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Elicitor-based strategy for shelf-life enhancement of oranges during cold storage

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Abstract

Postharvest losses are a citrus production challenge, requiring effective, low-cost strategies to maintain fruit quality during storage. This study evaluates the effects of L-phenylalanine application on the shelf life of Westin oranges stored in a cold incubator. We used a completely randomized split-plot design with four concentrations of L-phenylalanine (0, 3, 6, and 9 mM) and five evaluation time points (0, 10, 20, 30, and 40 days). We evaluated qualitative indicators and rot incidence. The 3 mM L-phenylalanine concentration was the most effective in balancing physical quality and appearance without deterioration. These results suggest that L-phenylalanine is a promising technique for orange cold storage. Oranges can be marketed up to 15-20 days after harvest with healthy appearance, uniform size, shiny skin, maintained firmness, and maximum juice yield.

Keywords: *Citrus sinensis* (L.) Osbeck; ascorbic acid; weight loss; soluble solids; colorimetry.

Practical application: Post-harvest innovation improves citrus sustainability and efficiency.

1 INTRODUCTION

Current demand for citrus fruits includes sensory and nutritional properties, as well as attractive appearance, which directly influence consumer purchasing decisions (Blasco et al., 2009; Lv et al., 2015; Sdiri et al., 2012). Among all cultivated citrus species worldwide, orange [*Citrus sinensis* (L.) Osbeck] is the most important, characterized by its sweet, pleasant taste and delicate aroma (Roussos, 2016). The Westin cultivar, an early-cycle orange, is widely cultivated in the state of São Paulo (Fundecitrus, 2024) and produces fruit of high commercial value, with an average weight of 145 g, thin peel, and high juice yield (JY). However, fruit persistence on the plant after ripening is low (Oliveira et al., 2025).

Citrus fruits do not ripen after harvesting and may deteriorate during post-harvest storage, with softening due to dehydration and the incidence of diseases (Berk, 2016b; Ejaz et al., 2022). In developing countries such as Brazil, post-harvest losses of citrus fruits range from 25 to 30% (Ladaniya, 2022), with fungi responsible for 25 to 50% of post-harvest losses (Nunes, 2015; Solanki et al., 2024; Zhang et al., 2017). Fungal pathogens, especially green mold [*Penicillium digitatum* (Pers.:Fr.) Sacc.], are the main cause of losses in fresh fruit (Benato et al., 2018; Spadaro & Droby, 2016). Controlling this disease is difficult because fungal propagules can remain dormant in healthy fruit and emerge progressively after harvest (Terao et al., 2020). Synthetic fungicides predominantly control fungi, but consumer markets question their use. Nevertheless, new, ecological, and more effective techniques are under development globally (Bhatta, 2022; Ladaniya, 2022).

Keeping the desired characteristics and standards, like even color, firmness (F), and no defects during post-harvest storage, is a big challenge (Ritenour, 2016). Coating fruits with carnauba wax is the most common method, protecting fruits from damage, improving appearance, extending shelf life, intensifying peel shine, and minimizing post-harvest waste. This method does not compromise fruit sensory quality and may contain fungicides mixed into the composition (Babarabie et al., 2024; Malgarim et al., 2007). Despite benefits, carnauba wax-based coatings have complex thermal crystallization. This requires adequate application and thermal control structures (Müller et al., 2018), which make their use challenging for small-scale growers without technical expertise.

Elicitor-based strategies for post-harvest disease control are advancing as sustainable, cost-effective alternatives that enhance consumer safety (Caicedo-López et al., 2021; Zhou & Wang, 2018). L-Phenylalanine (Phe), a natural elicitor and essential amino acid, can trigger fruit defense mechanisms by activating secondary metabolism at low concentrations (Moreno-Escamilla et al., 2018). It acts as a substrate for phenylalanine

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ammonia-lyase (PAL), a key enzyme in the phenylpropanoid pathway, which contributes to plant resistance through the synthesis of compounds like salicylic acid, lignin, flavonoids, tannins, and phytoalexins (Guest et al., 2017; Iqbal & Poór, 2025; Kumar et al., 2020; Mierziak et al., 2014; Pinto et al., 2011; Saidi et al., 2021; Tripathi et al., 2019).

Therefore, this study aimed to evaluate the effects of applying different concentrations of Phe and times of cold storage on the qualitative indicators and the incidence and severity of fungal diseases in 'Westin' oranges.

1.1 Relevance of the work

This study evaluates L-phenylalanine (Phe) as a sustainable postharvest alternative for maintaining fruit quality and controlling diseases. Phe, a precursor of secondary metabolites, can activate defense responses at low concentrations. The effects of Phe concentrations and cold storage durations were assessed on orange quality and rot incidence. Results indicate that Phe improves resistance, preserving fruit firmness, peel brightness, and juice yield. Its low cost, safety, and efficacy highlight its potential in postharvest management. Phe-treated fruits remained marketable for 15–20 days, maintaining uniformity and health, suggesting a viable ecological strategy for extending orange shelf life.

