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Effect of putrescine coating and cinnamon essential oil on the quality improvement of Washington Navel orange fruit

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Abstract

Objective: Agricultural losses in fruits and vegetables during postharvest operations, storage, transportation, processing, and packaging are one of the main contributors to food waste. Washington Navel orange is one of the main varieties of oranges and an important economic citrus fruit worldwide. This study aimed to investigate the effects of putrescine polyamine and cinnamon essential oil on the postharvest life and quality of Washington Navel oranges.

Methods: This experiment was conducted as a factorial experiment based on a completely randomized design with three replications. The factors were: Cinnamon essential oil at the concentrations of 150 and 300 mg/L, putrescine at the concentrations of 0.5 and 1.5 mM, and three storage times (0, 30, and 60 days. On the first day of the experiment, the following characteristics were measured in this study: Ion leakage, fruit firmness, pH, titratable acidity, total soluble solids, total phenols, flavonoids, antioxidant capacity, and ascorbic acid content. In addition, after 30 and 60 days, the decay percentage, physiological weight loss, fruit firmness,

Results: The results of this study showed that treatment with cinnamon essential oil and putrescine reduced weight loss and softening, slowed decay rate, and preserved total soluble solids, titratable acidity, and pH in the oranges. Additionally, the tested treatments, by maintaining phenols, flavonoids, and ascorbic acid, increased the antioxidant capacity of the plants. The results showed that both putrescine treatments at three concentrations and cinnamon essential oil treatments at two concentrations had a positive impact on the storage life and postharvest quality of the oranges during a 60-day storage period, compared to the control treatment.

Conclusion: Among the treatments evaluated, the putrescine with the concentration of 1.5 mM and cinnamon essential oil with the concentration of 300 mg/L proved most effective. These findings demonstrate that natural and bio-based compounds can mitigate quality loss in oranges when the cold-chain control is limited.

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Introduction

Global predictions estimate that by 2050, the Earth's population will reach approximately 9 billion people, reducing production losses and postharvest damage critically, risking global food security (Porat *et al.* 2018; Spagnol *et al.* 2018; Becerra-Sanchez and Taylor 2021). Suppliers and vendors face the challenge of meeting this growing demand while maintaining product quality throughout the year. Agricultural losses in fruits and vegetables during postharvest operations, storage, transportation, processing, and packaging are one of the main causes of food loss and waste. This issue is generally addressed through product management and the proper use of pre- and postharvest technologies. However, despite this, postharvest losses remain a significant cause of food wastage for fresh produce. While determining the full extent of postharvest losses is difficult due to the variability of spoilage, which is widely influenced by fruit type, production region, and season, it is estimated that in developing countries, these losses range from 30 to 45 percent (Mathabe *et al.* 2020).

Among the traditional varieties of oranges grown worldwide, the Valencia and Washington varieties hold a prominent place (Roussos 2015). Washington Navel orange is one of the main orange varieties and an important economic citrus fruit worldwide. The characteristic feature of the Washington Navel orange is its seedless fruit with a very large size, orange-colored skin (in most varieties), and a sweet and pleasant taste. The cultivated area of Washington Navel oranges reached 181,092 tons, representing 53.55% of the total cultivated area of oranges among other varieties (El-Khalifa *et al.* 2022).

Postharvest physiological disorders, decay, and weight loss negatively impact the shelf life and marketability of fruit (Ennab *et al.* 2020). Therefore, the use of appropriate technologies to reduce losses and increase postharvest shelf life during fruit storage is important (Aloui and Khwaldia 2016). In the global market, the citrus industry is constantly searching for new methods to extend the marketing period and offer fruits with high nutritional value and numerous health-related properties (Zacarías-García *et al.* 2023). To enhance the natural resistance of fresh horticultural products to postharvest stresses and preserve the sensory and nutritional quality of fresh products, the use of environmentally friendly technologies is essential (Champa and Gamage 2020). The application of

various coatings on fruit surfaces can increase gloss and skin health, reduce water loss, and extend shelf life (Khorram and Ramezanian 2021).

Polyamines are natural compounds with an aliphatic nitrogen structure, low molecular weight, and poly-cationic metabolites that are found in all living organisms. They play a significant role in many physiological processes related to plant growth and development, including mitotic division, embryogenesis, flower initiation and development, fruit growth, ripening, aging, and response to environmental stresses (Chen *et al.* 2019; Ennab *et al.* 2020). The major forms of polyamines found in plants are putrescine, spermidine, and spermine, which have been reported to affect postharvest fruit physiology. Putrescine is the main product in polyamine biosynthesis and a synthetic precursor of spermidine and spermine (Masson *et al.* 2017). The use of polyamines can prevent aging, reduce respiration rate, delay ethylene production and color changes, enhance fruit firmness, create mechanical resistance, and reduce cold damage to horticultural products (Habibi and Ramezanian 2017). It has been established that polyamines are used in most fruits as a postharvest treatment to preserve the fruit's quality characteristics due to ripening and aging during storage (Hanif *et al.* 2020). Tas *et al.* (2024) reported that the external application of putrescine could be used as a postharvest tool to maintain the quality and storage life of cherry fruit. Other researchers examined the effects of putrescine and methyl jasmonate on lipid peroxidation and hydrogen peroxide changes in the skin and pulp of Valencia orange fruit and reported that fruits treated with 5 mM putrescine combined with 10 μM methyl jasmonate had the best effects on the evaluated traits during storage (Mohammadrezakhani *et al.* 2017). Research findings showed that salicylic acid and putrescine effectively delayed the postharvest deterioration rate and increased the storage life of mandarin fruit with acceptable quality (Ennab *et al*. 2020). The evaluation by Kucuker *et al.* (2023) showed that applying different concentrations of putrescine postharvest reduced decay and weight loss in fig fruit, and the application of putrescine also reduced the decrease in fruit acidity during storage in a cold room.

