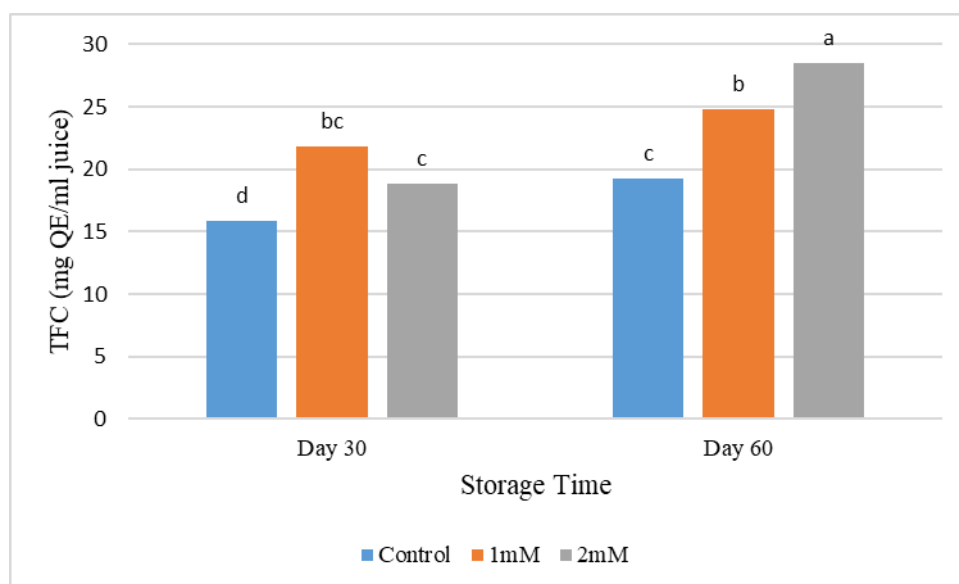


Fig. 6. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Total Phenolic Content (TPC)

The total phenolic content (TPC), expressed in mg GAE/ml juice, showed significant differences among treatments and storage periods. On Day 30, the 2 mM treatment recorded the highest TPC (84.68 mg GAE/ml), significantly surpassing the control (54.35 mg GAE/ml) and the 1 mM treatment (68.48 mg GAE/ml), indicating the positive impact of higher concentrations on phenolic retention during short-term storage. By Day 60, TPC decreased among all groups, with the 2 mM treatment maintaining the highest value (76.78

mg GAE/ml), significantly higher than the 1 mM (62.77 mg GAE/ml) and control (58 mg GAE/ml) groups. The control exhibited the steepest decline in TPC, underscoring the protective effect of treatments in reducing phenolic degradation over time. These results demonstrate that higher concentrations, such as 2 mM, are more effective in preserving TPC during extended storage, supporting improved antioxidant capacity and product quality (Fig. 7).

Melatonin has demonstrated a beneficial effect on enhancing the total phenolic content

in various treatments during orange fruits post-harvest storage (Fig. 7). Melatonin treatments (0.1 mM) applied pre-harvest significantly increased the total phenolic content at harvest and maintained higher levels during 60 days of storage compared to control (Lorente-Mento *et al.*, 2021). Melatonin treatment ($1000 \mu\text{mol L}^{-1}$) effectively maintained higher levels of total phenolics and antioxidant activity during 40 days of storage (Bal, 2021). Application melatonin can lead to maintain the total

phenolic content and enhanced antioxidant enzyme activities, which helped delay senescence and maintain fruit quality during storage (Shang *et al.*, 2021). Melatonin treatments among various fruits consistently show an increase in total phenolic content and enhanced antioxidant activity during post-harvest storage. This results in delayed senescence, improved fruit quality, and extended shelf-life.