2 MATERIALS AND METHODS

Fruits were harvested from 'Westin' orange trees grown at AlfaCitrus Anhumas Farm, located in the municipality of Anhembi, São Paulo (22°56'17" S and 48°16'05" W). The treatments were T1–control (0 mM Phe); T2–(3 mM Phe); T3–(6 mM Phe); T4–(9 mM Phe), with all treatments diluted in distilled water. A completely randomized design was used, with split plots (4x5), where the plots are the four Phe concentrations and the subplots are the five evaluation time points (0, 10, 20, 30, and 40 days after treatment), with four replicates and ten fruits per experimental subplot, totaling 40 fruits per treatment at each evaluation time point and 200 fruits evaluated in each study treatment (n = 800).

Fruit selection criteria included an average weight of more than 170 g and a yellowish-green color. After harvesting, fruits were distributed in plastic trays, sanitized in running water, and immersed in their respective treatments.

The amino acid Phe from Dinâmica Química® was used in the treatments. For fruit immersion, Phe concentrations were prepared by dilution in distilled water and homogenized by stirring with a TECNAL® Te-0853/1 Magnetic Stirrer. The orange fruits were immersed for 30 seconds in the respective Phe concentrations (Kumar et al., 2020). After immersion, the fruits were dried naturally and stored in cold storage for 40 days in a Biochemical Oxygen Demand (BOD) incubator with temperature and humidity control (3°C ± 1° / 85% RH) (Oliveira & Scivittaro, 2011).

Weight loss (WL) was quantified by selecting ten fruits from each treatment group and measuring their individual weights on specified evaluation dates. The value is expressed as a percentage (%) according to Equation 1:

$$WL = \left[\left(\frac{(FWinitial - FWfinal)}{FWinitial}\right) * 100\right] \quad (1)$$

Peel color was analyzed using a colorimeter (CR 400, Konica Minolta). The following parameters were represented by the CIE65 (International Commission illuminant) model: luminosity - darkness/lightness (L), greenness/redness (a), and blueness/yellowness (b). Coloring intensity Chroma (C) and hue angle (H) were calculated from a and b (Konica Minolta Sensing Americas, Inc., 2025; McGuire, 1992).

JY, expressed as a percentage, was calculated as the ratio of juice weight to fruit weight (Equation 2):

$$JY = [(\frac{JW}{FW}) * 100] \quad (2)$$

F was measured with a texture analyzer (10 mm compression at 1.0 $mm \cdot s^{-1}$) and expressed in N (Stable Micro Systems, 2025). Soluble solids (SS) (°Brix) and titratable acidity (TA) (% citric acid) were determined from homogenized juice using a refractometer and NaOH titration, respectively (Instituto Adolfo Lutz, 2008). Ripeness index (RI) was calculated as the ratio of °Brix to acidity. Ascorbic acid (AA) ($mg \cdot 100\ mL^{-1}$) was quantified by 2,6-Dichlorophenol Indophenol (DCPIP) titration (AOAC International, 2005). All analyses were performed in triplicate.

Rot incidence was visually assessed in all fruits from each treatment to detect post-harvest disease symptoms (Fischer et al., 2008). Incidence (%) was calculated as in Equation 3 (Campbell & Madden, 1990):

$$IR = \left(\frac{n}{N}\right) * 100 \quad (3)$$

Where:

N: the total number of fruits;

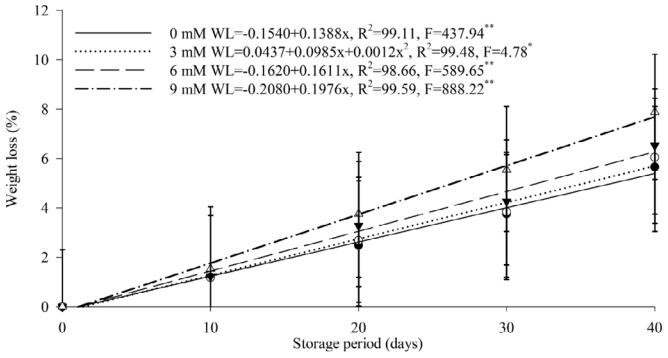
n: the number showing rot.

No post-harvest rot symptoms were observed during the evaluation period; therefore, symptom severity was not assessed.

Data were analyzed using Minitab® normality tests, variance and regression analyses using Sisvar® software, and graphs created using SigmaPlot® (Ferreira, 2019; Minitab, 2017; Systat, 2025; Zadocks, 1985). Weight loss data were transformed to x0.5. The multivariate and correlation analyses were performed using XLSTAT® software (Lumivero, 2025). Principal component analysis (PCA) and hierarchical clustering were used to describe interactions between Phe concentrations and storage times on the analyzed variables.

