



# Sodium Alginate Coating with Pomegranate Seed Oil: A Novel Approach to Preserve Acid Lime (*Citrus aurantifolia*) Fruit

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## ABSTRACT

Postharvest weight loss and color changes are common issues affecting the quality of lime fruit. This study aimed to evaluate the effectiveness of sodium alginate (SA) coatings (0.5% and 1%) both alone and in combination with pomegranate seed oil (PSO) in preserving the quality and freshness of Mexican lime fruits over a 24-day storage period at  $20 \pm 2^\circ\text{C}$  and 50-60% relative humidity. Results indicated that fruits in the control group exhibited dehydration and browning of peels by the end of storage. In contrast, the SA (0.5%) + PSO treatment significantly maintained visual quality, reducing weight loss to 12% compared to 19.8% in the control group. PSO alone reduced weight loss to 12.5% but led to increased fruit browning and reduced visual quality. The treatments effectively inhibited polyphenol oxidase (PPO) activity, with the SA (0.5%) + PSO treatment demonstrating the lowest PPO activity, a reduction of approximately 41% compared to the control. Peroxidase (POD) enzyme activity was around four times higher in the SA (0.5%) + PSO and SA (1%) treatments compared to the control. All treated fruits, except those treated solely with PSO, displayed a significant difference in the  $a^*$  value compared to the control group. Additionally, with the exception of the PSO treatment, all other treatments resulted in higher levels of total phenols, total flavonoids, and antioxidant capacity compared to the control at the end of storage. Overall, these results indicate that the SA (0.5%) + PSO treatment is effective in preserving the quality and freshness of Mexican lime fruits when stored at ambient temperature.

## Introduction

The Mexican lime (*Citrus aurantifolia* Swingle) is one of the world's most significant citrus varieties, valued for its fresh consumption globally (Rivera-Cabrera et al., 2010). The green color and freshness of lime peel signal quality and determine market value, with chlorophyll degradation being the main cause of visible deterioration. This degradation leads to browning, primarily driven by the enzyme polyphenol oxidase (PPO) (Adams and Brown, 2007). Additionally, postharvest water loss is common in limes, primarily through

transpiration or evaporation, influenced by environmental factors like temperature and humidity. As limes lose water, they lose firmness and may shrivel, with damage to the cuticle layer exacerbating water evaporation, shriveling, and texture changes (Riederer and Schreiber, 2001). Thus, browning and peel drying are key deterioration issues for limes during storage (Champa and Gamage, 2020). The typical shelf life of limes under ambient conditions is about 6–9 days post-harvest (Samaradiwakara et al., 2019). Given the economic impact of lime quality

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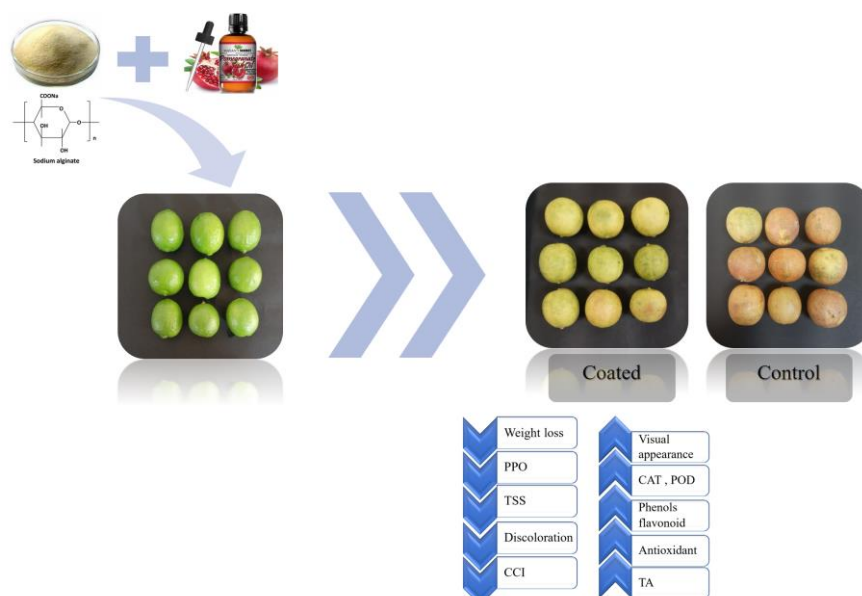
decline, developing strategies to mitigate these postharvest losses is critical for the fresh-cut produce sector.

Edible coatings have emerged as an effective postharvest technology to extend the shelf life of fresh produce. Acting as semi-permeable barriers, these coatings maintain structural integrity, reduce deterioration, and preserve quality (Kumar and Sethi, 2018). A key advantage of coatings is their edible, biodegradable nature. Sodium alginate coatings, in particular, have demonstrated significant efficacy in fruit preservation (Wang et al., 2020). For instance, coatings incorporating sodium alginate and tangerine peel essential oil improved the color, firmness, and reduced weight loss of strawberries during refrigerated storage (Utami et al., 2023). Similarly, postharvest coatings with sodium alginate and ascorbic acid extended strawberry storage life, reducing water loss and preserving fruit quality (Nazoori et al., 2020). For mangoes, sodium alginate coatings significantly reduced weight loss and retained higher firmness, total phenols, and flavonoids under storage at  $15 \pm 1$  °C with  $85 \pm 1\%$  relative humidity (Rastegar et al., 2019). Additionally, a 1.5% sodium alginate coating preserved the firmness of fresh-cut nectarines at 4 °C and 95% relative humidity (Chiabrando and Giacalone, 2016).

Pomegranate seed oil (PSO), rich in conjugated linolenic acid and other beneficial fatty acids, is gaining attention for its health-promoting properties and functionality in food preservation (Zielińska et al., 2022). Lipid-based coatings

create a hydrophobic barrier that minimizes water vapor transmission, reducing moisture loss and extending freshness and shelf life (Soliman and Zahran, 2022). PSO-enriched carboxymethyl cellulose coatings, for instance, effectively prolonged strawberry shelf life by reducing moisture loss and maintaining phenolic content (Melikoğlu et al., 2022). Combined coatings of tamarind seed starch and PSO similarly preserved guava quality during storage (Onias et al., 2018).

Notably, the application of sodium alginate, both alone and with PSO, on fresh Mexican lime fruits remains underexplored. Consequently, the specific effects and benefits of these treatments on the quality and postharvest attributes of Mexican limes are largely uncharted. Further research is essential to investigate these treatments' impact on Mexican limes comprehensively. This study aims to examine the innovative application of sodium alginate, enriched with PSO as an edible coating, to enhance the postharvest quality of Mexican lime fruits. Specifically, it assesses the effect of sodium alginate coating combined with PSO on fruit appearance, weight loss, and biochemical properties such as antioxidant enzyme activity, phenol content, and flavonoid content. This approach aims to establish a sustainable and effective postharvest strategy that may transform Mexican lime management, benefiting producers and consumers by reducing postharvest losses, enhancing quality, and extending the market availability of fresh limes (Fig. 1).



**Fig. 1.** Graphical abstract of the experiments process.

## Materials and Methods

The study utilized *Citrus aurantifolia* (Mexican lime) fruits sourced from a commercial orchard located in Rodan City, Hormozgan Province, at the geographical coordinates of 57° 29' E and 27° 59' N. The fruits were harvested at the mature green stage, with careful selection to ensure consistent size and freedom from damage. Prior to treatment application, the fruits underwent thorough washing and disinfection using a 0.05% sodium hypochlorite solution to ensure optimal quality for testing.