Essential oils are natural substances extracted from medicinal and aromatic plants. These compounds play a crucial role in food preservation and help enhance the safety and shelf life of food products. The effectiveness of essential oils is attributed to the presence of natural phenolic compounds. They are an important and healthy alternative to artificial preservatives and chemical additives (Shehata *et al.* 2020). Cinnamon, scientifically known as *Cinnamomum zeylanicum*, belongs to the Lauraceae family and grows wild in India, Sri Lanka, Indochina, and Madagascar. Its inner bark is used as a potent therapeutic agent in traditional medicine and as a flavoring agent in food. Cinnamon extract and essential oil exhibit antioxidant, mutagenic, and antimicrobial activities

(Alizadeh Behbahani *et al.* 2020). Research evidence suggests that cinnamon bark essential oil, as an antimicrobial agent, can extend postharvest life and reduce the risk of pathogen infections in many fresh fruits and vegetables (Champa *et al.* 2020). González *et al.* (2021) reported that the application of 500 mg per liter of cinnamon essential oil under refrigerated storage conditions (5 °C) was the most effective treatment for reducing fungal decay and maintaining fruit quality. According to the results of another study, adding cinnamon essential oil to orange peels can be an effective treatment to preserve the quality of citrus fruits (Khorram and Ramezanian 2021).

Today, one of the biggest challenges in fruit cultivation is preserving quality after harvest (Spagnol *et al.* 2018). Given that in recent years, in line with the development of organic farming and the production of healthy products, the use of natural compounds to increase the storage life of horticultural products has attracted the attention of researchers, and studies show that limited research has been conducted on the combined effect of polyamines and cinnamon essential oil on the postharvest life of Washington Navel oranges. Therefore, this experiment was conducted to investigate the effect of polyamines and cinnamon essential oil on increasing the postharvest life of Washington Navel oranges.

Materials and Methods

Washington Navel oranges at commercial maturity were obtained from a local orchard and selected for uniform size, color, and absence of defects. This experiment was carried out in a factorial arrangement based on a completely randomized design with three replications, and each replication included three fruits. The factors included cinnamon essential oil and putrescine treatments [control (without cinnamon essential oil and putrescine), 150 and 300 mg/L cinnamon essential oil, and 0.5, 1, and 1.5 mM putrescine] and storage time (0, 30, and 60 days). The oranges were collected from the Arzooie district, Valiabad area, Iran, and were transferred to the Plant Physiology Laboratory at Hormozgan University, Iran. The cinnamon essential oil was obtained from the Ayat Essences Company, Iran.

The oranges were immersed in putrescine solutions at three concentrations (0.5, 1, and 2 mM) and cinnamon essential oil at two concentrations (150 and 300 mg/L) for two minutes, after which, they were transferred to 1-liter closed plastic containers. The samples were kept at room temperature (±20 °C) and relative humidity (±75%) for the entire storage period. Every 30 days, the fruits were evaluated based on the traits under study.

Treatments

Solutions of putrescine (0.5, 1.0, and 1.5 mM) were prepared in distilled water. Cinnamon essential oil solutions (150 and 300 mg/L) were prepared by dissolving the essential oil in 1% methanol and then diluting to the final volume with distilled water. Methanol was used as the solvent because of its ability to disperse hydrophobic essential oil components. The final concentration of methanol in all cinnamon treatments did not exceed 1%. Fruits were dipped in each solution for two minutes, air-dried at room temperature, and then placed into storage. The control group was dipped in distilled water containing the same methanol concentration as in the essential-oil solutions.

Traits

On the first day of the experiment, the following characteristics were measured: Ion leakage, fruit firmness, pH, titratable acidity (TA), total soluble solids (TSS), total phenols, flavonoids, antioxidant capacity, and ascorbic acid. After 30 and 60 days, in addition to the above traits, fruit decay and weight loss percentage were also measured.

Decay percentage: To determine the percentage of decay, an observational scale with six degrees was used at each time point; Grade 0 (no decay), Grade 1 (10-20% decay), Grade 2 (20-30% decay), Grade 3 (30-40% decay), Grade 4 (40-50% decay), and Grade 5 (50-60% decay).

Decay index was calculated using the following formula (Ehtesham Nia *et al.* 2022):

Decay Index = [(Decay degree) × (Number of fruits in each grade)] ÷ (Total number of fruits in each replication)

Physiological weight loss: To determine the percentage of physiological weight loss, the weight of all oranges in each container at the start of the experiment was measured and recorded using a scale with a precision of 0.01g (initial weight). After 30 and 60 days, the oranges in each container were weighed again using the same scale (final weight). Finally, the percentage of physiological weight loss was calculated using the following formula (Ehtesham Nia *et al.* 2022):

$$PWL = \frac{W1-W2}{W1} \times 100$$

In the above formula, W1, W2, and PWL represent the initial weight, the final weight, and the percentage of physiological weight loss, respectively.

Firmness: To measure the hardness or firmness of the samples, a Lutron model FS-1001 firmness tester with an 8-mm diameter cylindrical probe was used. The pressure was reported in Newton.

Total soluble solids (TSS): To measure the TSS, the oranges were first squeezed by hand, and then a drop of orange juice was placed on the prism of the refractometer. The TSS value was determined in terms of Brix. Additionally, to calibrate and clean the device, distilled water was used after each reading (Ayala-Zavala *et al.* 2007).

pH of the samples: To measure the pH, a digital pH meter was used. The electrode of the device was placed in Falcon tubes (50 ml volume) containing orange juice, and the pH value reported by the device was read and recorded.

Titratable acidity (TA): To measure the TA, 27 ml of distilled water was poured into a beaker, and 3 ml of orange juice was added to it. The pH electrode was then placed in the beaker containing the sample, and titration was performed by gradually adding 0.1 normal NaOH until the pH reached 8.2. The amount of NaOH used for each sample was recorded. Finally, the TA was calculated using the following formula (Ayala-Zavala *et al.* 2007):

$$\mathrm{TA} = \left(\frac{\text{Amount of NaOH consumed} \times \text{ Molar acid equivalent of the fruit}}{\text{Sample volume}}\right) \times 100$$

Ascorbic acid: To measure ascorbic acid, fresh fruit juice was used. 100 µL of orange juice was added to a test tube containing 10 mL of 1% metaphosphoric acid and mixed thoroughly for 10 seconds, using a vortex at a constant speed of 2600 rpm. Then, 1000 µL of this mixture was taken and added to 9 mL of indophenol, and the mixture was vortexed again for 10 seconds. Finally, the samples were read at a wavelength of 515 nm using an ELISA reader (Etemadipoor *et al.* 2019). In this experiment, 1% metaphosphoric acid was used as a blank.

