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Chitosan based coating enriched with *Spirulina platensis* and moringa leaf extracts preserved the postharvest quality of Mexican Lime (*Citrus aurantifolia*)

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ABSTRACT

Purpose: The limited shelf life of Mexican lime fruits when stored under ambient conditions is a significant challenge. The progressive color alteration and loss of freshness can lead to reduced marketability and increased its waste. **Research Method**: The objective of this research was to preserve the storage quality of Mexican lime fruit by employing chitosan 1.5%, chitosan 1.5% + spirulina algae (Sp) (1%), and *Moringa oleifera* (Mo) leaf extracts (1%) at 20 ± 2 °C and 50-60% relative humidity for 24 days. **Finding**s: The findings indicated that the samples coated with chitosan + Sp experienced a significantly lower weight loss compared to the control (19.8%) fruit after 24 days of storage, with a weight loss of 16.4%. A significant difference was observed between the control and treated fruit in terms of *a** color parameter, with the highest value found in the control group (2.5) and the lowest value found in the chitosan-treated group (-6.7). The treated fruit exhibited significantly higher levels of phenol and flavonoid content compared to the control group. After the 24 days of storage, the chitosan 1.5% + Sp treatment displayed the highest antioxidant activity (88.66%), followed closely by the chitosan + Mo treatment (88.76%), while the control group exhibited the lowest antioxidant activity (78.75%). The treatments exhibited a significant decrease in polyphenol oxidase (PPO) enzyme activity compared to the control group, accompanied by an increase in the activity of peroxidase (POD) and catalase (CAT) enzymes. **Research limitations**: There was no limitation. **Originality/value**: Generally, the utilization of chitosan edible coatings, specifically chitosan combined with spirulina algae, has shown promising results in preserving the quality and extending the shelf life of Mexican lime fruit stored at 20 ± 2 °C.

INTRODUCTION

Lime (*Citrus aurantifolia*) is a citrus fruit that is widely grown after oranges and mandarins. It has many economic, nutritional, and health benefits. Mexican lime is native to Southeast Asia and countries such as India, Mexico, Brazil, China, and Iran are recognized as major producers of limes (Khan et al., 2017). The appearance of the fruit is a key factor that determines its quality and marketability (Raddatz-Mota et al., 2019). However, green lime easily turns into yellow and green uneven fruits after harvest, which seriously affects the appearance quality of the fruit (Zhang & Zhou, 2019). External color is one of the key factors that define the external quality of citrus fruits (Wang et al., 2022). The challenges that restrict the postharvest life of limes, such as the reduction in weight and the discoloration of the peel, are indeed significant factors contributing to postharvest losses (Khan et al., 2017). It is important to address these challenges to minimize losses and extend the shelf life of limes. By addressing these challenges through appropriate postharvest treatments, the postharvest life of limes can be extended, reducing losses, and ensuring a consistent supply of high-quality fruit to the market. Research has explored the use of melatonin, methyl jasmonate, gamma-aminobutyric acid, and commercial wax enriched with tea seed oil as treatments to improve the postharvest quality of citrus fruits (Firozi et al., 2021; Rastgoo et al., 2024)

Recent studies have revealed that the use of edible coatings can effectively slow down the physiological processes associated with fruit ripening. These coatings create a barrier that partially blocks the pores of the fruit, leading to a reduction in respiration and transpiration rates (Maringgal et al., 2020). It has been reported that a wax treatment containing palm oil, guar gum, sorbitol, and glycerol preserved the quality of lime fruits stored under cold conditions (13 ± 2°C and 85% RH) by reducing weight loss and maintaining other important characteristics (Wijewardane, 2022).