3 RESULTS AND DISCUSSION

Fruit weight loss increased linearly with cold storage time in the control and treatment groups using 6 and 9 mM Phe. A quadratic effect was observed in the 3 mM Phe treatment. The lowest fruit weight loss was observed in the control group (5.65%), with acceptable standard fruits up to 37 days of storage. The highest concentration of Phe (9 mM) accelerated fruit weight loss and reduced storage time, with 7.87% loss after 26 days of storage (Figure 1), exceeding the maximum defined limit of 5% weight loss (Rêgo et al., 2023).



WL: weight loss; R^2: R-squared; F: F-test value.

Figure 1. Weight loss in 'Westin' orange fruits under different L-phenylalanine concentrations during refrigerated storage (3 ± 1°C / 85%RH).

Phe acts as a precursor to various phenolic compounds via the phenylpropanoid pathway (Mamari, 2021). Ideal Phe concentrations, which vary by fruit species, can be used to extend shelf life (Kumar et al., 2020). One hypothesis for the negative effect of 9 mM Phe on orange fruits is that this concentration caused disordered stimulation through phenylpropanoids, leading to increased production of defense compounds, cell membrane damage, and accelerated water loss (Figure 1). The phenylpropanoid pathway improves defense mechanisms against biotic and abiotic stresses, contributing to plant acclimatization to adverse conditions (Dong & Lin, 2021; Gray et al., 2012). In addition to contributing to the neutralization of reactive oxygen species, these metabolites are also responsible for the organoleptic and qualitative properties of food, are used in the cosmetic and pharmaceutical industries, and are beneficial to human health due to their antioxidant activity (Ruiz-García & Gómez-Plaza, 2013). Phe can also induce ethylene synthesis through a non-enzymatic pathway, promoting PAL activation because of the increased synthesis of cinnamic acid, which acts as a direct substrate for this hormone biosynthesis (Talukder, 2020).

Different concentrations of Phe significantly influenced peel colorimetric indicators in 'Westin' oranges (Figure 2). Negative quadratic effects were observed for L and C. L decreased to a minimum at 3.76 mM Phe, followed by an increase at higher concentrations, reaching 70.81 at 6 mM and 73.80 at