### Preparation of coating solution

A sodium alginate solution (0.5% and 1% w/v) was prepared by gradually adding sodium alginate powder (Temad Kala company) to deionized hot water under continuous magnetic stirring to prevent clumping, stirring until complete dissolution to form a homogeneous

solution. Glycerol (0.5% w/v) was subsequently added to the alginate solution, followed by pomegranate seed oil (PSO, 0.05% w/v) from the Dal Sin brand. Table 1 details the experimental treatments along with abbreviations for the respective edible coatings used.

To ensure thorough application, Mexican lime fruits were submerged in each treatment solution for 5 minutes, then air-dried at ambient temperature. Control fruits were immersed in distilled water for the same duration. Each treatment was replicated three times, with each replication containing 10 fruits stored in plastic containers at  $20 \pm 2$  °C for 24 days. For the assessment of physical attributes, such as color and weight, individual fruits were evaluated and averaged. In contrast, the evaluation of biochemical attributes was conducted on pooled juice samples from each replication, measuring a range of quality factors.

**Table 1.** Experimental treatments and corresponding abbreviations for edible coatings.

Treatment	Abbreviation
Distilled water	Control
Sodium alginate 0.5%	SA 0.5%
Sodium alginate 1%	SA 1%
Sodium alginate 0.5% + Pomegranate seed oil	SA 0.5% + PSO
Sodium alginate 1% + Pomegranate seed oil	SA 1% + PSO
Pomegranate seed oil	PSO

### Overall visual acceptability (OVA)

A four-point scoring system was employed to conduct a subjective assessment of the lime fruits' quality, facilitating the evaluation of their overall visual acceptability (Mohammadi et al., 2024).

This system enabled a comprehensive analysis based on a range of attributes, thereby providing a structured framework for gauging the aesthetic appeal and perceived freshness of the fruits (Table 2).

**Table 2.** Visual acceptability scoring criteria.

Score	Description
4	Excellent quality, characterized by their freshness, firmness and glossy peel.
3	Good quality and still suitable for marketing. However, they exhibited slight shriveling and softness.
2	Fruits displayed symptoms such as shriveling, loss of greenness, dryness, and browning progression.
1	Poor quality, as indicated by decay, severe shriveling, and darkening of the peel color.

### Physiological loss in weight (PLW)

The reduction in the lime's mass was calculated by measuring the weight of lime fruits at the start and end of a 24-d storage period using a digital scale accurate to 0.1 g. The decrease in mass can be expressed as a percentage using the following equation.

$$PLW (\%) = \frac{w_0 - w_1}{w_0} \quad (1)$$

Where  $w_0$  represents the initial mass and  $w_1$  represents the final mass.

### Determination of fruit color

To quantitatively assess color changes in both control and coated lime fruits, a colorimeter CR-

400 (Konica, Tokyo, Japan) was used to measure the CIE Lab color space coordinates ( $L^*$ ,  $a^*$ ,  $b^*$ ). Color readings were taken from the fruit's central region and two lateral sides, with the mean of these measurements serving as the representative color value for analysis.

The  $L^*$  value denotes lightness on a scale from 0 (black) to 100 (white), where higher values indicate increased brightness and lower values indicate a darker appearance. The  $a^*$  value measures the position along the red-green axis, with positive values indicating red tones and negative values suggesting green hues. The  $b^*$  value represents the position along the yellow-blue axis, where positive values suggest a yellowish tint and negative values indicate a bluish tone. The citrus color index (CCI) was calculated as follows:

$$CCI = \frac{1000a^*}{L^* b^*} \quad (2)$$

### ***Determination of antioxidant enzyme activity***

#### ***Enzyme extraction***

Fruit peel (2.0 g) was blended with phosphate buffer (20 mL, 50 mM, pH 7.4) containing 1 mM PEG (polyethylene glycol) and 4% w/v PVPP (polyvinylpolypyrrolidone) according to relevant methods (Li et al., 2021) with some modification. The blending process was carried out in an ice bath to maintain a low temperature. The homogenized sample was then centrifuged at  $6000 \times g$  for 10 min at 4 °C.

#### ***PPO assay***

The reaction solution was synthesized through a combination of 100  $\mu$ L of phosphate buffer (0.05 M, pH 6.5) and 80  $\mu$ L of 0.5 M catechol, thereby forming the initial mixture. This mixture was subsequently incubated at a temperature of 35 °C for a duration of 5 min to ensure proper reaction conditions. Following the incubation phase, 20  $\mu$ L of extract was introduced into the reaction solution. The resultant changes in absorbance were meticulously monitored at a wavelength of 420 nm utilizing a spectrophotometer (CECIL-2501, England). Enzyme activity was quantified as U  $\text{mg}^{-1}$  FW. Each sample was assayed in triplicate to ensure reproducibility and accuracy of the results (Serradell et al., 2000).

#### ***POD assay***

To evaluate the POD activity in the fruit peel samples, a modified spectrophotometric method was utilized (Change and Maehly, 1955). The composition of the reaction mixture included 60

$\mu$ L of 0.05 M guaiacol, 20  $\mu$ L of hydrogen peroxide, and 20  $\mu$ L of the enzyme solution. The dynamic changes in absorbance within the reaction mixture were meticulously measured at a wavelength of 470 nm. Enzyme activity was quantified as U  $\text{mg}^{-1}$  FW. Each sample was assayed in triplicate to ensure reproducibility and accuracy of the results.

#### ***CAT assay***

The CAT activity within the fruit peel samples was quantified through a methodology established in the literature (Aebi, 1984). This analytical procedure involved preparing a reaction mixture consisting of 50 mM sodium phosphate buffer (pH 7.0), 0.2 mL of the enzymatic extract, and 150  $\mu$ L of 20 mM  $\text{H}_2\text{O}_2$ . To assess the catalytic breakdown of hydrogen peroxide facilitated by catalase, the resultant decrease in absorbance was meticulously measured at 240 nm using a spectrophotometer. The CAT enzyme activity was expressed in U  $\text{mg}^{-1}$  FW.

### ***Determination of the total phenolic content (TPC) and the total flavonoid content (TFC)***

During the extraction process, fruit juice underwent homogenization with 80% methanol. The homogenized sample was subsequently centrifuged at  $4000 \times g$  for 10 min, resulting in a supernatant that served as the basis for determining the total phenolic content, total flavonoid content, and antioxidant activity. The quantification of total phenolic content was conducted employing a modified methodology (Singleton et al., 1999). In this procedure, 0.3 mL of the methanol extract was combined with 1.2 mL of 7% sodium carbonate solution and 1.5 mL of diluted Folin-Ciocalteu reagent. After an incubation period of 90 min, the absorbance of the reaction mixture was measured at 750 nm utilizing a spectrophotometer. The resultant phenolic content in the extracts was quantified and expressed as mg GAE  $\text{g}^{-1}$  FW. Gallic acid was used for standard curve. Total flavonoid measurement was conducted according to Chang et al. (2002). This involved combining the methanol extract with a solution that consisted of 10%  $\text{AlCl}_3$  and potassium acetate solution (1 mM), and subsequently incubating the mixture for a period of 30 min. Upon completion of the incubation process, the absorbance of the reaction mixture was measured at 415 nm, utilizing a UV-vis spectrophotometer. Quercetin was used for standard curve.

