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Sodium nitroprusside as an effective postharvest treatment to mitigate quality deterioration in Mexican lime (*Citrus aurantifolia* Swingle)

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A B S T R A C T

Purpose: Mexican lime (*Citrus aurantifolia* Swingle) is a highly valued citrus fruit, renowned for its nutritional and therapeutic properties. However, its postharvest life is considerably limited due to rapid senescence-related color changes and weight loss. This study evaluated the effectiveness of postharvest immersion in sodium nitroprusside (SNP) in preserving the quality of Mexican lime fruits during 30 days of storage. **Research Method:** A completely randomized factorial design was employed, testing four SNP concentrations (0, 30, 60, and 90 μ M) across three storage intervals (10, 20, and 30 days). Fruits were dipped in SNP solutions for five minutes, air-dried, packed in perforated plastic bags, and stored at 7 °C with $80 \pm 5\%$ relative humidity. Each treatment included four replicates of 15 fruits. Evaluations were performed at 10-day intervals. **Findings:** Sodium nitroprusside treatment effectively preserved the postharvest quality of Mexican lime during the storage period. Compared to the control, it significantly reduced weight loss and electrolyte leakage, maintained chlorophyll content, and delayed carotenoid accumulation in the peel, thereby slowing yellowing and visual senescence. In addition to these preservative effects, SNP treatment also enhanced juice quality by increasing titratable acidity (TA) and ascorbic acid content, and by reducing the total soluble solids to titratable acidity (TSS/TA) ratio, resulting in a more favorable taste index. Among the tested concentrations, 30 μ M SNP proved to be the most effective in maintaining external fruit characteristics and improving internal juice quality. **Research Limitations:** No specific limitations were identified in this study. **Originality/Value:** This research demonstrates that postharvest immersion in 30 μ M SNP is a safe and promising strategy for extending the shelf life and preserving the quality of Mexican lime, thereby enhancing its economic value.

Keywords:

Ascorbic acid, Chlorophyll retention, Nitric oxide, Taste index, Weight loss

INTRODUCTION

Mexican lime (*Citrus aurantifolia* Swingle), also known as Key lime, acid lime, or Omani lime in various regions, is a valuable member of the Rutaceae family that has been cultivated for centuries (Al-Abadi et al., 2016). The fruit is recognized for its diverse biochemical composition and associated health benefits, containing key bioactive compounds such as citric acid, ascorbic acid, flavonoids, and essential minerals (Mohammadi et al., 2024). Notably, it is a rich source of vitamin C, providing approximately 35% of the recommended daily intake per 100 grams (Pimsorn et al., 2022). Its juice also contains exceptionally high levels of citric acid—nearly twice that of grapefruit juice and five times more than orange juice—contributing to its distinct tartness (Penniston et al., 2008; Pimsorn et al., 2022).

Beyond its nutritional value, Mexican lime has a long history in traditional medicine. It has been used as an antiseptic, anthelmintic, mosquito repellent, tonic, antiscorbutic, astringent, and diuretic. Traditionally, it has also been employed to relieve symptoms of headaches, arthritis, digestive disorders, colds, coughs, and sore throats (Asowata-Ayodele et al., 2019).

Although Mexican lime is a non-climacteric fruit with low respiration rate, it has a notably short postharvest shelf life (Lerslerwong et al., 2023). Despite its nutritional, medicinal, and economic importance, the lime industry faces significant challenges due to postharvest losses, estimated at 18% to 25% of total global production—approximately 21 million tons annually (Mohammadi et al., 2024). This issue is particularly severe in tropical regions, where high temperatures and humidity limit the shelf life to just 6–9 days under ambient conditions (Mohammadi et al., 2024).

Postharvest, the lime fruits undergo physiological changes that impair their marketability and visual appeal. One of the most prominent changes is chlorophyll degradation in the peel, resulting in yellowing and the loss of the characteristic bright green color associated with freshness (Al-Qurashi & Awad, 2023). This discoloration is especially problematic, as green peel is a key indicator of fruit quality in the market (Al-Qurashi & Awad, 2023). Another major issue is weight loss, primarily due to the thin epicarp of the fruit, which makes it highly susceptible to moisture evaporation during storage (Zea-Hernández et al., 2016). These challenges underscore the urgent need for effective and safe postharvest treatments to extend shelf life and preserve fruit quality. Developing such strategies is essential for maintaining a stable year-round supply of fresh limes for both domestic use and export markets (Mohammadi et al., 2024).

Nitric oxide (NO) is a highly reactive free radical gas that functions as a signaling molecule, playing crucial roles in various physiological and biochemical processes in plants, particularly during fruit ripening (Bambalele et al., 2021; Freschi, 2013; Ranjbari et al., 2018). As a ubiquitous molecule found in microbes, plants, and animals, NO has emerged as a key regulator in postharvest fruit preservation (Liu & Simmons, 2021; Steffens et al., 2022). Its significance in postharvest management primarily stems from its ability to inhibit ethylene biosynthesis, thereby delaying fruit ripening and senescence (Bambalele et al., 2021). In addition to its anti-senescence effects, NO treatment has been shown to enhance fruit quality by maintaining higher levels of vitamin C and organic acids during storage (Li & Liao, 2022). Furthermore, NO boosts antioxidant enzyme activities, which modulate reactive oxygen species (ROS) and reduce oxidative damage (Pang et al., 2024). This enhancement in antioxidant capacity contributes to preserving cell membrane integrity, thus minimizing electrolyte leakage and water loss (Yang et al., 2023).

The effectiveness of NO treatment depends on several factors, including application method, fruit type, and NO concentration (Liu et al., 2023). Common application methods

include fumigation with gaseous NO, immersion in NO-releasing solutions, and spraying with NO donors such as sodium nitroprusside (SNP) or S-nitrosoglutathione (GSNO) (Zhu et al., 2022). However, due to the highly unstable nature of NO gas and its rapid degradation—especially in the presence of oxygen, where it can convert into the potentially harmful nitrogen dioxide (NO₂)—its use is largely limited to low concentrations in controlled laboratory conditions (Liu et al., 2023).