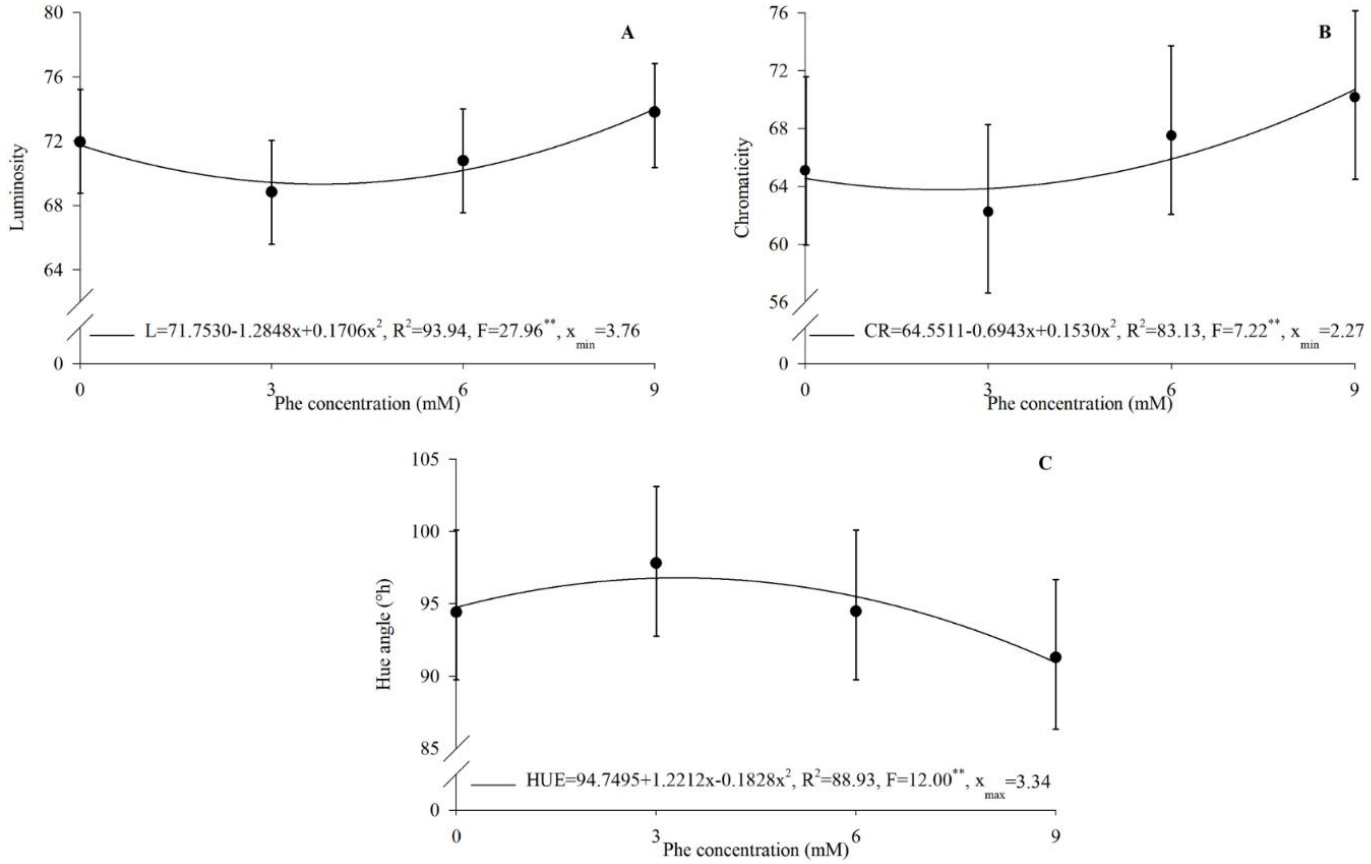


Figure 2. (A) Luminosity, (B) chromaticity, and (C) Hue angle in 'Westin' orange fruits under different Phe concentrations.

9 mM (Figure 2A). C also declined to a minimum at 2.27 mM Phe, then rose progressively at 3, 6, and 9 mM (62.24, 67.56, and 70.20, respectively) (Figure 2B).

H values remained close to 90°, corresponding to a yellow hue, but showed a quadratic response to Phe concentration, peaking at 3.34 mM (Figure 2C). Beyond this point, H values declined, indicating a shift in peel coloration (Figure 2C). Colorimetric analysis revealed complementary trends during cold storage. L increased from 69.36 to 73.52, indicating gradual peel lightening (Figure 2A). C rose from 62.26 to 70.52, reflecting greater C (Figure 2B), while H decreased from 98.53°h to 89.19°h, indicating a shift from green to yellow-orange tones (Figure 2C).

Color changes in fruit peel during development and ripening are linked to pigment synthesis and degradation (Choo, 2019; Kou et al., 2021; Melo, 2021; Nascimento & Bender, 2018; Siqueira & Salomão, 2017). In the highest Phe concentration treatment, fruits were visually lighter than the others. Higher Phe concentrations intensified peel color saturation, promoting a more vibrant, attractive appearance. This effect is associated with the action of Phe as a precursor in the flavonoid pathway, which directly contributes to citrus fruit peel coloration.

Peel color and uniformity are quality parameters in oranges (Ritenour, 2016). During storage, increases in L, C, and H (H ≈ 90°) reflected a shift toward a lighter, more vivid, and orange-colored peel. Intermediate Phe concentrations had minimal impact on degreening, maintaining green coloration, whereas higher concentrations promoted chlorophyll degradation and carotenoid accumulation, enhancing yellow-orange coloration and overall visual quality (Kou et al., 2021).

Several factors influence peel coloration, including phenolic compound contents, oxidative enzyme activity, and chlorophyll breakdown. While Phe may contribute to color changes, its effect is modulated by interactions with water loss, oxidation, and peel maturation, which can sometimes reduce yellow pigmentation (Rodrigo et al., 2013). Ethylene, a plant hormone, regulates senescence, ripening, and stress responses, and high Phe concentrations may stimulate its production (Falcone et al., 2012; Fatma et al., 2022). Though this relationship is complex and species-dependent, increased ethylene can promote chlorophyll degradation (degreening) and the synthesis of other pigments such as anthocyanins, contributing to the dynamic color changes observed during fruit ripening (Kou et al., 2021; Neves et al., 2024).

Cold storage had a quadratic effect on JY, with a maximum of 54.10% observed around 15 days, followed by a gradual decline (Figure 3A). The initial increase in JY is likely due to preferential water loss from the peel, reducing its relative weight and increasing the juice proportion. Beyond this point, water loss shifts to the pulp, associated with changes in membrane permeability and tissue integrity, leading to reduced juice extraction. These findings align with previous reports indicating high JY in the 'Westin' cultivar (50–56%) (Oliveira et al., 2025; Schwarz et al., 2018), with even higher values (67.67%) under different experimental conditions (Melo, 2021). Thus, for optimal JY under the conditions evaluated, storage should be limited to approximately two weeks.