### Determination of antioxidant activity

DPPH radical scavenging activity was assessed using a modified method as described by Brand-Williams et al. (1995). Initially, to prepare the DPPH solution, 0.025 g of DPPH was dissolved in 100 mL of 85% methanol. Subsequently, 30  $\mu$ L of the methanol extract from the samples was combined with 150  $\mu$ L of the prepared DPPH solution. This mixture was then incubated in darkness for 40 min to allow for the reaction to occur. Following incubation, the absorbance of the solution was meticulously measured at 517 nm employing a UV-VIS spectrophotometer. The percentage of DPPH radical inhibition ( $I_n$ ) was calculated using the specific equation provided.

$$I_n (\%) = \frac{C_a - S_a}{C_a} \times 100 \quad (2)$$

Where  $C_a$  and  $S_a$  represent the control absorbance and sample absorbance, respectively.

### Determination of the total soluble solid (TSS), potential of hydrogen (pH) and titratable acidity (TA)

TSS concentration in fruit juice was quantified using a digital refractometer (DBR 95), following a method outlined by Kumar et al. (2021). The results for TSS content were expressed as percentages, providing a measure of the soluble sugar content in the juice. For the determination

of TA, we utilized a titration method involving a 0.1 M NaOH solution, adjusting the pH of the lime juice samples to 8.2.

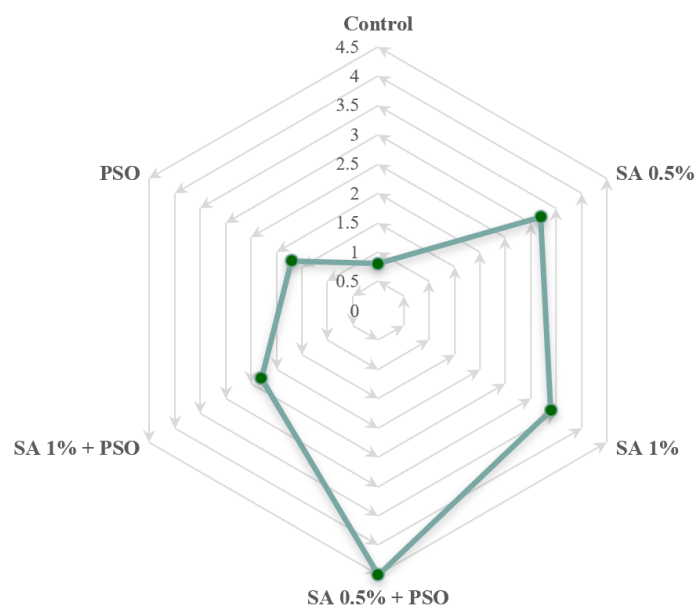
### Statistical analysis

The study's data analysis was conducted using ANOVA based on a factorial arrangement within a completely randomized design (CRD), with three repetitions per treatment, each including 10 fruits. Results are expressed as the mean of three values along with the standard error. The data from the experiment were analyzed with an LSD test to determine significant differences ( $p < 0.05$ ). Statistical analyses were performed using SAS software (version 9.4). Principal component analysis (PCA) was conducted with XLSTAT (version 2020, developed by Addinsoft SARL; accessible at [www.xlstat.com](http://www.xlstat.com)). Hierarchical cluster analysis and Pearson correlation were performed using R software (available at [www.r-project.org](http://www.r-project.org)).

## Results

### Overall visual acceptability (OVA)

As shown in Figure 2, the SA 0.5% + PSO treatment yielded the highest marketability levels for fruits after 24 days of storage, followed closely by SA 1%. In contrast, fruits in the control group demonstrated the lowest marketability compared to all other treatments.

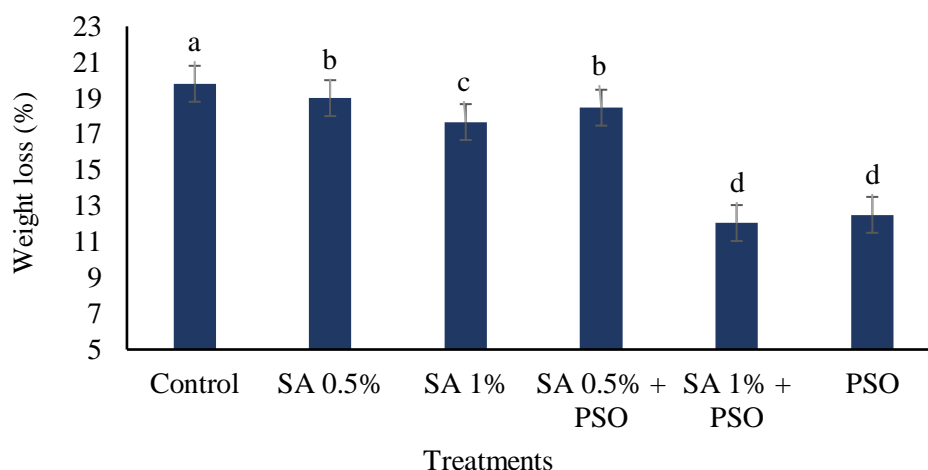


**Fig. 2.** Impact of treatments (distilled water-control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on the overall visual acceptability of Mexican lime fruit stored for 24 d at  $20 \pm 2$  °C and  $64 \pm 5\%$  RH.

### Weight loss

A comparison of control and treated fruits after 30 days of storage at ambient temperature revealed a significant difference between the control group and fruits treated with SA 1%, SA 1% + PSO, and PSO. Notably, the PSO and SA 1% + PSO treatments effectively prevented weight

loss in lime fruits, showing a significant difference from fruits treated with SA 1% alone. As illustrated in Figure 3, these applied coatings substantially reduced fruit weight loss after 24 days of storage. The treatments with only PSO and with SA 1% + PSO demonstrated the least weight loss, at 12.5% and 12.04%, respectively.



**Fig. 3.** Impact of treatments (distilled water-control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on the weight loss of Mexican lime fruit stored for 24 d at  $20 \pm 2$  °C and 50-60% RH. The data represent mean values of  $n = 3$ , and the error bars indicate standard errors (SE) of the means. Statistical analysis was performed using the LSD test at the  $P \leq 0.05$  level.