Among the various methods, immersion using NO donors like SNP has shown promising results in preserving fruit quality and delaying senescence, primarily by suppressing ethylene production and reducing respiration rates (Zhu et al., 2022). Sodium nitroprusside is particularly advantageous because it is easy to use, can be stored at room temperature (~20 °C) in the dark, and can be dissolved in neutral distilled water without requiring special handling or storage conditions (Liu et al., 2023).

Several studies have demonstrated the efficacy of SNP in maintaining postharvest fruit quality. For instance, Zhou et al. (2016) demonstrated that exogenous NO, applied as 50 µM SNP, effectively mitigated postharvest quality loss in ‘Valencia’ orange during storage at 20 °C. Sodium nitroprusside treatment reduced weight loss, preserved ascorbic acid and titratable acidity (TA) levels, and delayed the increase in total soluble solids (TSS) in the juice. Moreover, NO enhanced the antioxidant capacity of citrus fruit by regulating hydrogen peroxide levels, stimulating phenolic compound synthesis, and inducing the activities of key antioxidant enzymes, including catalase and enzymes of the ascorbate–glutathione cycle in the peel. In another study, Adhikary et al. (2021) demonstrated that postharvest treatment with SNP significantly improved the storability of ‘Patharnakh’ pear under cold storage (0–1 °C, 90–95% RH). Sodium nitroprusside reduced weight loss, browning, and respiration rate, while maintaining firmness, color, and key quality parameters such as TSS, TA, phenolic content, and ascorbic acid. The treatment also modulated enzyme activities by enhancing superoxide dismutase and suppressing polyphenol oxidase and fruit-softening enzymes. Among the tested concentrations, 2 µM SNP was most effective, extending storage life and preserving sensory quality for up to 60 days.

The objective of the present study was to evaluate the effects of postharvest dipping treatments with different concentrations of SNP on the quality parameters of Mexican lime fruit during a 30-day storage period.

MATERIALS AND METHODS

Plant material

Mature-green Mexican lime fruits were handpicked from a commercial orchard situated in the village of Maniyan, located in the Jolghah Rural District of the Central District in Jahrom County, Fars Province, Iran (53°12'57"E, 28°34'38"N). The orchard comprised 8-year-old trees grafted onto Mexican lime rootstocks. Fruits were randomly harvested from the outer portions of the upper and middle canopy of several trees to ensure representativeness. In general, harvested fruits were at the optimal harvest stage for export market limes, characterized by a peel color transition from dark to light green, a smooth surface, slight softness to the touch, and a juice content of 30% or higher. Immediately after harvest, the fruits were transported to the laboratory. There, they were sorted to ensure uniformity in size, shape, and color, and any fruit showing signs of mechanical injury, disease, or pest infestation was discarded. Prior to treatment, fruits were surface-disinfected using a 2% sodium hypochlorite solution for two minutes, rinsed thoroughly with distilled water, and air-dried at ambient temperature.

Experimental design and treatments

The experiment was arranged in a completely randomized factorial design, with two factors: SNP immersion at four concentrations (0, 30, 60, and 90 μM) and sampling time at three intervals (10, 20, and 30 days into storage). Each treatment was replicated four times, with 15 fruits in each replicate. For treatment application, fruits were immersed in SNP solutions (Sigma-Aldrich, St. Louis, MO, USA) for five minutes. Control fruits were dipped in distilled water. All fruits were then left to dry at room temperature for one hour. Following this, they were packed into perforated zip-lock plastic bags (perforation ratio: 3%) and stored at 7 °C under relative humidity of $80 \pm 5\%$ for a 30-day period. Evaluations were performed at 10-day intervals. To document initial conditions (not for statistical comparison), all measured parameters were also evaluated on day 0, prior to the start of experiment.

Fruit weight loss

Fruit weight loss during storage was monitored using a mass difference method (Habibi et al., 2020). The fruits were weighed at designated time intervals throughout the storage period using a digital balance (Pand PT206B, Iran) with a precision of 0.1 g. Weight loss was determined by calculating the difference between the initial weight and the recorded weight at each sampling point, and was expressed as a percentage of the initial weight using the following formula (1):

$$\text{Weight Loss (\%)} = [(\text{Initial Weight} - \text{Weight at Sampling}) / \text{Initial Weight}] \times 100 \quad (1)$$

Ascorbic acid

Ascorbic acid levels in the fruit juice were quantified through titration using a 2,6-dichlorophenolindophenol (DCPIP) reagent (AOAC, 2000). The outcomes were reported in milligrams per 100 grams ($\text{mg } 100 \text{ g}^{-1}$) of juice.

Titrateable acidity (TA)

Titrateable acidity of the fruit juice was assessed through titration using a 0.1 N sodium hydroxide (NaOH) solution (Habibi et al., 2020). The titration was completed when the pH reached 8.2, and the TA values were expressed as a percentage of citric acid.

Total soluble solids (TSS)

The total soluble solids content of the fruit juice was determined at ambient temperature using a digital refractometer (MA871, Milwaukee, Hungary) and expressed as a percentage (%) (Habibi et al., 2020).

Taste index (TSS/TA ratio)

The taste index was obtained by calculating the ratio of TSS to TA.

Peel electrolyte leakage

Electrolyte leakage was determined based on the procedure outlined by McCollum and McDonald (1991), with slight adaptations. For each replicate, ten uniform peel discs (10 mm in diameter) were randomly selected and immersed in 25 ml of 0.4 M mannitol solution. The samples were incubated under constant shaking at room temperature (approximately 25 °C) for 4 hours. Following incubation, the initial electrical conductivity of the solution was measured using a conductometer (Model 644, Metrohm, Herisau, Switzerland). Subsequently, the vials were autoclaved at 121 °C for 20 minutes to completely release cellular electrolytes.