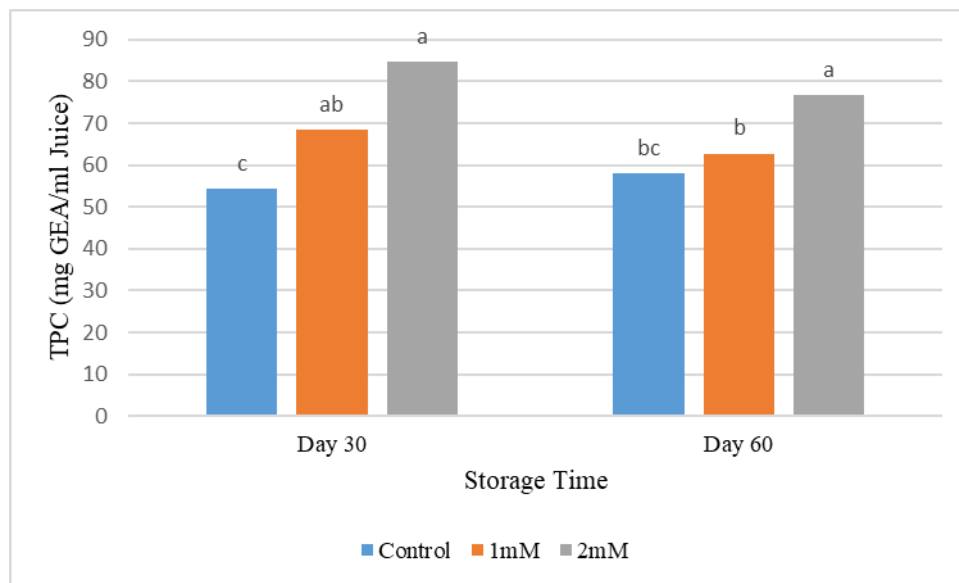


Fig. 7. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Phenylalanine Ammonia-Lyase (PAL)

The activity of PAL, measured in mg/g FW/min, varied significantly among treatments and storage periods. On Day 30, the 2 mM treatment showed the highest PAL activity (18.3 mg/g FW/min), significantly exceeding the 1 mM treatment (16.4 mg/g FW/min) and the control (13.45 mg/g FW/min), highlighting the role of higher concentrations in enhancing PAL activity during short-term storage. By Day 60, PAL activity declined in all treatments, with the 2 mM and 1 mM treatments maintaining similar levels (11.1 and 10.2 mg/g FW/min, respectively), both significantly higher than the control (6.4 mg/g FW/min). The control group experienced the steepest reduction in PAL activity, emphasizing the protective effect of treatments in sustaining enzyme function

during extended storage. These findings underscore the effectiveness of higher concentrations (1 mM and 2 mM) in preserving PAL activity, which is crucial for secondary metabolite production and stress response, thereby enhancing product quality and stability during storage (Fig. 8).

Melatonin treatment significantly enhances PAL activity in several fruits, which is associated with improved disease resistance and delayed senescence. For instance, in peaches, melatonin treatment increased PAL activity, contributing to enhanced disease resistance and maintenance of fruit quality (Dong *et al.*, 2024; Lei *et al.*, 2022). In apricots, melatonin treatment increased PAL activity (Zhang *et al.*, 2024). Similarly, in litchis, melatonin enhanced PAL activity, which was

associated with increased resistance to *Peronophythora litchii* and improved post-

harvest quality (Zhang *et al.*, 2021).

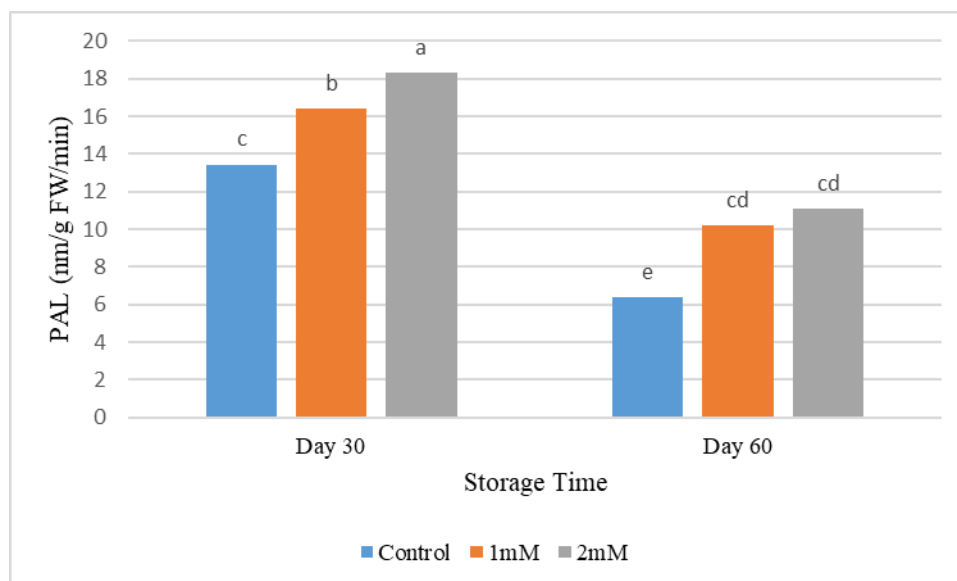


Fig. 8. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Catalase (CAT)

The activity of catalase (CAT) enzyme was evaluated during two storage periods (30 and 60 days) under three treatment conditions: Control, 1 mM, and 2 mM. On day 30, the Control group exhibited the lowest CAT activity compared to the treatments, although the difference was not statistically significant compared to the 1 mM treatment. Both the 1 mM and 2 mM treatments resulted in a higher CAT activity, with no significant difference between them. By day 60, a distinct trend was observed, where the Control group showed the lowest enzyme activity, while the 1 mM treatment moderately increased CAT activity. However, the 2 mM treatment significantly enhanced CAT activity, achieving the highest value among all groups. These findings indicate that the 2 mM treatment effectively boosts catalase activity during prolonged storage, suggesting its potential role in enhancing oxidative stress resistance and maintaining product quality over time (Fig. 9).