Chitosan edible coating is a promising technology for postharvest preservation of fruits and vegetables, as it is environmentally friendly, biologically safe, and cost-effective (Shiekh et al., 2013). Chitosan edible coating is a type of biodegradable and biocompatible film that can be applied to the surface of fruits and vegetables to extend their postharvest shelf life and quality. It has many beneficial properties, such as antimicrobial, antioxidant, anti-browning, and moisture barrier effects. Chitosan edible coating can prevent or delay the microbial decay, enzymatic browning, water loss, softening, and ripening of fruits and vegetables during storage and transportation (Kore et al., 2017). Chitosan edible coating can be used alone or in combination with other natural additives, such as essential oils, plant extracts, nanoparticles, or wax (Saxena et al., 2020). Plant extracts contain a wide range of bioactive compounds such as antioxidants, antimicrobials, and phytochemicals, which can provide numerous benefits when incorporated into edible coatings. Spirulina is a nutrient-rich blue-green algae that is known for its high protein content, as well as its vitamins, minerals, and antioxidants (Ashoush & Mahdy, 2019) . In addition, spirulina is widely recognized as a natural and healthy ingredient, and its addition to edible coatings can enhance the perceived value and appeal of the coated products. Ramji and Vishnuvarthanan (2022) have proposed that due to its significant phytonutrient value and the presence of salicylic, chlorogenic, caffeic acids, and tocopherol, this microalga demonstrated great potential for its application as a pharmaceutical agent and nutritional supplement. In addition, the chitosan film spirulina exhibited promising features such as high tensile strength, low oxygen and water vapor transmission rate (Ramji & Vishnuvarthanan, 2022). There have been numerous reports on *Moringa oleifera* that have shed light on its considerable protein content, β-carotene, vitamins, phenolics, flavonoids, fatty acids, and other bioactive compounds (Saucedo-Pompa et al., 2018). Moringa leaves contain a high concentration of phenolic acids, flavonoids, glucosinolates, and

isothiocyanates. Multiple investigations have demonstrated the potential of moringa leaves as a functional additive in food products and food applications (Kubheka et al., 2020). By incorporating spirulina and moringa extract into edible coatings, the products can benefit from the nutritional value and health-promoting properties associated with spirulina. This can make coated products more attractive to health-conscious consumers who are seeking functional foods with added nutritional benefits (Budak & Sarıkaya, 2022). Spirulina algae and moringa extract were added to chitosan to produce a coating with enhanced functionality and improved properties.

In our previous experiments, we demonstrated that the application of guar (Ebrahimi & Rastegar, 2020) and alginate (Rastegar & Atrash, 2021) edible coatings enriched with spirulina effectively preserved the quality of mango fruit during storage. It has been demonstrated that gum Arabic (GA) 15% + moringa and Carboxy methylcellulose (CMC) 1% + moringa retained fruit firmness and lowered weight loss of avocado fruit (Kubheka et al., 2020). Previous research has provided evidence that the implementation of CMC coatings can effectively contribute to the preservation of Kinnow mandarin fruit quality throughout cold storage. Specifically, these coatings have been shown to mitigate weight loss, sustain desirable textural attributes, and minimize adverse physiological changes (Baswal et al., 2020).

Given the recent advancements in utilizing edible coatings for preservation of fruit quality, this study seeks to employ an innovative method by incorporating chitosan with moringa and spirulina extracts to maintain the quality of Mexican lime fruits (*Citrus aurantifolia*) during storage. To the best of our knowledge, there is limited research conducted on the combination of chitosan with spirulina and moringa extracts, highlighting a gap in combinatorial studies in this specific context. The primary objective is to examine the impact of this formulation on prolonging the shelf life of the Mexican lime fruits at a temperature of 20 ± 2 °C. Building upon existing scientific discussions highlighting the potential advantages of edible coatings in preserving fruit quality and reducing postharvest losses, we aim to explore a novel combination of chitosan, spirulina, and moringa extracts to enhance the mechanical and barrier properties of the coatings.