Antioxidant capacity: To prepare the methanolic extract for measuring antioxidant capacity, fruit juice and 85% methanol were used in a 1:3 ratio. The antioxidant capacity was measured using the DPPH free radical assay. For preparing the DPPH solution, 0.0048 g of DPPH powder was weighed using a sensitive scale (with a precision of 0.01 mg) and dissolved completely in 200 mL of 85% methanol using a magnetic stirrer on an alpha hot plate device. 950 µL of the DPPH solution was added to 50 µL of the methanolic extract. The samples were kept in the dark at room temperature for 30 minutes. After resting for 30 minutes, the absorbance of the samples was measured at a wavelength of 517 nm using an ELISA reader. In this experiment, 85% methanol was used as a blank, and the DPPH solution served as the control for the experiment (Sheng *et al.* 2018). The free radical scavenging percentage was calculated using the following formula:

$$TAC = \frac{AC-OD}{AC} \times 100$$

In the above formula, TAC, AC, and OD represent the antioxidant capacity, the control absorbance, and the sample absorbance, respectively.

Total phenols: To measure total phenols, the Folin-Ciocalteu reagent was used. 150 µL of methanolic extract was added to 750 µL of 10% Folin-Ciocalteu reagent from Merck. After 5 minutes of resting, 600 µL of 7% sodium carbonate was added. The samples were placed on a magnetic shaker in the dark for 1.5 hours. The absorbance of the samples was then measured at a wavelength of 760 nm using an ELISA reader. The total phenol content was expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g) (Singleton and Rossi 1965).

Flavonoids: The flavonoid content was measured using the aluminum chloride (AlCl3) colorimetric method. 100 µL of methanolic extract was added to 300 µL of 85% methanol. Then, 20 µL of 10% aluminum chloride and 20 µL of 1 M potassium acetate were added. Finally, 560 µL of distilled water was added to the mixture. The samples were kept on a shaker in the dark for 30 minutes. The absorbance of the samples was then measured at a wavelength of 415 nm using an ELISA reader. In this experiment, 85% methanol was used as the blank. The flavonoid content was expressed as milligrams of quercetin equivalent per gram of fresh weight (mg QE/g) (Chang *et al.* 2002).

Ion leakage: The ion leakage was measured using the method of Masoomi *et al.* (2012). 0.5 grams of orange peel, along with 20 mL of distilled water, were placed in a 50 mL Falcon tube for 24 hours. After 24 hours, the initial electrical conductivity (EC1) of the samples was measured using an EC meter. Then, the samples were placed in a water bath at 100 °C for one hour. After cooling the samples to 25 °C, the secondary electrical conductivity (EC2) was measured using the EC meter. The ion leakage percentage (IL) was then calculated using the following formula:

$$IL = (EC_1/EC_2) \times 100$$

Statistical analysis

Analysis of variance was performed based on the factorial arrangement under a completely randomized design. Assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene tests. Means were compared using Duncan's multiple range test at $p \leq 0.05$. The data were analyzed using the SAS software.

Results and Discussion

Based on the analysis of variance, the treatment (putrescine and cinnamon essential oil), time, and their interactions significantly affected the decay, firmness, weight loss, TSS, pH, TA, ascorbic acid, antioxidant capacity, total phenols, and flavonoids of the orange fruit (Tables 1 and 2).

Table 1. Analysis of variance for the effects of putrescine and cinnamon essential oil treatments and storage time on physiological traits of orange.

Source of variation	df	Mean Squares				df	Mean Squares	
		Firmness	TSS	pH	TA		Weight loss	Decay
Treatment	5	10.33**	2.95**	0.011**	0.0003**	5	8.28**	436.43**
Time	2	358.99**	1.01*	2.96**	0.0032**	1^{+}	256.48**	2277.31**
Treatment × Time	10	6.20**	1.053**	0.016**	0.000084**	5	2.45**	170.19*
Error	36	1.02	0.55	0.02	0.000016	24	0.22	1.59
Total	53	-	-	-	-	35	-	-
CV (%)	-	5.6	5.61	2.93	4.83		5.30	14.46

*,**: Significant at 5% and 1% probability levels, respectively; TSS; total soluble solids, TA: Titratable acidity; Only for 30 and 60 days of storage.

Table 2. Analysis of variance for the effects of putrescine and cinnamon essential oil treatments and storage time on biochemical traits of orange.

Source of variation	df	Mean Squares				
		Ion leakage	Total phenols	Flavonoids	Antioxidant capacity	Ascorbic acid
Treatment	5	23.07*	745.27**	.0038**	2.69**	4.47**
Time	2	35.12*	15677.04**	3.870**	534.47**	1150.92**
Treatment × Time	10	40.53*	385.19**	0.017**	0.77*	1.85**
Error	36	18.02	8.67	0.029	0.17	2.32
Total	53	-	-	-	-	-
CV (%)	-	5.30	2.43	14.21	1.47	5.33

*,**: Significant at 5% and 1% probability levels, respectively.

Decay

The highest level of decay was observed in the control treatment and 0.5 mM putrescine at 60 days after the start of the experiment, while the lowest level of decay was found in the cinnamon treatments at concentrations of 150 and 300 mg/L and putrescine at the concentrations of 1 and 1.5 mM at 30 and 60 days (Figure 1). The susceptibility of fruit to postharvest diseases is accelerated by ripening and aging, as the peel softens, requiring less force for pathogens to attack the skin (Ehtesham Nia *et al.* 2022). Polyamines bind to phenolic compounds and hydroxycinnamic acid amides (HCAA), and

studies have shown a strong correlation between HCAA accumulation and pathogen resistance (Ehtesham Nia *et al.* 2022). In this study, the cinnamon treatment showed the most significant positive effect on reducing the decay. Reports suggest that cinnamon essential oil damages the cytoplasmic membrane of the fungus, causing green decay, leading to a decrease in electron transfer, loss of membrane integrity, and leakage of proteins, potassium, and phosphate from epidermal cells of the skin (Alizadeh Behbahani *et al.* 2020; Khorram and Ramezanian 2021). Our results are also consistent with previous reports on lemons, oranges, and other citrus fruits (Pérez-Alfonso *et al*. 2012; Khorram and Ramezanian 2021; Yang *et al.* 2021).