### Color index

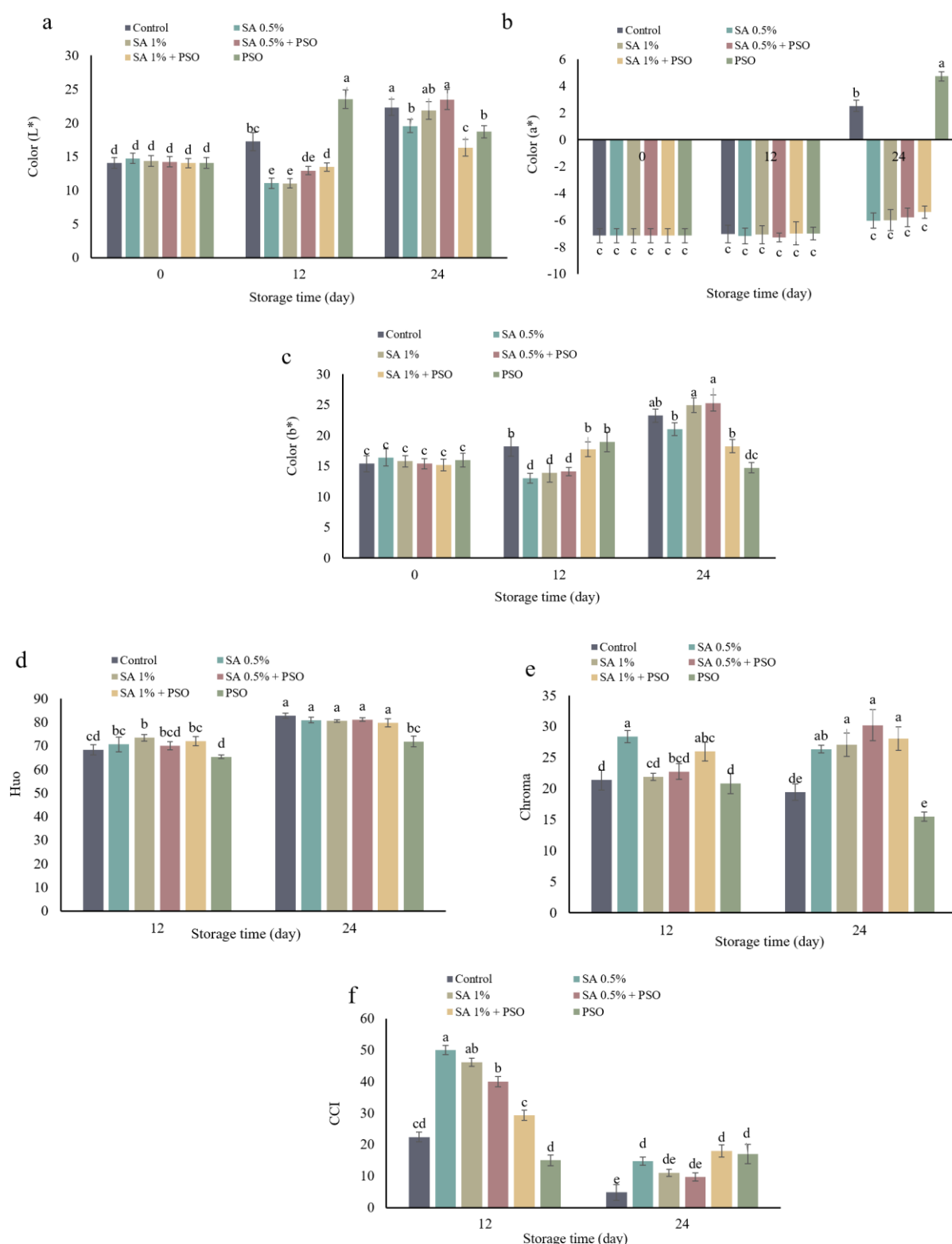
In Figure 4a, after 12 days of storage, the PSO treatment exhibited the highest  $L^*$  index among all treatments, indicating a significant increase compared to the others. After 24 days of storage, however, the SA 1% + PSO treatment showed the lowest  $L^*$  index, which was significantly different from both the control group and other treatments. According to Figure 4b, the  $a^*$  index remained stable in the treated fruits throughout the storage period, with the exception of those treated with PSO. By the end of 24 days, both the PSO-treated fruits and control fruits exhibited a noteworthy increase in the  $a^*$  index compared to the other treatments.

Figure 4c visually represents that the  $b^*$  index also significantly increased after the 24-day storage period, with the PSO treatment displaying the lowest  $b^*$  index. This distinction was statistically significant when compared to the control and other treatments, highlighting the notable color parameter differences attributed to the PSO treatment. However, no significant difference in the  $b^*$  index was detected between the control and other treatments.

Similarly, Figure 4d illustrates a significant increase in the Hue index after 24 days of storage, with the PSO treatment showing the lowest Hue index, a statistically significant difference compared to the control and other treatments. Nevertheless, there was no notable distinction observed in the Hue index between the control group and the other treatment groups.

Figure 4e shows that the chroma index remained relatively stable after 24 days in the control group, as well as in the alginate 1% and alginate 2% + PSO treatments. In contrast, the PSO treatment experienced a significant decrease in the chroma index, while significant increases were observed in the other treatments.

Finally, Figure 4f indicates that the CCI index remained relatively stable after 24 days in the PSO treatment. In contrast, the other treatments and the control group showed a significant reduction in the CCI index. No significant distinctions were observed among the treatments (except for the PSO treatment) and the control group regarding the CCI index. No significant distinctions were observed among the treatments and the control group showed the lowest CCI index.



**Fig. 4.** Impact of treatments (distilled water: control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on a) L\*, b) a\*, c) b\*, d) Chroma, e) Hue, and f) CCI of Mexican lime fruit stored for 24 d at  $20 \pm 2$  °C and 50-60% RH. The data represent mean values of  $n = 3$ , and the error bars indicate standard errors (SE) of the means. Statistical analysis was performed using the LSD test at the  $P \leq 0.05$  level.



### **Enzyme activity**

Figure 5a indicates that at 0 d of storage, all treatments have similar CAT values, around 86.6 U mg<sup>-1</sup> FW. However, by 24 d of storage, the treatments show significant differences. The SA 1% and SA 0.5% + PSO treatments have the highest CAT activity (303 and 296 U mg<sup>-1</sup> FW) values, while the control, SA 1% + PSO, and PSO treatments have lower values (69, 90 and 77 U mg<sup>-1</sup> FW respectively).

According to the data presented in Figure 5b, the activity of the PPO enzyme remained consistent throughout the storage period in sodium alginate 0.5% + and PSO treatment. This indicates that this treatment had a notable impact on controlling PPO enzyme activity and effectively preventing peel browning. In contrast, the other treatments displayed an increase in PPO enzyme activity during storage. However, apart from PSO treatment, the remaining treatments exhibited a significant decrease in enzyme activity.

Figure 5c also shows the POD activity during 24 d of storage. At 0 d, all treatments have similar POD activity (65 U mg<sup>-1</sup> FW). But by 24 d, the SA 0.5% treatment having the highest POD (89.3 U mg<sup>-1</sup> FW) activity, while the control, SA 1% + PSO, and PSO treatments have significantly lower values (71.3, 76 and 75 U mg<sup>-1</sup> FW).

### **Total phenolic content (TPC), flavonoid content (TFC), and antioxidant activity**

Figure 6a illustrates a decline in the total phenolic content of the fruits over the storage period. Nonetheless, the treated fruits maintained superior phenolic content compared to the control group, with the exception of the PSO treatment. As shown in Figure 6b, the flavonoid content in the control group decreased significantly during storage. In contrast, the treated fruits exhibited higher flavonoid levels than the control. Among the treatments, the highest flavonoid content (1.59 mg g<sup>-1</sup>) was recorded in the group treated with 1% alginate, followed by the alginate (0.5 and 1%) + PSO treatments, which showed high flavonoid levels of 1.57 and 1.49 mg g<sup>-1</sup>, respectively.