After being held overnight at room temperature, the final (total) conductivity was recorded. Electrolyte leakage was then calculated as a percentage using the following equation (2):

$$\text{EL (\%)} = (\text{Initial Conductivity} / \text{Total Conductivity}) \times 100 \quad (2)$$

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid content in fruit peel

The concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid in fruit peel were assessed based on the protocol established by Wellburn (1994). Fresh peel tissue was first cleaned thoroughly and cut into uniform segments (approximately 1–2 cm²). A 0.5 g sample of the peel was then accurately weighed and ground into a fine paste using a mortar and pestle in the presence of a small volume of 80% (v/v) acetone. The homogenate was transferred to a centrifuge tube, and the volume was brought up to 10 ml with 80% acetone. To prevent pigment degradation, the mixture was kept in the dark for 10 minutes before being centrifuged at 4000 rpm for 10 minutes. The supernatant was carefully decanted into a clean tube, avoiding any pellet at the bottom, and filtered through filter paper to remove residual particles. Absorbance readings of the clear extract were taken at 663, 645, and 470 nm using a PhotoLab® 7600 UV-Vis spectrophotometer (WTW, Germany). Based on the absorbance values, the levels of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were calculated using standard equations:

$$\text{Chlorophyll } a \text{ (mg g}^{-1} \text{ FW)} = 12.25 \times A_{663} - 2.79 \times A_{645} \quad (3)$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1} \text{ FW)} = 21.50 \times A_{645} - 5.10 \times A_{663} \quad (4)$$

$$\text{Total chlorophyll (mg g}^{-1} \text{ FW)} = \text{Chlorophyll } a + \text{Chlorophyll } b \quad (5)$$

$$\text{Carotenoid (mg g}^{-1} \text{ FW)} = (1000 \times A_{470} - 1.82 \times \text{Chlorophyll } a - 85.02 \times \text{Chlorophyll } b) / 198 \quad (6)$$

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) at a significance level of $P \leq 0.05$, and mean comparisons were carried out with the least significant difference (LSD) test. All statistical analyses were conducted using SAS software version 9.4.

RESULTS

Weight loss

Analysis of variance revealed that the effects of dipping treatment ($P \leq 0.01$), storage duration ($P \leq 0.01$), and their interaction ($P \leq 0.01$) on fruit weight loss were statistically significant. Weight loss increased progressively over time across all treatments, with control fruits consistently exhibiting the highest percentage of weight loss at each evaluation point. After 10 days of storage, fruits treated with 30 and 60 μM SNP exhibited the lowest weight loss (4.79% and 5.15%, respectively), with no significant difference between the two concentrations. At 20 and 30 days of storage, the minimum weight loss (6.23% and 6.75%, respectively) was recorded in fruits treated with 30 μM SNP. During the final 10 days of storage, no further increase in weight loss was noted in fruits treated with the higher SNP concentrations. Although fruits treated with 60 and 90 μM SNP experienced significantly less weight loss than the control, their losses remained significantly higher than those observed in the 30 μM treatment. Overall, 30 μM SNP was the most effective in minimizing postharvest weight loss throughout the storage duration (Fig. 1).

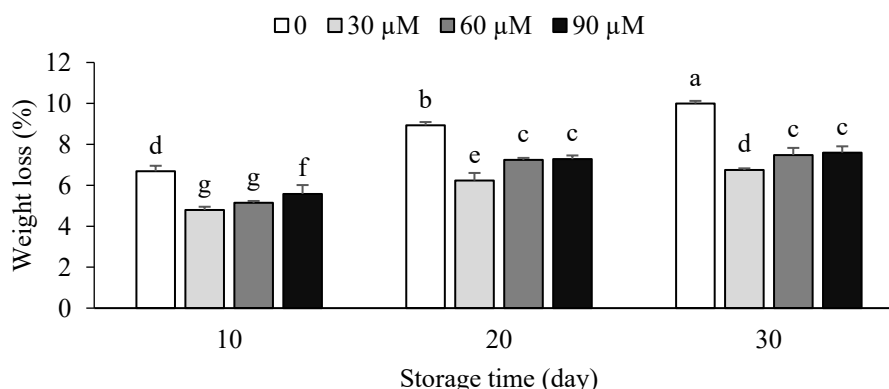


Fig. 1. Changes in the weight loss of Mexican lime fruits during 30 days of storage at 7 °C and 80 ± 5% RH. Fruits were dipped in sodium nitroprusside (SNP) solutions at 0, 30, 60, or 90 µM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

Ascorbic acid

Analysis of variance revealed that the dipping treatment ($P \leq 0.01$), storage duration ($P \leq 0.01$), and their interaction ($P \leq 0.01$) significantly influenced the ascorbic acid content of fruit juice. After 10 days of storage, fruits treated with 30 and 60 µM SNP exhibited higher ascorbic acid levels (118.30 and 109.56 mg 100 g⁻¹, respectively) compared to the initial value (107.70 mg 100 g⁻¹). At this stage, all SNP-treated fruits showed significantly greater ascorbic acid content than the control (93.49 mg 100 g⁻¹), with statistically significant differences among the concentrations; the highest and lowest values were observed in fruits treated with 30 and 90 µM SNP, respectively (118.30 and 104.15 mg 100 g⁻¹). Over the subsequent 20 days of storage, ascorbic acid levels initially declined and then increased across all treatments. Notably, in fruits treated with 60 µM SNP, the final value returned to a level comparable to that measured on day 10. After 30 days of storage, fruits treated with 30 and 60 µM SNP exhibited statistically higher ascorbic acid contents (109.15 and 109.37 mg 100 g⁻¹, respectively) compared with the control, which showed the lowest value (86.89 mg 100 g⁻¹) (Fig. 2).