Cold storage had a linear negative effect on F, which progressively declined with increasing storage duration, from 97.58 N to 55.12 N (Figure 3B). F is a critical quality attribute that strongly influences consumer preference. Its reduction, commonly observed as fruit softening, significantly compromises postharvest shelf life and increases susceptibility to mechanical injury and pathogen invasion during handling and transportation. This F loss is primarily associated with the degradation of cell wall components—particularly pectin, hemicellulose, and cellulose—which leads to reduced structural integrity and cellular turgor. Additionally, the decline in water content exacerbates tissue softening, often resulting in visible signs such as wilting, surface wrinkling, and reduced fresh weight (Melo et al., 2016; Melo, 2021; Rêgo et al., 2023).

L increased with storage time, ranging from 69.36 to 73.52 (Figure 3C), indicating progressive peel lightening. C (Figure 3D) increased from 62.26 to 70.52 during storage, reflecting an increase in color brightness and purity. H decreased from 98.53°h to 89.19°h, characterizing a spectral transition from green to yellow-orange (Figure 3E). These parameters reflect an improvement in visual quality, which is crucial for market acceptance (Kou et al., 2021).

There was a significant interaction between Phe concentrations and storage time ($p < .05$) for SS contents. The control showed an increase in SS during storage (13.42º–13.8ºBrix), while the other treatments showed a reduction in the same period, with values of 3 mM (14.02º–13.42ºBrix), 6 mM (14.15º–13.15ºBrix), and 9 mM (14.17º–13.42ºBrix) (Figure 4A). The absence of an increase during the storage period indicates that citrus fruits show little variation and a decrease in SS content during cold storage (Tanaka et al., 2008). A linear decrease in TA was observed for 3 mM (2.8–0.67% citric acid), while a quadratic decrease was observed for 0 mM (2.0–0.79% citric acid), 6 mM (1.63–0.80% citric acid), and 9 mM (2.56–0.73% citric acid). This shows an initial increase followed by a decline in values for 0 mM and 6 mM, respectively, with a maximum occurring around days 12 and 15 of storage (Figure 4B).

The acidity of citrus fruits is mainly determined by citric acid, which accounts for 80–85% of TA. During initial fruit development, this acid accumulates until it reaches its maximum content, remaining practically constant until harvest. During ripening, the reduction in acid concentration mainly reflects the metabolic consumption of organic acids during storage and ripening. This decrease in acidity, which gives the fruit its characteristic sour taste, improves its sensory quality, since excessive acidity compromises its acceptance for fresh consumption (Kluge et al., 2007; Siqueira & Salomão, 2017). Dilution caused by the increase in juice volume reduces acid concentration during ripening (Siqueira & Salomão, 2017). As the orange fruit ripens, the volume of juice within the fruit's cells tends to increase. This can occur due to the accumulation of water in the vacuoles of the cells. This dilutes the substances present, including organic acids. During ripening, in addition to dilution from higher juice volume, acid production, such as citric acid, can also decrease. This is common, as many acidic

compounds found in orange fruits tend to decrease with ripening, resulting in a less acidic taste. Reduced acid concentration due to dilution can influence the flavor of the fruit, making it sweeter as acidity decreases (Anese & Fronza, 2015; Siqueira & Salomão, 2017).

RI showed a quadratic increase over the storage period, with all treatments displaying a similar response. The minimum desirable RI of 9.5 was reached between days 26 and 29, depending on the treatment (Figure 4C). The maximum RI of 16 was achieved by the 6 mM and 9 mM treatments on day 38, and by the 0 mM and 3 mM treatments on day 40. This increase in RI was primarily associated with a decrease in acidity rather than an increase in SS content, as SS remained relatively stable throughout storage, while acidity loss became more evident in the later stages.

RI is the most widely used indicator to determine the ideal harvest stage for citrus fruits because it indicates flavor, showing balance between organic acids and sugars. This value varies for table oranges, with a minimum of 9.5 recommended, while values above 16 indicate very ripe fruit, unsuitable for sale due to compromised flavor (Nascimento & Bender, 2018; Siqueira & Salomão, 2017). Although the RI index reached the established commercial threshold of 9.5 only after 26–29 days, demonstrating that higher concentrations exceed the maximum value faster than lower concentrations (Siqueira & Salomão, 2017), the selected