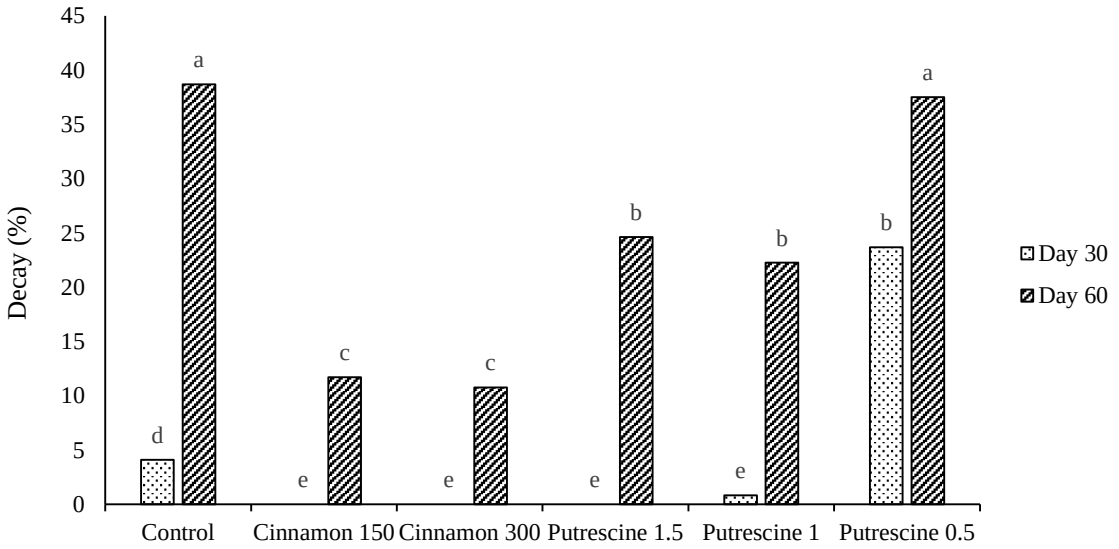

Figure 1. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning decay incidence; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range test.

Physiological weight loss

After 30 days from the start of the experiment, the highest level of weight loss was observed in the control treatment, while the lowest level of weight loss was found in the cinnamon essential oil treatments at both concentrations and in the putrescine treatments at concentrations of 1 and 1.5 mM (Figure 2). After 60 days, the lowest level of weight loss was observed in the cinnamon essential oil treatment at the concentration of 300 mg/L and in the 1.5 mM putrescine. It is likely that the use of putrescine helps maintain membrane integrity and delays the removal of the epicuticular wax, reducing ethylene release and respiration, which minimizes weight loss and preserves fruit firmness during storage. Our results are consistent with other reports on the effect of putrescine on fruit quality in mangoes (Wannabussapawich and Seraypheap 2018).

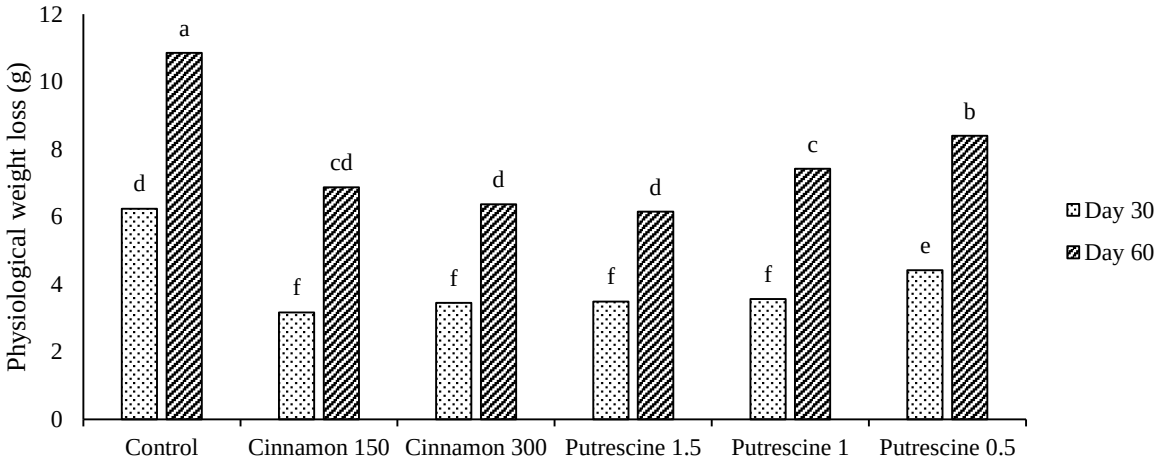

Figure 2. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning physiological weight loss; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range test.

Firmness

The results showed the highest firmness in the putrescine treatment at the concentration of 1.5 mM at both 60 and 30 days after the start of the experiment, which did not show a significant difference from the other treatments on day 0. The lowest firmness was found in the control treatment at 60 days after the start of the experiment (Figure 3). It has been established that the application of polyamines reduces softening progress by inhibiting the activity of cell wall-modifying enzymes (Singh *et al.* 2019). Evidence suggests that putrescine strengthens the cell wall by forming cross-links with carboxyl groups of pectin in the cell wall, thus maintaining fruit firmness. Additionally, it reduces the activity of cell wall-degrading enzymes such as pectin methylesterase, pectin esterase, and polygalacturonase (Valero *et al.* 2002). Similar results were reported (Habibi and Ramezanian 2017) in blood oranges, by Ennab *et al.* 2020) in tangerines, and by Pachuau *et al.* (2024) in oranges.

TSS

After 60 days of storage, the highest amount of TSS was found in the control treatment, while the lowest amount of this trait was observed for the putrescine treatment at the concentration of 1 and 1.5 mM, and for the cinnamon essential oil at the concentration of 300 mg/L (Figure 4). The lower sugar accumulation in the putrescine treatment is likely due to the delay in aging and ripening processes, which occur as a result of the suppression of amylase and phosphorylase enzyme activities. Treating the fruit with putrescine increases the polyamine levels within the fruit, which may reduce sugar accumulation and respiration rate (Hanif *et al.* 2020). The results of this experiment are in agreement with findings regarding the effect of putrescine on TSS in apples (Sedaghati *et al.* 2020), papaya

(Hanif *et al.* 2020), and the effect of cinnamon essential oil on TSS, as reported by Sotelo-Alcántara *et al.* (2022).