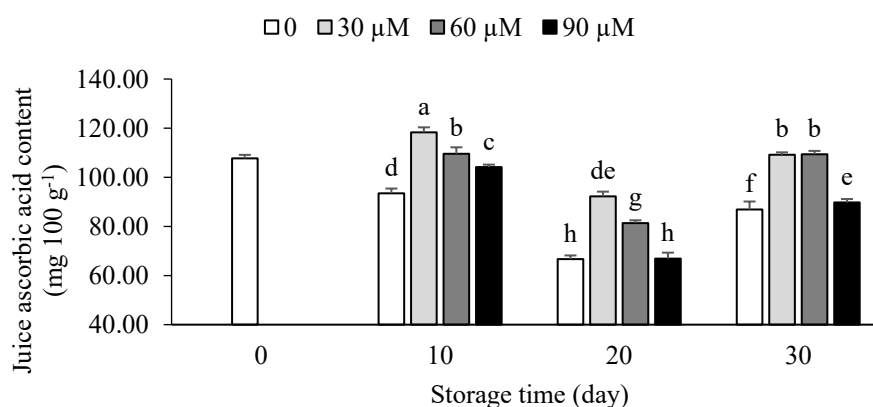


Fig. 2. Changes in the juice ascorbic acid content of Mexican lime fruits during 30 days of storage at 7 °C and 80 ± 5% RH. Fruits were dipped in sodium nitroprusside (SNP) solutions at 0, 30, 60, or 90 µM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

Titrateable acidity (TA)

duration ($P \leq 0.01$) on TA of fruit juice were statistically significant, while their interaction had no significant effect. An initial increase in TA was observed after 10 days of storage. Mean comparisons revealed a significant decline in TA during the first half of the subsequent 20-day period, which was followed by a notable increase in the latter half. By day 30, the TA level had returned to a value similar to that observed on day 10 (Fig. 3a). Furthermore, all concentrations of SNP treatment resulted in similarly higher TA levels compared to the control (Fig. 3b).

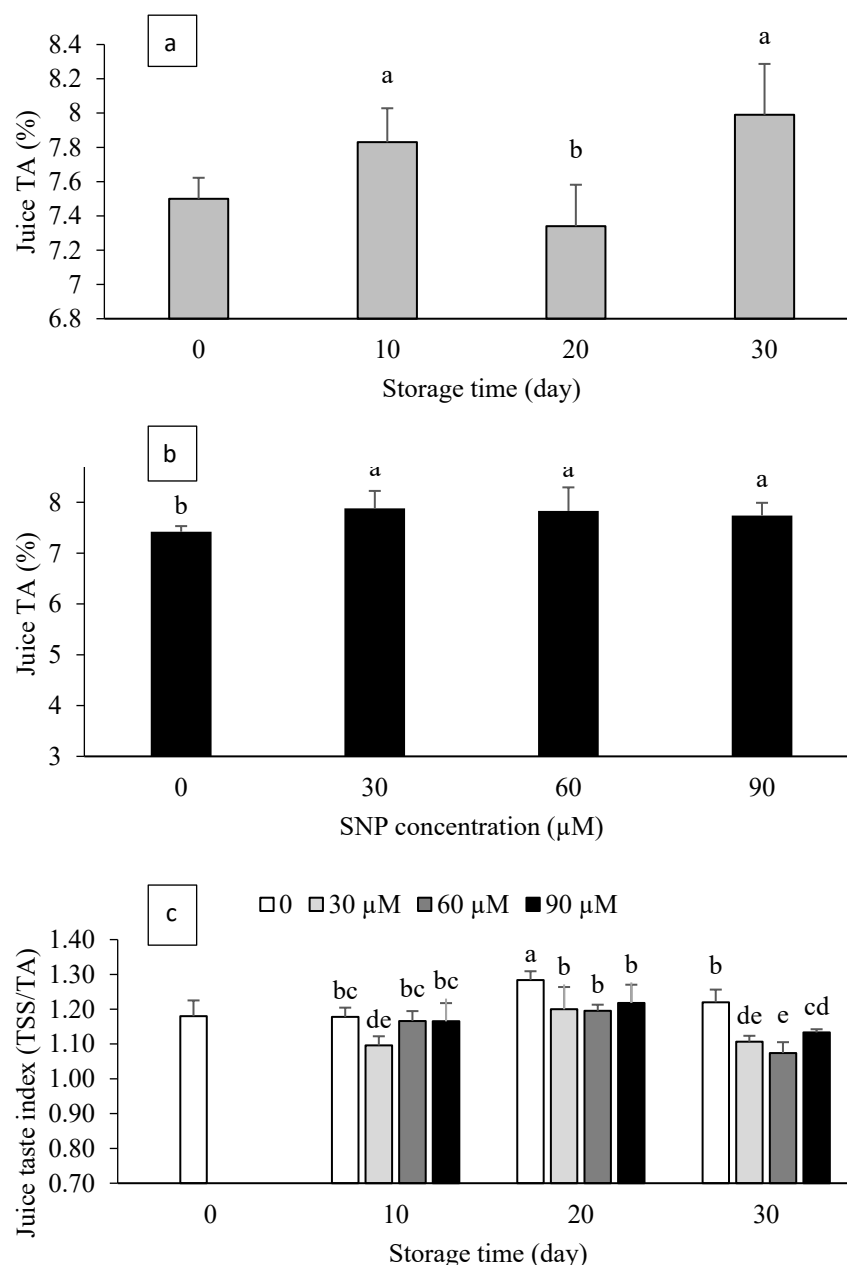


Fig. 3. Changes in juice titratable acidity (TA) of Mexican lime fruits under the main effects of storage time (a) and sodium nitroprusside (SNP) treatment (b). Changes in juice taste index (TSS/TA) (c) during 30 days of storage. Fruits were dipped in SNP solutions at 0, 30, 60, or 90 μM for 5 minutes prior to storage at 7 °C and 80 ± 5% RH. Measurements were taken on days 10, 20, and 30. Data are presented as means of 16 replicates for (a), 12 replicates for (b); and 4 replicates for (c) ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