During the storage period, the antioxidant capacity of all samples decreased, though the reduction was more pronounced in the control sample compared to the treatments. After 24 d of storage, the control sample and PSO treatment exhibited the lowest antioxidant capacity (approximately 77%). In contrast, the other treatments demonstrated significantly higher antioxidant activity (84%) (Fig. 6c).

### **Total soluble solid (TSS), titratable acidity (TA), and pH**

During the storage period, the TA of the fruits declined. After 24 days, fruits in the control and PSO-treated groups exhibited lower TA levels, while the other treatments showed comparatively higher TA, with the highest values recorded for the SA 1% (15.7%) and SA 0.5% + PSO (15.6%) treatments (Fig. 7a). As shown in Fig. 7b, the TSS content of the treated fruits, with the exception of those treated with PSO, remained stable throughout the storage duration. In contrast, the control fruits and those treated with PSO experienced a significant rise in TSS content. Fig. 7c indicates that, during storage, the pH of the control and PSO-treated fruits increased, whereas it remained relatively stable in the other samples. The highest pH values were observed in the control and PSO groups, at 2.25 and 2.24, respectively.

### **Pearson correlation, principal component analysis (PCA), and hierarchical clustering heatmap**

Figure 8 presents the results of the Pearson correlation analysis performed on the biochemical parameters of lime fruits throughout the storage period. The correlations among different parameters are visually represented using color-coding, where negative correlations are depicted in green and positive correlations in red. Notably, the analysis highlights the relationships between overall visual quality (OVQ) and other parameters.

According to the findings, OVQ shows a significant positive correlation with peroxidase (POD), catalase (CAT), phenolic content, flavonoid content, and antioxidant activity. This indicates that as OVQ values increase, the values of these biochemical parameters also tend to rise, reflecting a positive relationship. Conversely, OVQ demonstrates a negative correlation with the a\* color index, polyphenol oxidase (PPO), and total soluble solids (TSS). This suggests that as OVQ increases, the values for a\*, PPO, and TSS decrease, indicating a negative relationship.

Principal components analysis reveals that F1 and F2 correspond to the first and second principal components, respectively. F1 accounts for 61.25% of the variance, while F2 explains 28.6% of the variance in the dataset. F1 exhibits a strong positive loading for antioxidant activity, total phenolic content, titratable acidity (TA), and OVQ, indicating a positive relationship among these variables. In contrast, F1 shows a strong negative loading for TSS, PPO, and weight loss,



suggesting a negative relationship with these variables.

Figure 8 presents a normalized heatmap matrix evaluating the comprehensive responses of fruit biochemical parameters to various treatments over the storage period. The heatmap matrix likely contains rows representing treatments, columns representing different biochemical parameters, and the cell values representing the response or change in the parameters under each treatment.

The heatmap effectively illustrates patterns and trends in the data, highlighting how different treatments influence the biochemical parameters over the storage period. Notably, the maximum activity of catalase was observed at the end of the experiment in the treatments of SA (0.5%) + PSO and SA (0.5%). Three distinct clusters emerged from the analysis, each reflecting the varying impacts of treatments and storage durations on the biochemical parameters. Cluster I included fruits treated with SA (0.5%) and SA (1%) after 24 days of storage. Fruits in this cluster exhibited elevated to exceptionally high catalase (CAT) activity along with low  $a^*$  color values, indicating a positive effect on enzymatic activity and a potential influence on fruit coloration. Cluster II comprised fruits stored for 24 days, including the control group and the PSO treatment. Fruits within this cluster showed high polyphenol oxidase (PPO) activity coupled with low levels of phenolic compounds, suggesting that these treatments may not effectively mitigate enzymatic browning and preserve phenolic content. Cluster III demonstrated high to very high antioxidant capacity alongside low  $a^*$  values, indicating a strong correlation between antioxidant activity and the maintenance of fruit quality under specific treatments. Overall, the heatmap provides valuable insights into how treatments can differentially affect biochemical parameters in lime fruits during storage.

## Discussion

The combination of chemical and biochemical reactions, physical changes, and microbial activity during storage can lead to undesirable changes in the appearance of fruits and vegetables. Proper storage conditions and handling practices are essential to minimize these adverse effects and maintain the quality and visual appeal of produce (Toivonen and Brummell, 2008). Peel browning, a discoloration caused by various factors, includes enzymatic and non-enzymatic browning reactions. During storage, fruits and vegetables can be exposed to

oxygen, triggering these reactions and resulting in peel browning (Nath et al., 2022).

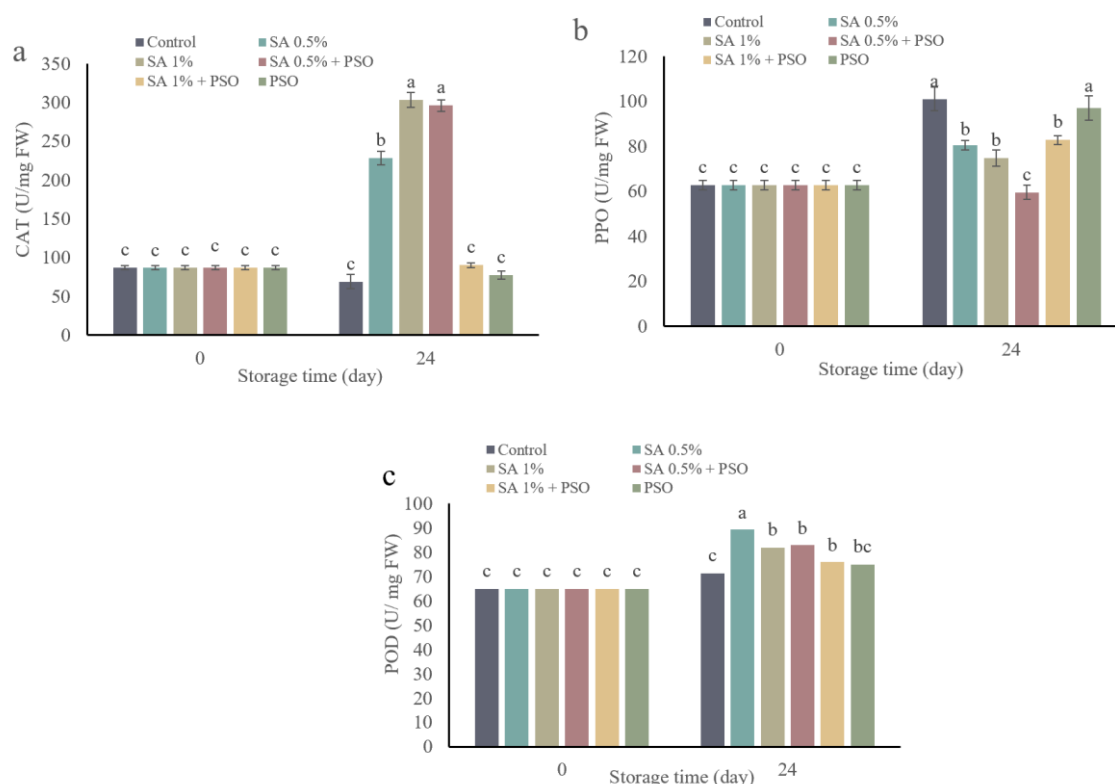
Edible coatings play a vital role in enhancing fruit preservation by reducing moisture loss and gas exchange, protecting against physical damage, and improving appearance through enhanced color and glossiness during storage (Panahirad et al., 2021). The use of edible coatings can help maintain the appearance of fruits, thereby increasing their marketability and consumer appeal. For instance, chitosan coatings have been shown to reduce peel browning in apples during storage (Qi et al., 2011).