Melatonin treatments have been consistently reported to increase catalase activity in post-

harvest fruits such as citrus, pitaya, apricots, raspberries, cassava, and litchi (Ba *et al.*, 2022; Guo *et al.*, 2021; Rahmanzadeh-Ishkeh *et al.*, 2024; Zhang *et al.*, 2021). This increase in catalase activity helps in scavenging reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2), thereby reducing oxidative stress and delaying senescence. For instance, in fresh-cut pitaya fruits, melatonin at $100 \mu\text{mol L}^{-1}$ significantly increased catalase activity, which contributed to lower H_2O_2 levels and delayed ripening (Ba *et al.*, 2022). Similarly, in apricots, melatonin treatments enhanced catalase activity, reducing active oxygen content and delaying fruit senescence (Guo *et al.*, 2021). The effectiveness of melatonin in increasing catalase activity and improving post-harvest quality appears to be dose-dependent. For example, in raspberries, the highest catalase activity was observed at a melatonin concentration of 0.1 mM (Rahmanzadeh-Ishkeh *et al.*, 2024). Similarly, in eggplants, $100 \mu\text{mol L}^{-1}$ melatonin was effective in maintaining higher catalase activity and reducing chilling injury (Song *et al.*, 2022).

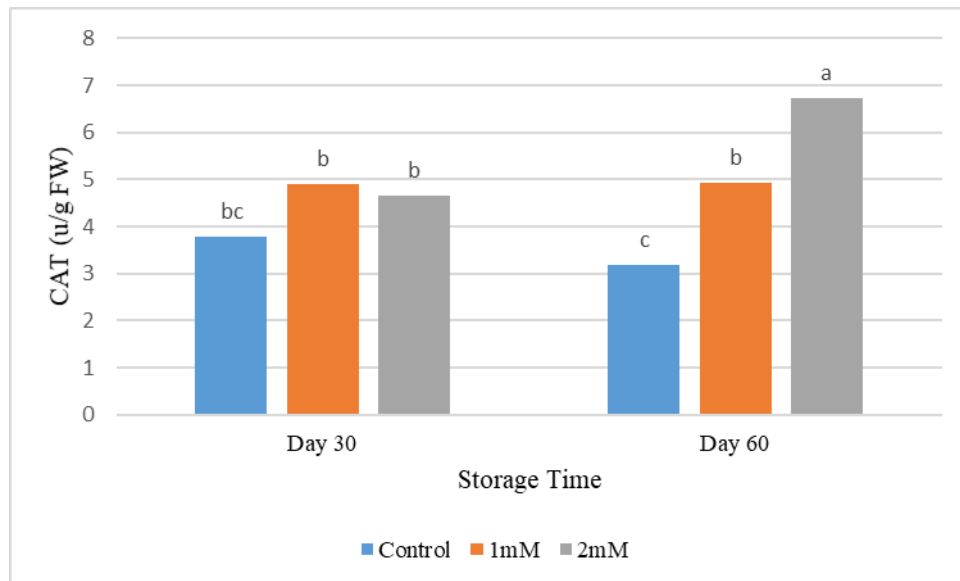


Fig. 9. Effect of different melatonin concentrations on the Vitamin C of orange juice over 30 and 60 days of storage

Color Changes

The color parameters of the samples, including a^* , b^* , L^* , Chroma, and Hue, were measured at two storage periods (30 and 60 days) under three treatment conditions: Control, 1 mM and 2 mM melatonin (Table 2).

On Day 30, the a^* and b^* values were generally lower in the Control and 1 mM treatments compared to the 2 mM treatment. Specifically, a^* showed the lowest value in 2 mM treatment (24.77), which was significantly lower than the Control and 1 mM treatment. The b^* value followed a similar pattern, with 2 mM exhibiting a slightly lower value than the other treatments. The L^* value (lightness) did not show a significant difference between the Control and 1 mM treatment, but the 2 mM treatment slightly reduced L^* value. For Chroma, the 1 mM treatment had the highest value (18.95), while the 2 mM treatment exhibited the lowest (16.13). The Hue angle increased with the melatonin concentration, with 2 mM showing the highest value (27.82), indicating a noticeable shift in the color attributes. On Day 60, the a^* value was

significantly higher in 2 mM treatment (31.78), followed by the Control (29.03) and 1 mM treatment (26.68). The b^* value was highest in the Control and 1 mM treatment, with no significant difference between them, whereas the 2 mM treatment exhibited a lower b^* value (32.44). The L^* value remained consistent among all treatments, with only minor variations. Chroma was highest in 1 mM treatment (19.13), while the Control and 2 mM treatment showed lower values. The Hue angle followed a similar trend as Day 30, with the 2 mM treatment showing the highest Hue angle (24.34), indicating a shift toward a more yellowish tone. These results suggest that melatonin treatments, particularly at 2 mM, significantly influence the color parameters of samples during storage, potentially enhancing visual quality attributes such as color stability and hue angle over time. The application of melatonin can enhance and promote the accumulation of fruit pigments during the storage period. Previous research findings on pear (Sun *et al.*, 2021), apple (Verde *et al.*, 2022), tomato (Sun *et al.*, 2015), and grape (Xia *et al.*, 2021) support these results.