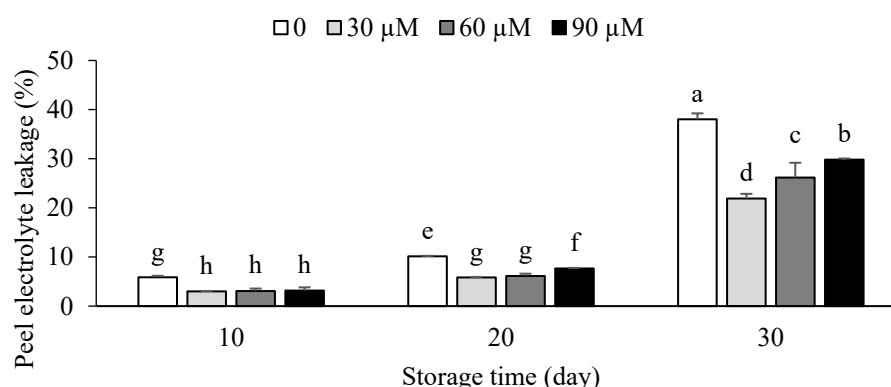


Fig. 4. Changes in the peel electrolyte leakage of Mexican lime fruits during 30 days of storage at 7 °C and 80 ± 5% RH. Fruits were dipped in sodium nitroprusside (SNP) solutions at 0, 30, 60, or 90 μM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

Peel electrolyte leakage

Analysis of variance indicated that the effects of dipping treatment ($P \leq 0.01$), storage duration ($P \leq 0.01$), and their interaction ($P \leq 0.01$) on fruit peel electrolyte leakage were statistically significant. A consistent and significant increase in electrolyte leakage was observed across all treatment groups throughout the storage period, with control fruits showing the highest levels at each sampling point. After 10 days of storage, no significant differences were found among fruits treated with different concentrations of SNP. By day 20, fruits treated with 30 and 60 μM SNP exhibited the lowest electrolyte leakage. However, at the end of the storage period, electrolyte leakage in SNP-treated fruits increased with increasing SNP concentration (Fig. 4).

Chlorophyll *a* in fruit peel

Analysis of variance showed that the effects of dipping treatment ($P \leq 0.01$) and storage duration ($P \leq 0.01$) on chlorophyll *a* content in fruit peel were statistically significant, while their interaction had no significant effect. Mean comparison indicated a significant decrease in chlorophyll *a* content over the course of storage (Fig. 5a). Moreover, SNP treatment resulted in higher chlorophyll *a* levels compared to the control group, with lower concentrations of SNP being more effective in preserving chlorophyll *a* in the fruit peel (Fig. 5b).

Chlorophyll *b* in fruit peel

Analysis of variance revealed that the effects of dipping treatment ($P \leq 0.01$), storage duration ($P \leq 0.01$), and their interaction ($P \leq 0.01$) on chlorophyll *b* content in fruit peel were statistically significant. Overall, chlorophyll *b* levels declined progressively during storage. At all three sampling points, fruits treated with the lowest concentration of SNP consistently exhibited the highest chlorophyll *b* content. A statistically significant difference between fruits treated with 60 μM SNP and the control emerged after 20 days of storage, and this difference persisted until the end of the storage period. Fruits treated with 90 μM SNP only showed significantly higher chlorophyll *b* content than the control at the end of the storage period (Fig. 5c).

Total chlorophyll in fruit peel

Analysis of variance showed that the effects of dipping treatment ($P \leq 0.01$) and storage duration ($P \leq 0.01$) on total chlorophyll content in the fruit peel were statistically significant, while their interaction had no significant effect. Mean comparisons indicated that the trend of total chlorophyll changes, as influenced by storage time (Fig. 6a) and SNP treatment levels (Fig. 6b), was similar to that observed for chlorophyll *a* (Fig. 5a, Fig. 5b).