Moreover, some edible coatings contain antioxidants that help prevent peel browning by reducing oxidative stress, which can trigger both enzymatic and non-enzymatic browning reactions. One of the main advantages of edible coatings is their ability to preserve the texture of crops during storage by minimizing moisture loss and physical damage (Panahirad et al., 2021).

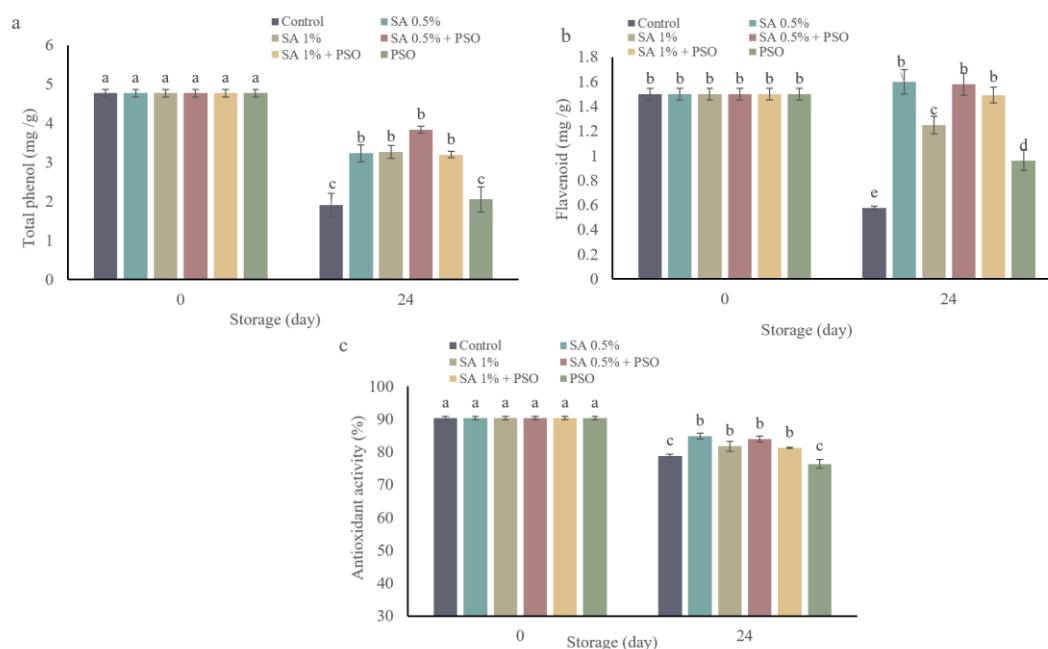
The color of coated products significantly impacts their visual perception. Coatings applied to fruits like apples and citrus can enhance their appearance and prolong shelf life by providing a glossy or waxy finish, making the fruit more visually appealing to consumers (Pathare et al., 2013; Perez-Gago et al., 2005).

Arroyo et al. (2020) noted that the modified atmosphere created between the fruit's outer layer and the coating can hinder pigment deterioration by reducing oxygen availability, thus slowing the development of undesirable colors. Coated guava fruits, for example, exhibited less yellowness compared to untreated control fruits, which had the highest  $b^*$  value, indicating a greater degree of yellow color (Hasan et al., 2022). Guava treated with alginate and chitosan, incorporated with pomegranate peel extract, displayed fewer changes in color and better retention of peel color during storage (Nair et al., 2018).

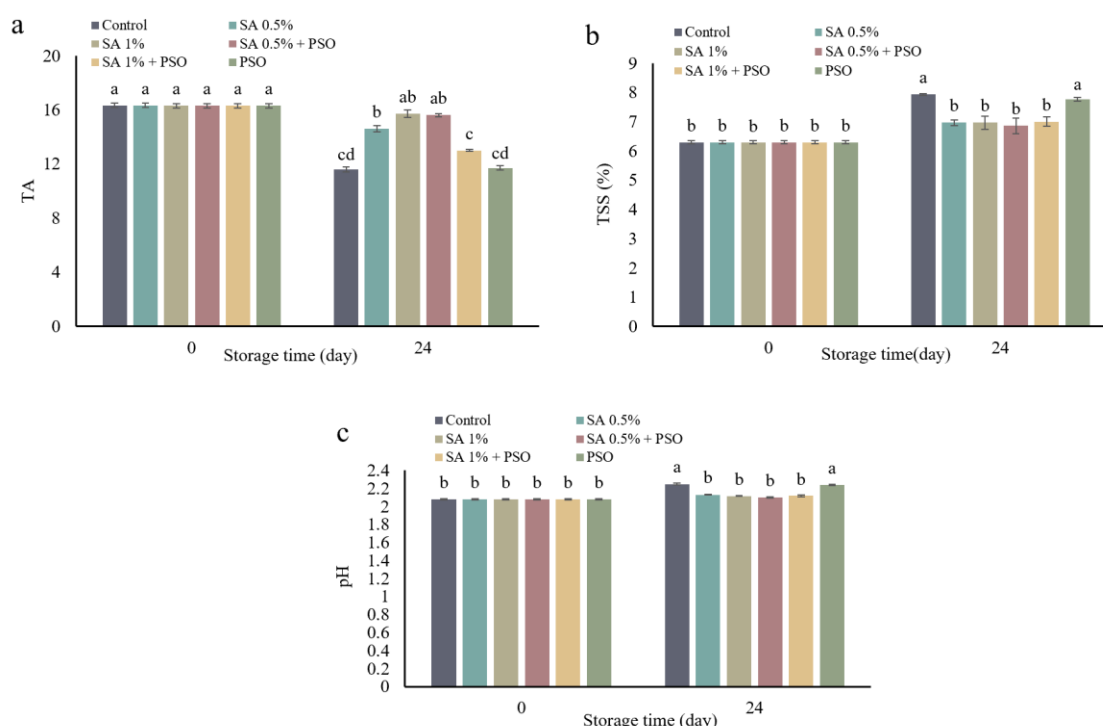
The coatings also create a modified atmosphere that slows pigment degradation due to the reduced oxygen levels, delaying the development of undesirable colors (Arroyo et al., 2020). Postharvest weight loss is a significant issue with harvested fruits and is a critical metric for determining freshness and storability (Ahmad et al., 2015). Weight loss occurs due to the difference in water vapor pressure between the fruit and the surrounding air, a process usually mitigated by the fruit's epidermis and cuticle layer (Maguire et al., 2010).



**Fig. 5.** Impact of treatments (distilled water-control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on a) CAT, b) PPO, and c) POD of Mexican lime fruit stored for 24 d at  $20 \pm 2^\circ\text{C}$  and 50-60% RH. The data represent mean values of  $n = 3$ , and the error bars indicate standard errors (SE) of the means. Statistical analysis was performed using the LSD test at  $P \leq 0.05$ .