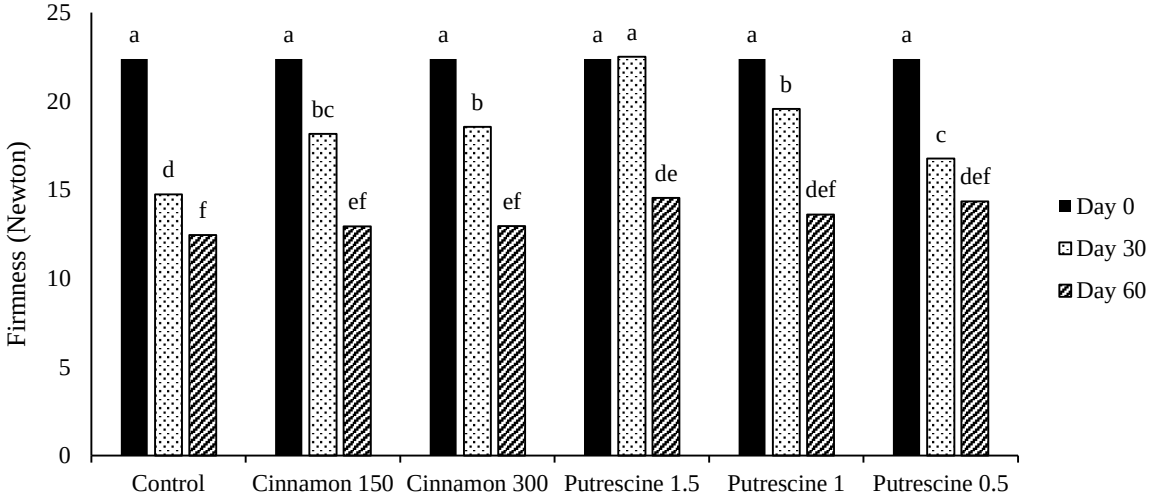

Figure 3. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the fruit firmness; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range test.

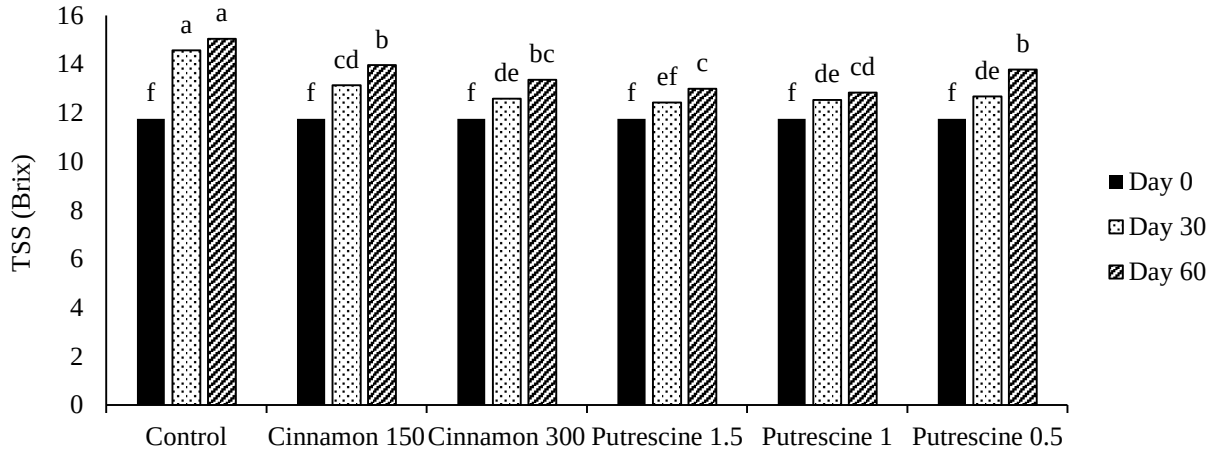

Figure 4. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the total soluble solids (TSS); Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range test.

pH and TA of orange juice

Sixty and 30 days after the start of the experiment, the highest pH and TA of orange juice were observed in all putrescine and cinnamon essential oil treatments as compared to the control, which showed the lowest pH (Figures 5 and 6). Thus, putrescine and cinnamon essential oil treatments had a positive effect on preserving the organic acids in the samples throughout storage. It appears that the effect of polyamine treatments is related to their ability to maintain the acidic conditions and total acidity of the fruit extract, as well as their ability to compete with ethylene and delay the ripening process (Hosseini *et al.* 2017). In respiration metabolism, organic acids serve as substrates in the

ripening process (Solís-Contreras *et al.* 2021). Cinnamon essential oil has an inhibitory effect on metabolism and prevents the breakdown of organic acids (Yu *et al.* 2021). These results are consistent with other findings in apples (Solís-Contreras *et al.* 2021), mangoes (Yu *et al.* 2021), oranges (Khorram and Ramezanian 2021), tangerines (Ennab *et al.* 2020), and grapes (Ehtesham Nia *et al.* 2022).

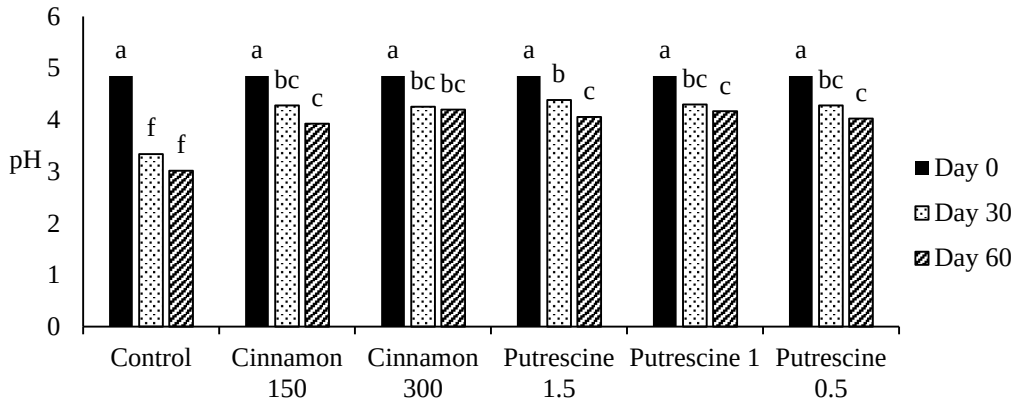

Figure 5. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the total soluble solids (TSS); Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range test.