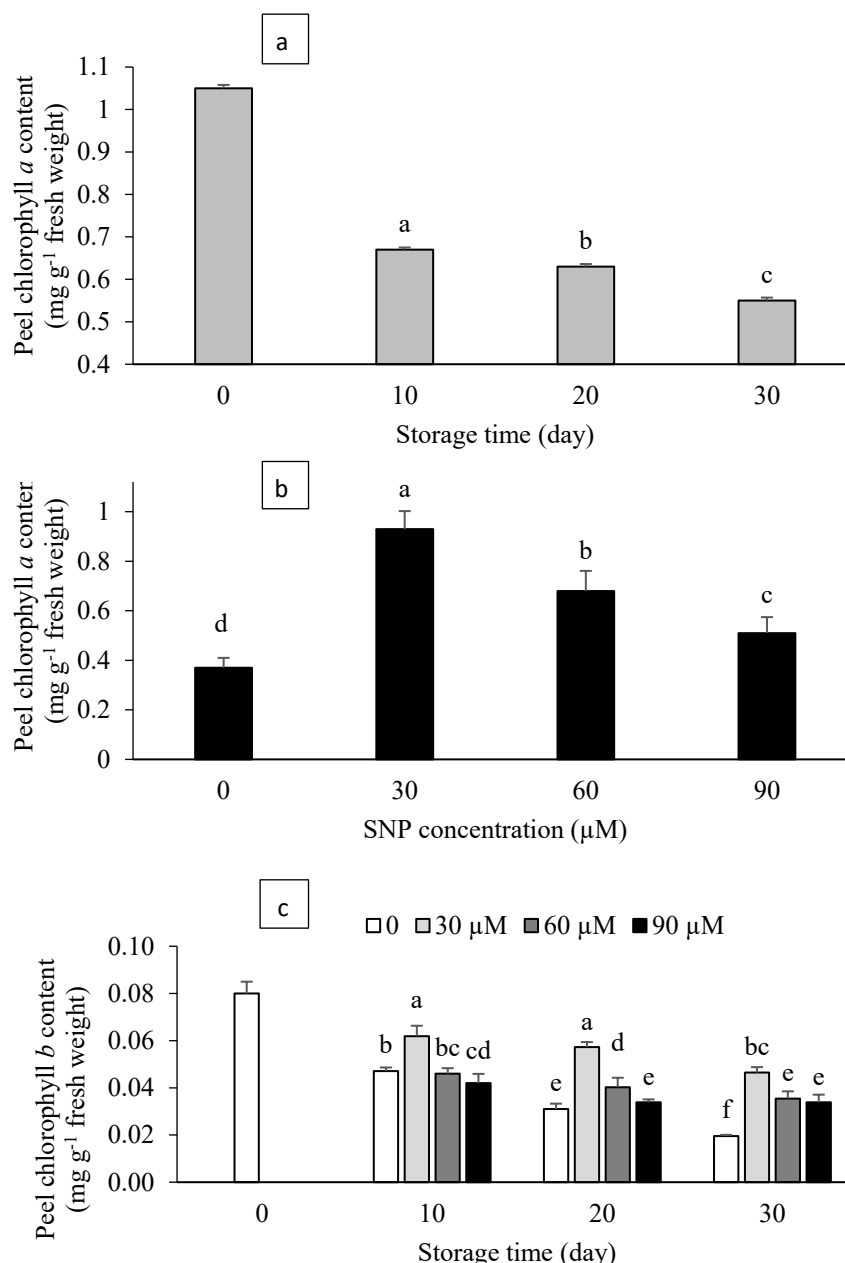


Fig. 5. Changes in peel chlorophyll *a* content of Mexican lime fruits under the main effects of storage time (a) and sodium nitroprusside (SNP) treatment (b). Changes in peel chlorophyll *b* content (c) during 30 days of storage. Fruits were dipped in SNP solutions at 0, 30, 60, or 90 μM for 5 minutes prior to storage at 7 °C and 80 ± 5% RH. Measurements were taken on days 10, 20, and 30. Data are presented as means of 16 replicates for (a), 12 replicates for (b); and 4 replicates for (c) ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

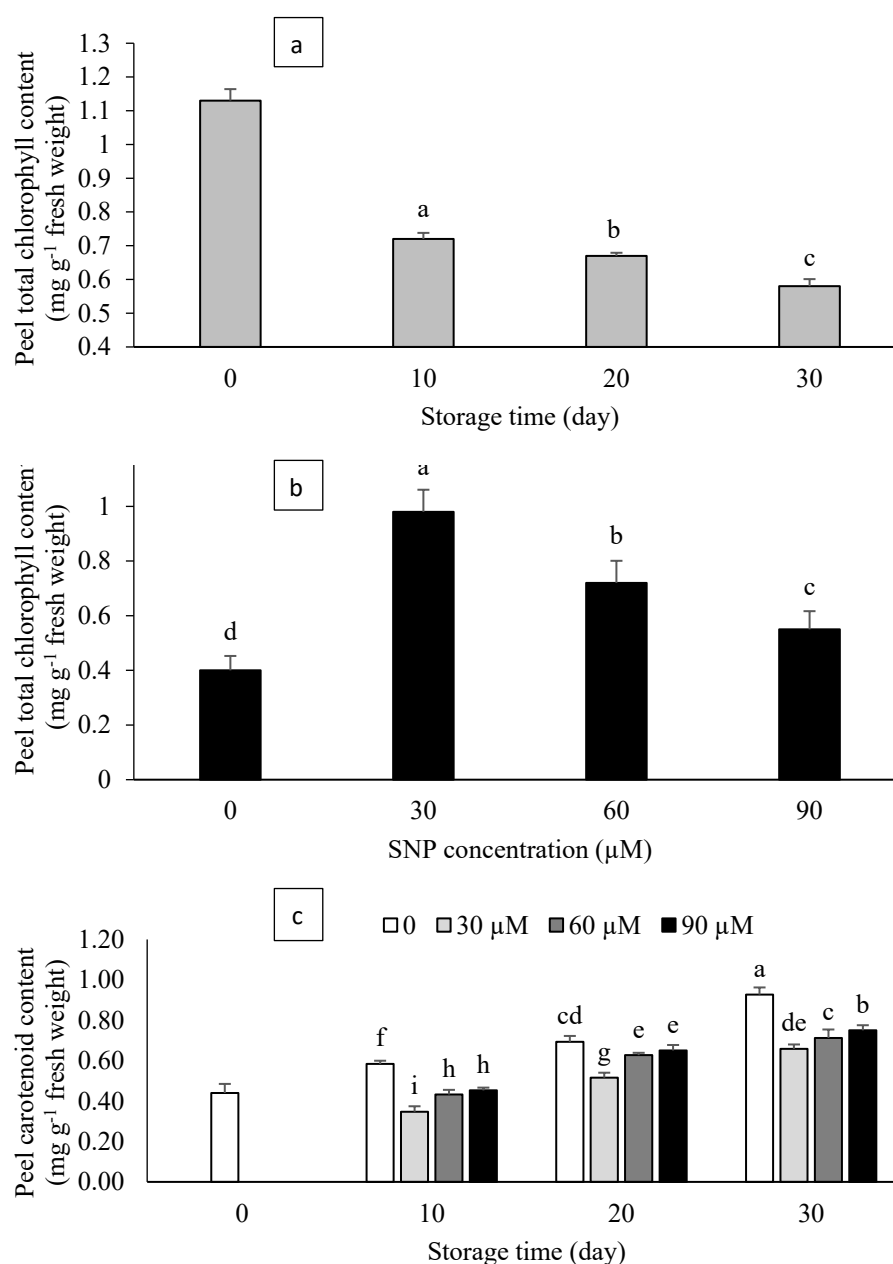


Fig. 6. Changes in peel total chlorophyll content of Mexican lime fruits under the main effects of storage time (a) and sodium nitroprusside (SNP) treatment (b). Changes in peel carotenoid content (c) during 30 days of storage. Fruits were dipped in SNP solutions at 0, 30, 60, or 90 μM for 5 minutes prior to storage at 7 °C and 80 ± 5% RH. Measurements were taken on days 10, 20, and 30. Data are presented as means of 16 replicates for (a), 12 replicates for (b); and 4 replicates for (c) ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