Table 2- Effect of Melatonin Treatments on Color Parameters (a*, b*, L*, Chroma, and Hue) During Storage Periods

Storage Period	Melatonin (mM)	a*	b*	L*	Chroma	Hue
Day 30	Control	26.04b	33.69b	57.33a	17.91b	17.93f
	1mM	26.51b	33.72b	56.49a	18.95a	22.77c
	2mM	24.77bc	32.17b	54.61ab	16.13c	27.82a
Day 60	Control	29.03a	35.69a	57.95a	17.59ab	17.85f
	1mM	26.68b	35.51a	57.08a	19.13a	19.86e
	2mM	31.78a	32.44b	55.43ab	17.99b	24.34b

Conclusion

This study demonstrates the potential of melatonin treatments in maintaining the post-harvest quality of orange fruit during storage. Both 1 mM and 2 mM melatonin concentrations effectively improved critical parameters such as total soluble solids, vitamin C content, antioxidant capacity, and enzymatic activities while reducing weight loss. The 2 mM treatment exhibited greater efficacy in enhancing nutritional and sensory attributes during early storage, while 1 mM melatonin provided better stability over prolonged storage. These findings highlight melatonin's role in extending shelf life and preserving quality, emphasizing its value as a natural preservative. Further research should explore

its broader applications and mechanisms.

Authors Contribution

Ansari A.: Conceptualization, Methodology, writing and revision of the manuscript. **Saadatian M.:** Formal analysis, Software, writing – original draft. **Haji-Taghilou R.:** Investigation, writing – review and editing. **Mohammad K.S.:** Visualization, Validation. **Abdollah R.A.:** Methodology, Data curation. **Majid Taha A.:** Validation, writing – review and editing.

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مقاله پژوهشی

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افزایش ماندگاری کیفیت میوه پرتقال (*Citrus sinensis*) با کاربرد ملاتونین خارجی از طریق بهبود برخی ویژگی‌های فیتوشیمیایی

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چکیده

این مطالعه تأثیر تیمارهای ملاتونین (۱ و ۲ میلی‌مولار) را بر کیفیت پس از برداشت میوه پرتقال در طی ۳۰ و ۶۰ روز نگهداری در دمای سرد بررسی کرد. در این پژوهش، پارامترهایی مانند اسیدیت قابل تیتر (TA)، مواد جامد محلول کل (TSS)، ویتامین C، ظرفیت آنتی‌اکسیدانی، ترکیبات فنولی کل (TPC)، فلاونوئیدهای کل (TFC)، فعالیت آنزیم‌های پال (PAL) و کاتالاز (CAT)، و رنگ مورد ارزیابی قرار گرفت. ملاتونین به‌طور معنی‌داری کیفیت میوه را با حفظ سطوح بالاتر TSS، ویتامین C و ظرفیت آنتی‌اکسیدانی بهبود بخشید. هر دو تیمار به‌طور مؤثری از کاهش وزن میوه جلوگیری کرده و فعالیت آنزیم‌های آنتی‌اکسیدانی را افزایش دادند. در حالی که تیمار ۲ میلی‌مولار ملاتونین در مراحل ابتدایی نگهداری اثربخشی بیشتری داشت، تیمار ۱ میلی‌مولار در حفظ کیفیت در بازه‌های طولانی‌تر عملکرد پایدارتری نشان داد. تیمارهای ملاتونین همچنین بر ویژگی‌های رنگ میوه تأثیر گذاشتند که نشان‌دهنده بهبود احتمالی جذابیت ظاهری میوه است. این یافته‌ها پتانسیل ملاتونین را به‌عنوان نگهدارنده طبیعی برای بهبود کیفیت پس از برداشت و افزایش ماندگاری میوه پرتقال نشان می‌دهند. برای دستیابی به مدیریت مؤثر و پایدار میوه، تحقیقات بیشتری برای بهینه‌سازی غلظت‌های ملاتونین و بررسی ترکیب آن با سایر روش‌های نگهداری مورد نیاز است.

واژه‌های کلیدی: آنتی‌اکسیدان، آنزیم PAL، پس از برداشت، کاهش وزن، کیفیت میوه

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