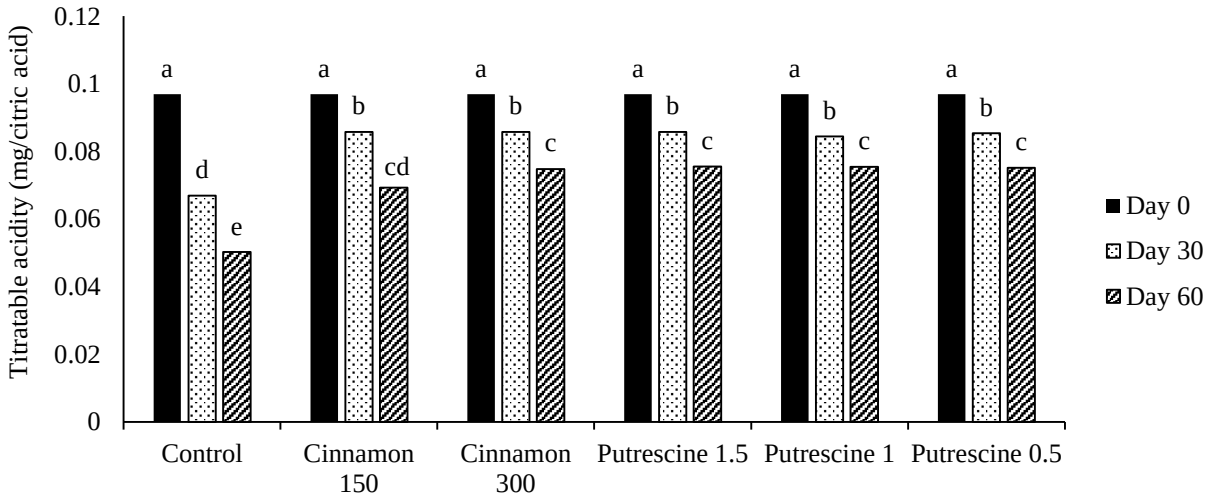

Figure 6. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the titratable acidity; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

Ascorbic acid and antioxidant capacity

All cinnamon and putrescine treatments had significantly higher ascorbic acid content compared to the control after 30 and 60 days from the start of the experiment (Figure 7). The highest antioxidant capacity was found in the cinnamon treatment at 300 mg/L after 60 days from the start of the experiment, while the lowest antioxidant capacity was recorded in the control (Figure 8).

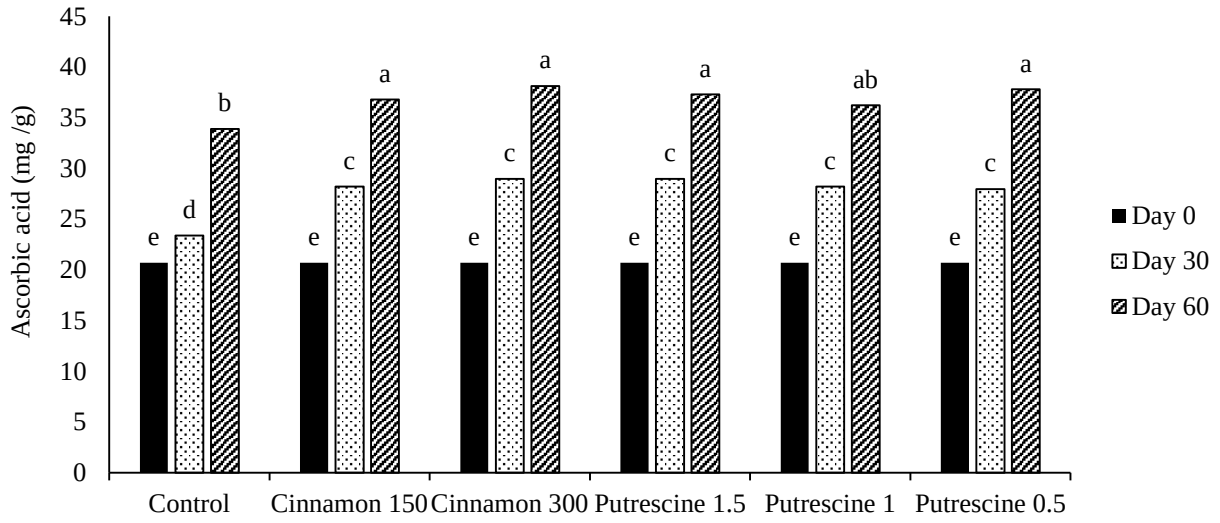

Figure 7. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the ascorbic acid; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

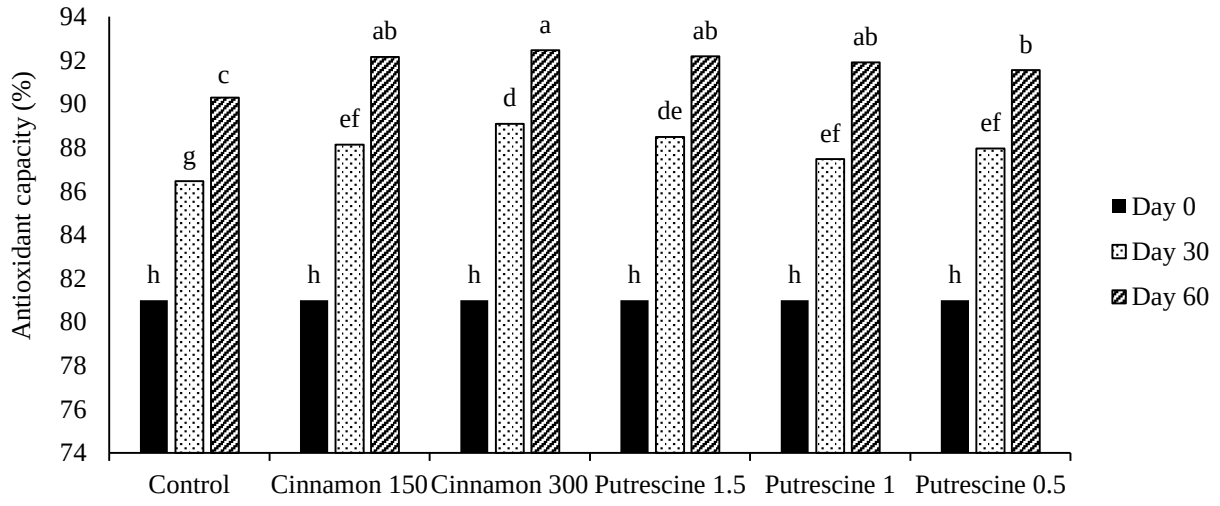

Figure 8. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the antioxidant capacity; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