Carotenoid in fruit peel

Analysis of variance showed that the effects of dipping treatment ($P \leq 0.01$), storage duration ($P \leq 0.01$), and their interaction ($P \leq 0.01$) on carotenoid content in the fruit peel were statistically significant. The carotenoid content increased significantly throughout the storage period across all experimental groups. At all three sampling points, the highest and lowest carotenoid levels were recorded in the control fruits and those treated with 30 μM SNP, respectively. A statistical difference between the carotenoid content of fruits treated with 60 and 90 μM SNP emerged only at the end of the storage period, whereas no significant

difference was observed between these two groups earlier. After one month of storage, fruits treated with the highest concentration of SNP exhibited significantly higher carotenoid content in the peel compared to those treated with lower concentrations (Fig. 6c).

DISCUSSION

Postharvest weight loss in Mexican lime fruits is predominantly related to transpiration and respiration (Mohammadi et al., 2024). One of the primary postharvest benefits of NO and its donor compound SNP is their effectiveness in mitigating fruit weight loss during storage a process that typically intensifies with increasing storage duration. Previous studies support the role of NO in reducing weight loss, largely through its influence on physiological and biochemical processes. For instance, NO treatments have been reported to reduce transpiration-related weight loss by 14–26% across various fruits and vegetables (Leshem et al., 1998). The application of NO or NO-releasing compounds has consistently resulted in significantly lower weight loss compared to untreated controls in crops such as papaya and orange (da Veiga et al., 2024; Zhu et al., 2021). This effect is particularly relevant for fruits like papaya, where a weight loss of as little as 7% can lead to visible deterioration in external quality (Paull & Chen, 1989). These findings are further supported by meta-analytical data (Madebo et al., 2022), reinforcing NO's value in maintaining postharvest quality.

The reduction in fruit weight loss mediated by NO is attributed to several interrelated mechanisms, with the delay of fruit senescence and the associated reduction in water loss being among the most significant (Machado et al., 2022; Yang et al., 2023). Senescence is typically accompanied by the excessive accumulation of ROS, which leads to oxidative damage to lipids, proteins, and nucleic acids (Kumar et al., 2025; Siddiqui et al., 2024). Nitric oxide alleviates this oxidative stress by upregulating antioxidant defense systems, particularly the ascorbate–glutathione cycle, which plays a central role in detoxifying hydrogen peroxide (Kumar et al., 2025; Siddiqui et al., 2024; Wang et al., 2024). For instance, in cucumber (*Cucumis sativus* L. cv. Deltastar), the application of NO increased the activity of key antioxidant enzymes, including superoxide dismutase, catalase, ascorbate peroxidase, and peroxidase (Yang et al., 2011). Similarly, in bananas, NO treatment reduced oxidative damage by modulating phenolic metabolism and limiting ROS generation (Siddiqui et al., 2024). By preserving cell membrane integrity and maintaining intracellular compartmentalization, NO minimizes electrolyte leakage and the diffusion of water vapor from the fruit surface, thereby reducing transpiration rates (Machado et al., 2022; Yang et al., 2023). Our findings also demonstrated that SNP treatment significantly reduced electrolyte leakage in the fruit peel, which was accompanied by a significant decrease in fruit weight loss.

Another critical mechanism by which NO mitigates postharvest weight loss is through suppression of respiration rate. Elevated respiration rate accelerates the degradation of stored carbohydrates, resulting in a loss of dry matter and overall fruit mass (Pang et al., 2024). Nitric oxide has been reported to reduce respiration in fruits such as wax apple and banana, thereby conserving energy reserves and extending shelf life (Siddiqui et al., 2024; Zhang et al., 2023). This respiratory inhibition may be mediated via modulation of key metabolic enzymes in pathways such as the pentose phosphate pathway and the alternative oxidase pathway, which are involved in energy metabolism and stress responses (Song et al., 2021).

Two key indicators of fruit flavor and nutritional quality during postharvest storage are TA and ascorbic acid content. Ascorbic acid plays a critical role as a non-enzymatic antioxidant in plants, contributing to stress tolerance, enhanced phenolic content, suppressed respiration, and delayed senescence (Madebo et al., 2022). In parallel, TA not only influences

the sensory characteristics of fruits but also plays a physiological role in maintaining postharvest metabolic balance. As a respiratory substrate, TA may help stabilize ascorbic acid levels during storage (Madebo et al., 2022).

Our results indicated that postharvest treatment with SNP significantly enhanced TA and ascorbic acid content in the fruit juice compared to the control. These findings align with previous studies reporting that NO consistently demonstrates strong potential in preserving and enhancing both ascorbic acid and TA (Xueping et al., 2014; Zhou et al., 2016). Nitric oxide appears to influence the temporal dynamics of ascorbic acid content during storage. For instance, in papaya fruits, ascorbic acid levels typically rise during the early stages of storage and gradually decline thereafter; however, this decline occurs more slowly in NO-treated fruits, suggesting improved retention of nutritional quality (Xueping et al., 2014). In Valencia oranges, exogenous NO application was shown to reduce weight loss, enhance both TA and ascorbic acid content, and delay the accumulation of total soluble solids (Zhou et al., 2016). Collectively, the ability of NO treatment to preserve or enhance ascorbic acid and TA highlights its potential as an effective strategy to improve both the nutritional and sensory quality of fruits during postharvest storage.