**Fig. 6.** Impact of treatments (distilled water-control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on a) total phenols, b) flavonoids, and c) antioxidant capacity of Mexican lime fruit stored for 24 d at  $20 \pm 2^\circ\text{C}$  and 50-60% RH. The data represent mean values of  $n = 3$ , and the error bars indicate standard errors (SE) of the means. Statistical analysis was performed using the LSD test at  $P \leq 0.05$  level.



**Fig. 7.** Impact of treatments (distilled water-control), sodium alginate 0.5% (SA 0.5%), sodium alginate 1% (SA 1%), sodium alginate 0.5% + pomegranate seed oil (SA 0.5% + PSO), sodium alginate 1% + pomegranate seed oil (SA 1% + PSO), pomegranate seed oil (PSO) on a) titratable Acidity (TA), b) total soluble solids (TSS), and c) pH, of Mexican lime fruit stored for 24 d at  $20 \pm 2$  °C and 50-60% RH. The data represent mean values of  $n = 3$ , and the error bars indicate standard errors (SE) of the means. Statistical analysis was performed using the LSD test at  $p \leq 0.05$  level.

The reduction in mass observed in fresh produce during storage primarily results from water loss due to metabolic activities such as respiration and transpiration (Brizzolara et al., 2020). This weight loss affects the fruit's volume and quality. The alginate coating forms a thick, semi-permeable barrier on the fruit's skin against gases like oxygen and carbon dioxide, which reduces respiration and transpiration rates (Riva et al., 2020). Similar results were noted in guava treated with black cumin extract-alginate edible coating, which demonstrated that sodium alginate coatings, especially when combined with essential oils, exhibit antimicrobial activity against fungi and bacteria. By inhibiting microbial growth, these coatings help prevent fruit decay and weight loss (Hasan et al., 2022).

Antioxidant enzymes are crucial in safeguarding cells against damage from free radicals, which can cause cellular harm and contribute to various diseases. The antioxidant enzyme system in fruit tissue is vital for reducing postharvest oxidative damage and maintaining structural integrity in response to senescence stress (Meitha et al., 2020). Edible coatings enhance the activity of key

enzymes like catalase (CAT) and peroxidase (POD), reducing oxidative damage by controlling reactive oxygen species (ROS) in produce, thus preserving quality (Huang et al., 2021). In the current study, antioxidant enzymes such as CAT and POD were found to be more active in alginate-coated fruits compared to those in the control group (Li et al., 2017).

Edible coatings can also mitigate the decline of antioxidant enzyme activity during storage by forming a barrier that slows gas exchange and oxidation, thereby preserving their protective capacity against oxidative damage (Nair et al., 2020). Research has demonstrated that edible coatings can help maintain the activity of antioxidant enzymes in various fruits and vegetables, such as kiwifruits treated with chitosan coatings (Kumarihami et al., 2022).

Edible coatings have been shown to effectively preserve and even enhance the phenolic content in fruits by limiting environmental exchange and influencing enzymatic activity during storage (Mahardiani et al., 2021). For example, applying an alginate-based edible coating infused with Loquat leaf extract on Nanfeng tangerine fruits

can potentially increase the abundance of phenolic compounds (Zhang et al., 2022). Supporting this, Martínez-Romero et al. (2019) found that rosehip oil-coated European and Japanese plums exhibited high levels of phenolic compounds. Edible coatings also play a significant role in maintaining antioxidant levels in fruits and vegetables by minimizing gas and moisture exchange, which slows oxidation and enhances nutritional quality during storage and transportation (Khaliq et al., 2019).

These coatings significantly influence the internal atmosphere of fruits, affecting their metabolic activities and the stability of bioactive compounds such as carotenoids, phenolics, flavonoids, and ascorbic acid. By preserving these antioxidants, edible coatings enhance the overall antioxidant capacity of fruits, contributing to prolonged shelf life and improved nutritional value (Riva et al., 2020). For instance, coated guava fruits show increased antioxidant activity, attributed to higher levels of ascorbic acid, flavonoids, and phenolic compounds (Etemadipoor et al., 2020). Our findings align with those of Dulta et al. (2022), which indicated that alginate-chitosan edible coatings enriched with ZnO nanoparticles improved antioxidant levels in orange fruits.

During storage, the total soluble solids (TSS) of fruits can decrease while titratable acidity (TA) may increase, negatively affecting fruit quality and taste (Lin and Zhao, 2007). Edible coatings can help maintain TSS and TA during storage by reducing moisture loss and gas exchange between the fruit and its environment (Ebrahimi and Rastegar, 2020). This reduction slows the biochemical reactions that can alter TSS and TA. Khaliq et al. (2019) suggested that changes in TSS are linked to a decrease in fruit respiration rates and lower concentrations of metabolites in

coated fruits. This indicates that the presence of coatings affects the metabolic processes of fruits, potentially influencing their overall quality and shelf life.

## Conclusions

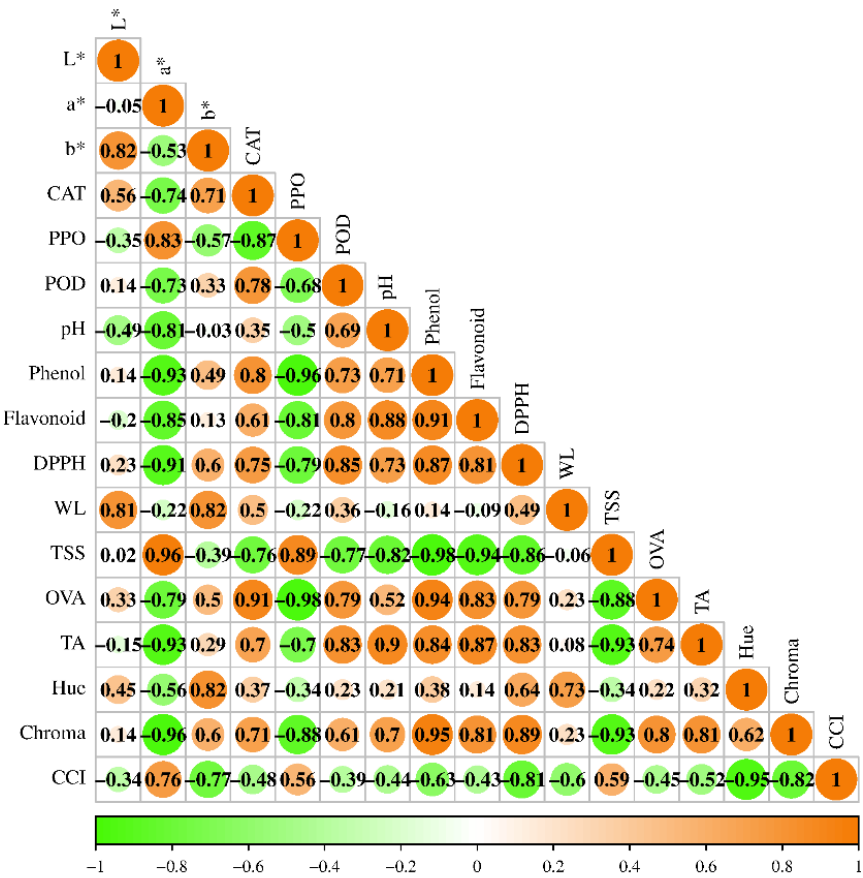
In conclusion, this study demonstrated that the application of sodium alginate combined with pomegranate seed oil effectively maintained the quality and freshness of Mexican lime fruit during a 24-d storage period at room temperature. The treated fruits exhibited enhanced visual quality, reduced weight loss, and inhibited polyphenol oxidase activity, which is responsible for browning in fruits. Furthermore, these fruits showed elevated activities of peroxidase and catalase enzymes, lower total soluble solids, higher titratable acidity, and increased concentrations of total phenolics and flavonoids, along with enhanced antioxidant capacity compared to the control group.