It appears that the effect of the putrescine treatments is related to its ability to maintain acidic conditions and the total acidity of the fruit extract, their ability to compete with ethylene, delaying the ripening process, and increasing the levels of phenolic compounds and flavonoids (Champa and Gamage 2020; Ehtesham Nia *et al.* 2022). Ramezanian *et al.* (2016) stated that the increase in ascorbic acid content in 'Washington Navel' oranges treated with essential oils, compared to the control, could be due to the antioxidant effects of the essential oils. Similar results were found in pomegranate

(Fawole *et al.* 2020), mango (Razzaq *et al.* 2014), oranges (Ramezanian *et al.* 2016), and grapes (Ehtesham Nia *et al.* 2022).

Total phenols and flavonoids

At 60 days from the start of the experiment, the highest total phenolic content was observed in the cinnamon treatment at the concentration of 300 mg/L, which was significantly higher than the control. This treatment, together with the 1.5 mM putrescine, also showed the highest total phenolic content after 300 days of storage (Figure 9). The flavonoid content of all cinnamon and putrescine treatments was significantly higher than that of the control at 60 and 30 days from the start of the experiment (Figure 10).

The role of the putrescine treatment in preserving phenolic content can be attributed to the delay in the aging process (Ehtesham Nia *et al.* 2022). The increase in phenolic compounds and antioxidant activity by the external application of putrescine aligns with the findings of Chen *et al.* (2019) and Collado-González *et al.* (2021). It appears that cinnamon essential oil increases the levels of phenolic compounds and flavonoids, which have antioxidant effects in plants, thereby enhancing the antioxidant capacity (da Costa Gonçalves *et al.* 2021). Similar results were also obtained in cherry tomatoes by El-Sayed *et al.* (2022).

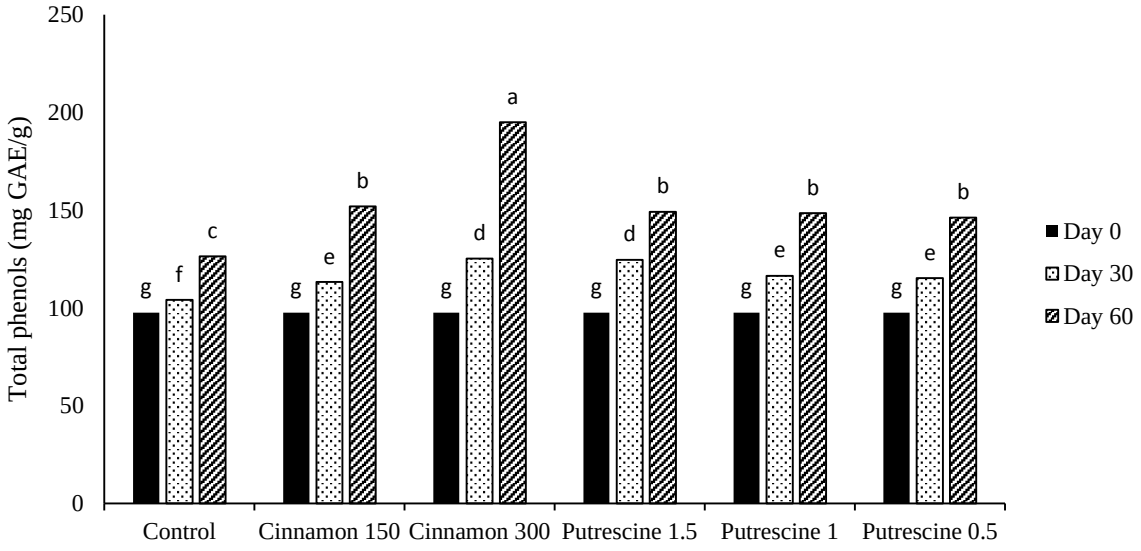

Figure 9. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the total phenol; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

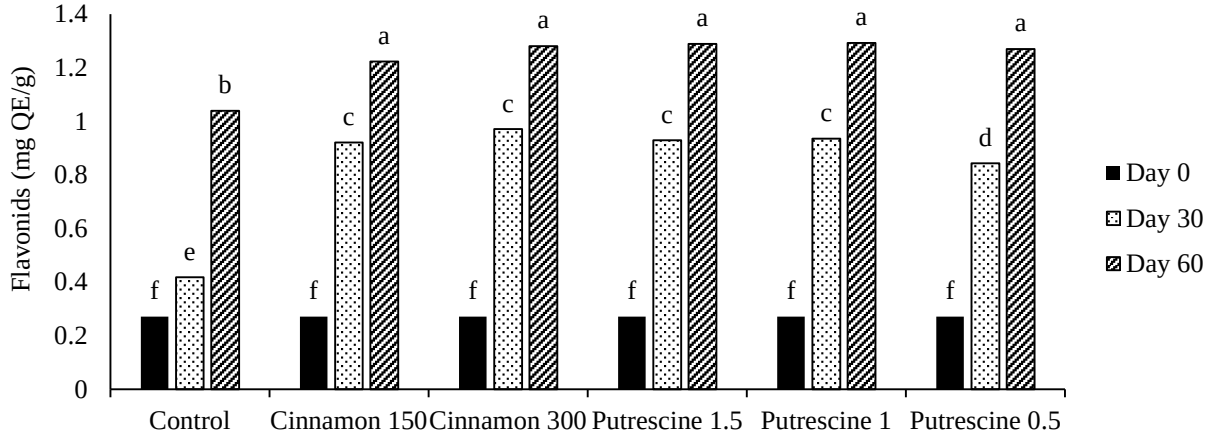

Figure 10. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the flavonoids; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