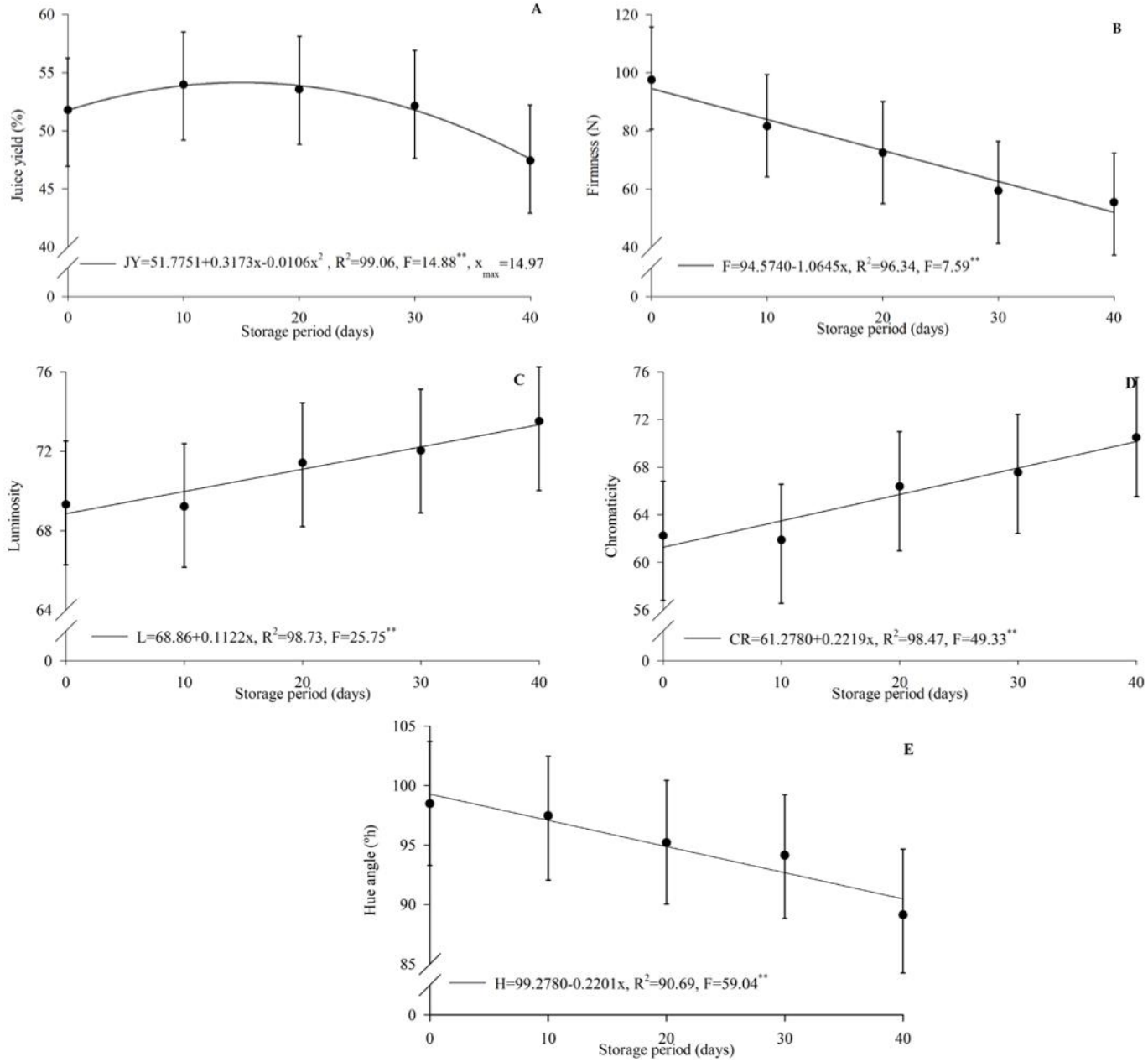


Figure 3. (A) Juice yield, (B) firmness, (C) luminosity, (D) chromaticity, and (E) Hue angle in 'Westin' orange fruits during cold storage (3 ± 1°C / 85% relative humidity).