Recent studies have begun to elucidate the biochemical mechanisms by which NO influences ascorbate metabolism. For instance, in cornelian cherry, treatment with 500 μ M SNP significantly elevated ascorbic acid content, likely due to enhanced activity of antioxidant enzymes such as ascorbate peroxidase and glutathione reductase (Rabiei et al., 2019). In mung bean sprouts, NO application enhanced the activity of key enzymes involved in both the L-galactose pathway for ascorbic acid biosynthesis and the ascorbate-glutathione cycle, leading to increased ascorbic acid levels and improved antioxidant capacity (Wang et al., 2024). In pepper fruits, treatment with 5 ppm NO led to approximately 40% higher ascorbic acid content, which was attributed to the upregulation of L-galactono-1,4-lactone dehydrogenase (GalLDH)—the enzyme responsible for the final step in ascorbic acid biosynthesis (Machado et al., 2022).

The taste index, commonly expressed as the ratio of TSS to TA, is a key indicator of fruit quality and a critical determinant of consumer acceptance. A higher TSS/TA ratio is generally associated with a sweeter, less acidic flavor profile, although the preferred balance varies depending on fruit type and consumer preferences (Nour, 2022). For fruits such as Mexican lime, which are primarily valued for their acidity, an increase in TSS/TA ratio during storage is typically undesirable. The findings of the present study indicated that SNP treatment had no significant effect on the content or temporal changes of TSS during storage, which is consistent with the results reported by Sahu et al. (2020) in guava. Moreover, SNP treatment markedly enhanced titratable acidity (TA) and ascorbic acid content in the juice, thereby improving the taste index, as indicated by a reduced TSS/TA ratio.

Several studies have reported that nitric oxide (NO) application can mitigate the typical postharvest rise in the TSS/TA ratio (Li & Liao, 2022). This effect is largely attributed to NO's ability to suppress respiration, as evidenced by lower CO₂ production rates in NO-treated fruits during storage (Xueping et al., 2014). By reducing respiration rate, the degradation of complex carbohydrates into simple sugars is slowed, and the metabolic depletion of organic acids is diminished, thereby helping to stabilize the TSS/TA ratio during storage (Bhardwaj et al., 2022).

Peel carotenoids are responsible for the yellow, orange, and red coloration in fruits, and their accumulation often coincides with chlorophyll degradation during postharvest storage (Peng et al., 2023). Notably, NO treatments have been shown to preserve chlorophyll content while concurrently suppressing carotenoid accumulation in fruit peels, thereby contributing to the maintenance of green coloration and visual quality during storage (Li & Liao, 2022). Our

findings, which showed that SNP-treated fruits had higher total chlorophyll content and a delayed carotenoid accumulation in the peels compared to the control, are consistent with these observations.

One of the primary mechanisms by which NO preserves chlorophyll is its ability to mitigate oxidative stress within chloroplast membranes, which in turn delays chlorophyll degradation and the yellowing typically associated with senescence. This protective effect is linked to reduce lipid peroxidation, as evidenced by significantly lower malondialdehyde levels in NO-treated broccoli florets compared to untreated controls (Eum et al., 2009). In addition to its antioxidant function, NO supports cellular homeostasis by maintaining osmotic balance and cytoplasmic viscosity, which helps stabilize chloroplast membrane integrity under stress conditions (Emamverdian et al., 2023).

Another critical mechanism underlying the NO-induced maintenance of green coloration is its inhibitory effect on ethylene biosynthesis and signaling. Ethylene is a well-established promoter of chlorophyll degradation and carotenoid accumulation; thus, its suppression by NO plays a significant role in delaying fruit senescence and extending shelf life (Li & Liao, 2022). For example, Barman et al. (2014) reported that postharvest dipping of mango fruits in SNP reduced ethylene production by 117–270% compared to control fruits treated with distilled water. Similarly, application of 1 mM SNP or 20 ppm NO gas to pear fruits effectively preserved green peel coloration (Hendges et al., 2016). In guava, SNP treatment not only reduced chlorophyll loss but also delayed carotenoid accumulation, a result attributed to NO's modulation of ethylene signaling (Sahu et al., 2020).

Nitric oxide functions as a crucial signaling molecule at physiological concentrations. While low to moderate concentrations of SNP can be beneficial as a postharvest treatment, higher concentrations may lead to reduced efficacy, and at even greater levels, phytotoxic effects that result in adverse physiological responses in fruits. The phytotoxicity observed at elevated SNP levels is believed to result from the excessive generation of reactive nitrogen species (RNS), which are derived from NO (Kumar et al., 2025). Overaccumulation of RNS may disrupt cellular homeostasis, inducing oxidative and nitrosative stress, enzyme inactivation through S-nitrosylation or other post-translational modifications, and ultimately, cell death (Intarasit et al., 2022; Kumar et al., 2025).

The findings of the present study indicate that all applied concentrations of SNP positively influenced the maintenance or improvement of fruit quality attributes. Among them, the application of 30 μ M SNP proved to be the most effective overall. Therefore, this concentration is recommended for the long-term storage of Mexican lime fruits.

CONCLUSION

The findings of this study highlight the effectiveness of SNP in preserving the postharvest quality of Mexican lime fruits during 30 days of cold storage at 7 °C and 80 $\pm$ 5% relative humidity. Postharvest immersion in SNP solutions significantly reduced fruit weight loss, suppressed electrolyte leakage, and maintained chlorophyll content while delaying carotenoid accumulation, thereby slowing peel yellowing and visual senescence. Additionally, SNP enhanced juice quality by increasing TA and ascorbic acid content and reducing the TSS/TA ratio, resulting in a more favorable taste index at the end of storage. Among the tested concentrations, 30 μ M SNP was the most effective in maintaining external appearance and improving internal quality parameters, compared to the untreated control. Overall, SNP at 30 μ M presents a safe, practical, and promising postharvest treatment for extending the shelf life and marketability of Mexican lime fruits.

Conflict of interest

The authors declare that there is no conflict of interest.

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