Ion leakage

The highest ion leakage was observed in the control treatment after 60 days from the start of the experiment, while at this date, the lowest ionic leakage was recorded for the putrescine at 1 and 1.5 mM concentrations (Figure 11). One of the characteristics of aging is an increase in the permeability of the cell membrane, which is expressed as an increase in the ion leakage. This is often used as an indicator of membrane damage; the greater the ion leakage, the more damage has occurred to the membrane (Ennab *et al.* 2020). The application of putrescine delays the lipid peroxidation of the membrane during storage and helps maintain the integrity of the membrane structure, leading to a reduction in ion leakage (Zhao *et al.* 2023). This finding is consistent with those reported in tangerines and blood oranges (Habibi and Ramezanian 2017; Ennab *et al.* 2020).

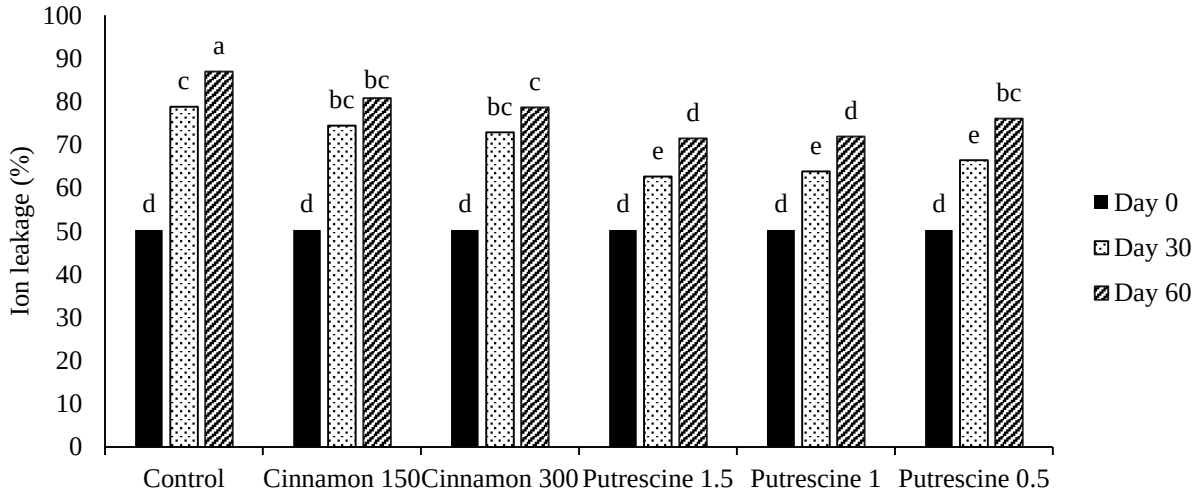

Figure 11. The interaction of putrescine (0.5, 1.0, and 1.5 mM) and cinnamon essential oil treatments (150 and 300 mg/L) with the sampling time concerning the Ionic Leakage; Means with different letters are significantly different at the 5% probability level, according to Duncan's multiple range.

The results of this study demonstrated that putrescine and cinnamon essential oil effectively delayed postharvest deterioration of Washington Navel oranges stored at 20 °C. Storage at this temperature accelerates respiration, senescence, and water loss in citrus fruits, which explains the rapid decline in the quality observed in the untreated control. However, both treatments significantly slowed these physiological changes.

The reduction in physiological weight loss is likely associated with the role of putrescine in stabilizing cellular membranes. Polyamines such as putrescine are known to interact with phospholipids and protect membrane integrity, thereby decreasing leakage and transpiration. In the present study, the higher firmness and lower decay in putrescine-treated fruits support the hypothesis that membrane stabilization reduces susceptibility to tissue softening and pathogen invasion (Alan *et al.* 2025).

Cinnamon essential oil also contributed to maintaining fruit quality. Its bioactive phenolic components, such as cinnamaldehyde and eugenol, exhibit well-documented antifungal and antibacterial properties. These compounds disrupt microbial cell membranes, inhibit spore germination, and suppress fungal growth, which explains the markedly lower decay percentage observed. Additionally, essential oils have been shown to reduce ethylene production and oxidative stress, which may contribute to the slower changes in TSS, TA, and pH (OuYang *et al.* 2019).

The selected storage temperature of 20 °C was intentional and important. Unlike many studies that focus exclusively on refrigerated storage, this experiment was designed to simulate ambient market conditions, where most postharvest losses occur due to inadequate cooling during transport, handling, and retail display. Evaluating the performance of natural compounds under these conditions provides practical value for growers and storage operators, offering insight into how fruits behave when cold-chain control is limited.

Our results confirmed that bio-based treatments can mitigate quality loss and extend shelf-life even at room temperature. This aligns with increasing interest in sustainable and residue-free postharvest technologies and highlights the potential of putrescine and cinnamon essential oil as accessible alternatives to synthetic chemicals.

Conclusion

This study showed that putrescine and cinnamon essential oil can significantly slow the deterioration of Washington Navel oranges stored under ambient market conditions (20 °C). These treatments reduced physiological weight loss, decay incidence, and changed the biochemical attributes, demonstrating their potential to maintain fruit quality in situations where cold-chain facilities are

limited. Among the treatments evaluated, the 1.5 mM concentration of putrescine and/or 300 mg/L cinnamon essential oil consistently produced the greatest positive effects on firmness, antioxidant capacity, and overall quality retention.

While our findings indicate that bio-based treatments may serve as practical alternatives to synthetic postharvest chemicals, further research under commercial-scale conditions and with additional cultivars is recommended. Such studies would help validate the broader applicability and economic feasibility of these treatments. For orchardists and citrus storage operators, the use of these natural dipping solutions may provide a simple and environmentally friendly option for reducing quality loss and extending marketability during room-temperature handling and short-term storage.

Conflict of Interest

The authors declare that they have no known competing interests with any individuals or organizations concerning the subject of this article.

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