Therefore, the implementation of the SA (0.5%) + PSO treatment can be considered a viable approach to extending the freshness of Mexican lime fruit under ambient storage conditions. Given the significant benefits of this coating in preserving postharvest quality, along with its eco-friendliness and potential to enhance product value, this technology offers a promising solution for reducing waste, increasing farmers' incomes, and improving food security. However, further research is needed to investigate the long-term effects of these coatings and their applicability to a broader range of fruits.

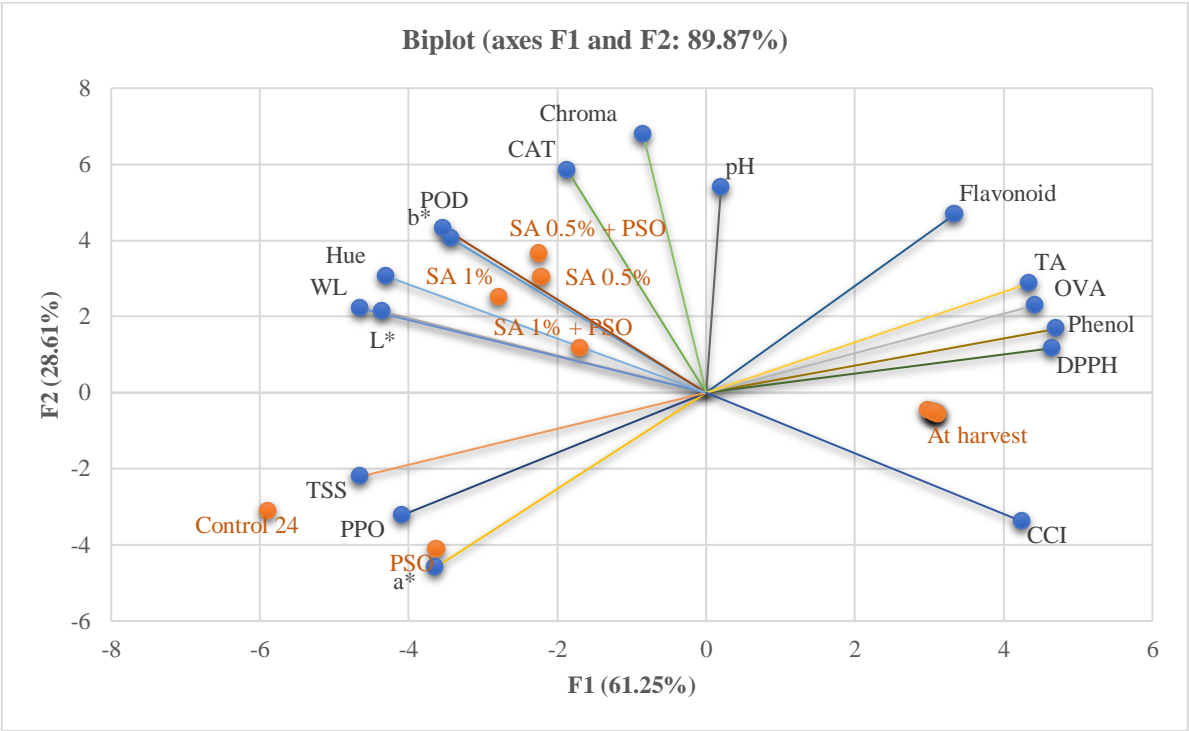
## Conflict of Interest

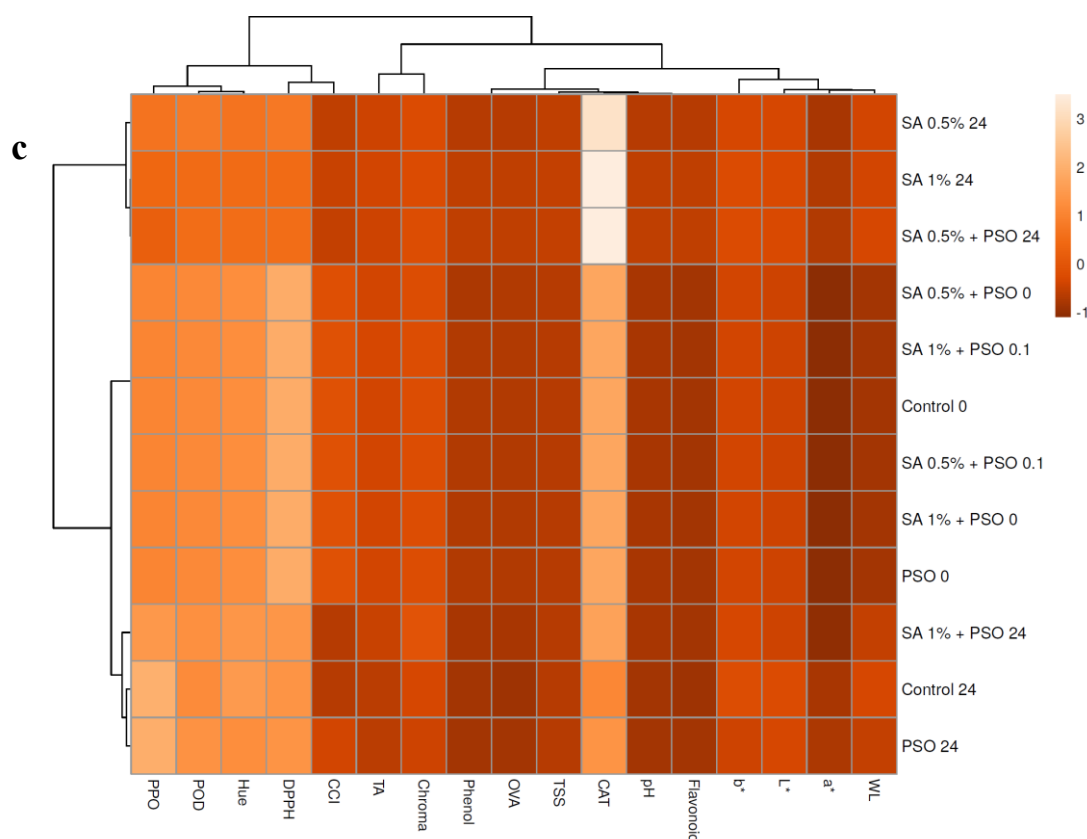
The authors indicate no conflict of interest in this work.

a



b





**Fig. 8.** a) Pearson's correlation coefficients between total traits that were determined. (b) Principal component analysis (PCA) of treatments and variable trait relationships in Mexican lime fruit, including PCA loading plots of the examined variable traits. (c) Hierarchical clustering analysis (HCA) of the edible coating treatments and variable trait relationships in Mexican lime fruit, including a heatmap of Pearson's correlation coefficients ( $r$  values) for variable traits. The colored scale shows the  $r$  coefficient values ( $r = -3$  to  $3$ ) indicating positive (light) and negative (red) correlations, respectively.

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