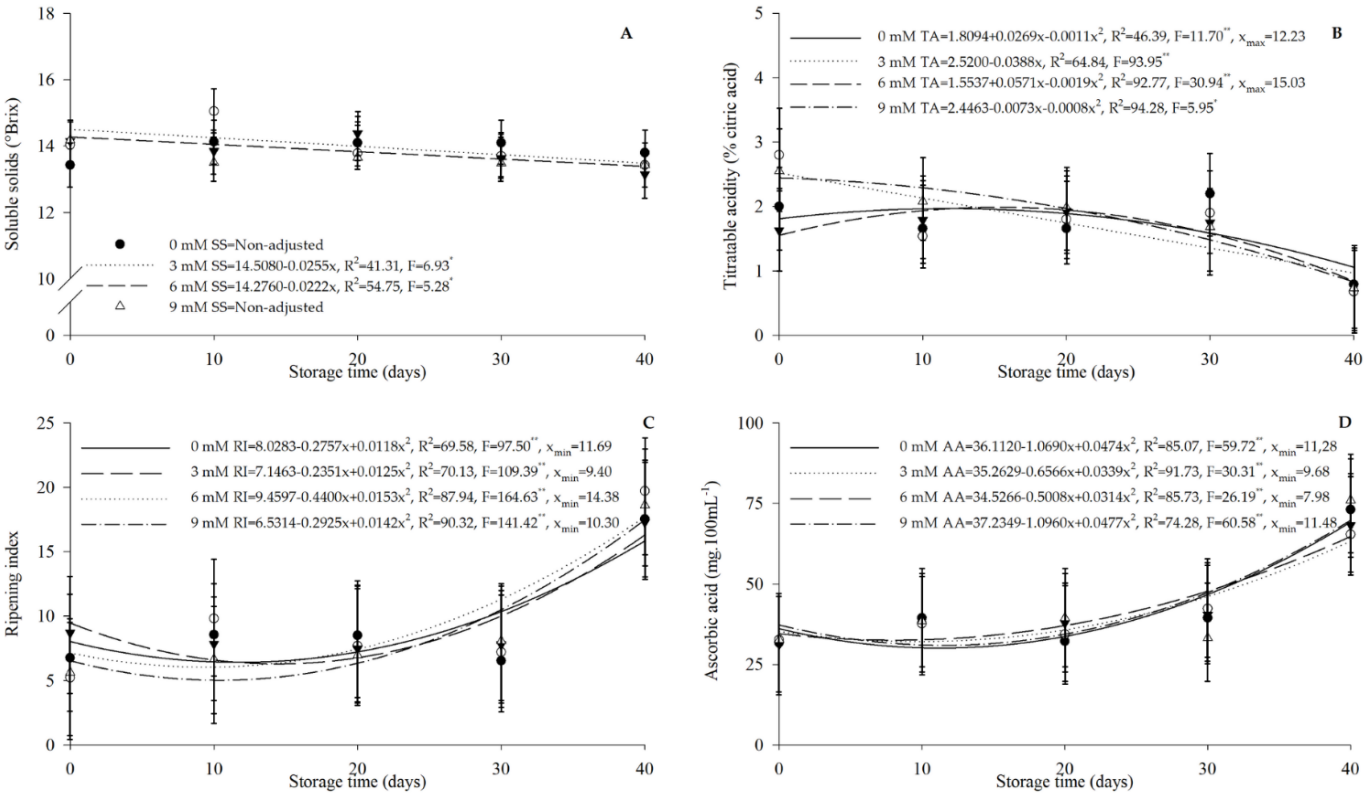


Figure 4. (A) Soluble solids, (B) titratable acidity, (C) ripening index, and (D) ascorbic acid in 'Westin' orange fruits under different Phe concentrations during cold storage times (3°C ± 1 / 85% RH).

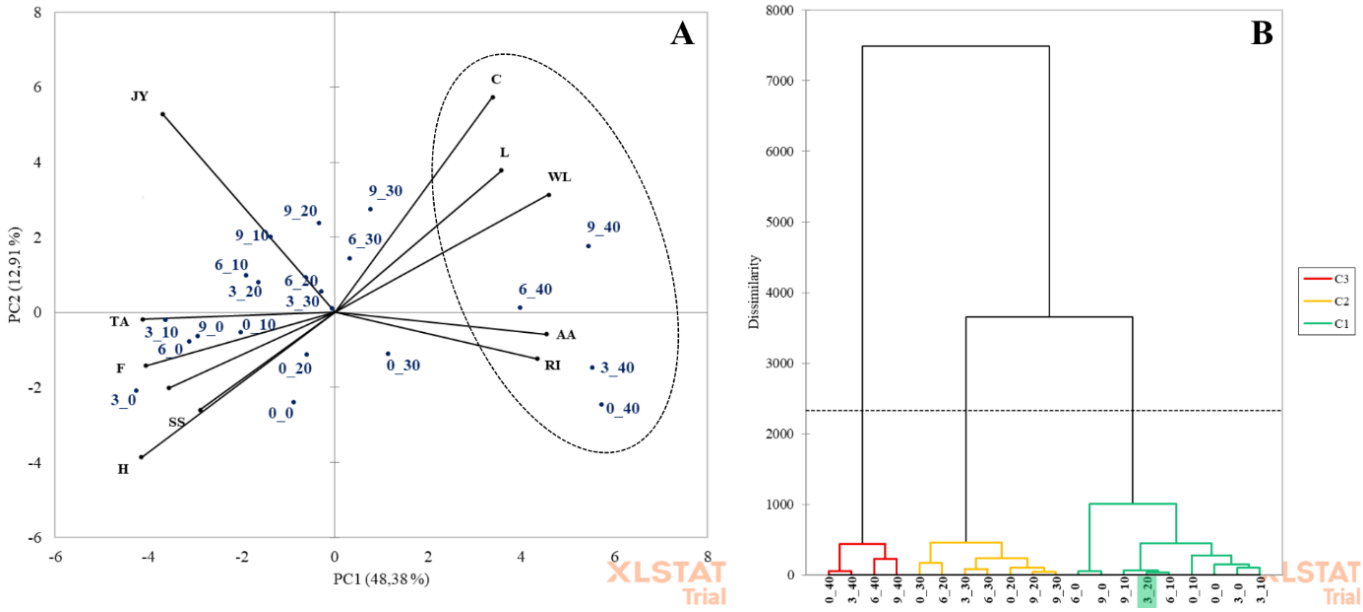
storage period optimally balances fruit quality preservation with market requirements.

There was a quadratic increase in AA contents (Figure 4D) throughout the storage time in all treatments, with values expressed in mg.100 mL^{-1}, ranging from 0 mM (31.73–73.08), 3 mM (32.69–65.38), 6 mM (31.25–68.27), and 9 mM (32.69–75.96). Different orange cultivars differ in AA content and post-harvest storage effects. AA is considered the main bioactive compound in citrus juice (Berk, 2016a). A study using edible coatings found that the AA concentration increased linearly throughout storage (Lisboa et al., 2023). This pattern of increase is similar to that observed in Figure 4D; although, in this case, the growth followed a quadratic effect, indicating a non-constant accumulation rate.

One objective of this study was to evaluate the impact of cold storage and Phe application on the incidence and severity of post-harvest rot in 'Westin' oranges. Contrary to expectations, no visible symptoms of rot were observed during the 40-day storage period. This absence may be attributed to a combination of factors, including optimal harvest maturity, effective pre-treatment sanitation (washing and drying), proper handling, and favorable storage conditions—particularly temperature, humidity, and fruit distribution within the BOD chamber. Well-spaced trays and no fruit overlap likely reduced microclimate formation conducive to fungal growth. Additionally, the orchard's high phytosanitary standard and its focus on fresh fruit production may have minimized field-level inoculum. While the presence of latent infections cannot be ruled out, the storage period may have been insufficient for symptom expression. Consequently, it was not possible to assess disease severity, highlighting the need for extended storage evaluations in future studies.

PCA (PC1 and PC2) explained 62.27% of the total variance in fruit attributes, with 46.96% from PC1 and 15.31% from PC2 (Figure 5A). The biplot indicated a clear separation of treatments based on storage duration and Phe concentration. Longer storage times (especially 40 days) correlated with increased WL, L, C, RI, and AA. In contrast, fruits stored for shorter periods (0 and 10 days) were associated with higher F, SS, and H. Cluster analysis (Figure 5B) supported these findings, grouping treatments into three distinct clusters: C1 (best quality preservation), C3 (higher deterioration), and C2 (intermediate performance).

PCA and cluster analysis revealed clear variation patterns influenced by both Phe concentration and storage duration in postharvest 'Westin' oranges. Extended storage, particularly with higher Phe concentrations, was associated with increased dehydration, lighter and more saturated peel color, and elevated AA content—indicators of bioactive compound accumulation, but also of advanced ripening and early senescence, potentially compromising F and marketability. In contrast, shorter storage durations preserved physical quality, with higher F and SS. Intermediate outcomes observed with 3 mM Phe after 20–30 days suggest a balance between maintaining structural integrity and enhancing nutritional quality. Cluster results supported this result, with C1 treatments showing superior preservation and C3 exhibiting greater degradation, underscoring the need



WL: weight loss; JY: juice yield; F: firmness; L: luminosity; C: chromaticity; H: Hue angle; SS: soluble solids; TA: titratable acidity; RI: ripening index; AA: ascorbic acid.

Figure 5. (A) Principal component analysis and (B) cluster dendrogram of the variables, treatments, and storage times of 'Westin' orange fruits.

to optimize Phe concentration and storage time for quality retention and functional enrichment.

The findings underscore the need for further investigation into the effects of Phe on the ripening physiology of 'Westin' oranges. To support safe and effective postharvest application, future studies should explore a broader range of Phe concentrations, immersion times, and storage conditions, including cold storage and artificial inoculation with rot-causing fungi to assess disease severity. Additionally, comparisons with commercial wax treatments are essential, as they represent standard postharvest practice in citrus preservation (Lisboa et al., 2023).

4 CONCLUSIONS

The application of 3 mM Phe during cold storage of 'Westin' oranges for 15–20 days effectively maintains essential postharvest quality parameters, including F, JY, and consistent peel coloration. This method offers a viable alternative for low-technology operations, circumventing the need for sophisticated equipment. Moreover, it is an environmentally sustainable strategy because it reduces reliance on synthetic postharvest agents.

DATA AVAILABILITY STATEMENT:

All the data are included in the published article.

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