MATERIALS AND METHODS

Preparation of chitosan and fruit materials

Mature green Mexican lime fruit was collected from a commercial orchard in Mosafr-Abad plain (27 °N, 57 °E), Ziarat-Ali village, Rodan City, Hormozgan province, Iran. In terms of climate, this region has an average temperature of 37 °C and humidity of 64%. The fruits were packed in a plastic box and transported to the laboratory within 2 hours. Fruits were selected based on the similarity in size, absence of physical damage, and microbiological contamination. The 120 fruits were separated into groups of four, for treatment in triplicate. Edible coating based on chitosan (Sigma-Aldrich) was prepared as described by Wang & Gao (2013). Chitosan solutions were made by dissolving 1.5 g chitosan in acetic acid (0.5 mL acetic acid/100 mL de-ionized H_2O). The pH values for control and all chitosan solutions were adjusted to 5.6 with 1.0 mol/L NaOH. Extracts of spirulina (1%) and moringa (1%) were also prepared. The aqueous extracts prepared by stirring 40 g of the *Spirulina platensis* and *Moringa oleifera* leave powders in 100 ml of distilled water as describe by Ebrahimi & Rastegar, (2020). The treatments consisted of immersing fruits for 5 min at room temperature (20°C) in: (a) chitosan 1.5% (b) chitosan (1.5%) + spirulina (Sp) (c) chitosan (1.5%) + moringa (Mo). The control fruits also were immersed in 0.5 mL acetic acid/100 mL de-ionized H_2O. Following the air-dried of fruits at 20 °C for 1 h, 10 fruits for each replication

(3n) were packed in transparent plastic container with lid at a temperature of 20 ±2 °C and 50-60% relative humidity (RH) for 24 days.

Determination of physiological loss in weight (PLW)

The mass difference method determined the physiological weight loss of lime fruits (coated and controlled). In this context, coated lime fruits were weighed on the first and last day of storage using a weighing scale (Singh & Reddy, 2006). The reduction in lime mass (as percentage reduction) was calculated using formula 1:

$$PLW\ (\%) = \frac{w_0 - w_1}{w_0} \times 100 \qquad (1)$$

Where w_0 represents the initial mass and w_1 represents the final mass at 24 days.

Total soluble solids (TSS) and titratable acidity (TA)

The fruit juice was examined utilizing a DBR95 handheld refractometer, which was manufactured in Thailand. The analysis was conducted at a temperature of 25 °C, and the TSS content of the juice was expressed as a °Brix (Gupta et al., 2022). For the determination of TA, the research team utilized a titration method involving a 0.1 M NaOH solution, adjusting the pH of the lime juice samples to 8.2.

Fruit color

The color of both the control and coated lime fruits was assessed using the CIE Lab color space, which includes three coordinates: L^* (lightness), a^* (red-green axis), and b^* (blue-yellow axis). The measurements were carried out using a Chroma meter CR-400 (Konica, Tokyo, Japan) (McGuire, 1992).

Total phenolic content (TPC), total flavonoid content (TFC) and radical scavenging activity (RSA)

The extraction process involved homogenizing fruit juice samples with an 80% methanol solution. Afterward, the homogenate was centrifuged at a speed of 4000 × g for 10 min at room temperature. The supernatant was further utilized for analysis to quantify the phenol, and flavonoid content and evaluate antioxidant activity (Mohammadi et al., 2023).

TPC content in the lime fruit was measured using the standard method with the Folin–Ciocâlteu (FC) reagent with some changes (Ordonez et al., 2006). The methanol extract (0.3 mL) was mixed with the diluted Folin–Ciocalteu reagent (1.5 mL) and after 5 min, the sodium carbonate solution (7 %, 1.2 mL) was added to prepare the reaction mixture. The absorbance at 750 nm was measured using a UV spectrophotometer after the reaction mixture was incubated for 90 min. The phenolic content was expressed as the equivalent of gallic acid (mg/g FW) in the lime fruit extract.

The aluminum chloride spectrophotometric method followed by Chang et al. (2002) was used to measure the TFC of the control and coated fruits that were stored. The methanol extract (0.5 ml) and the $AlCl_3$ solution (10 %, 0.1 mL) were mixed with the acetate potassium solution (1 mM, 0.1 mL). The mixture was left for 30 min at room temperature. The absorbance was measured at 415 nm using a UV spectrophotometer. The flavonoid content of fruit extract was calculated using quercetin as a standard and expressed in mg/g FW.

The lime fruit RSA was measured using the standard DPPH 2, 2-diphenyl-1-picryl-hydroxyl) assay method with some changes (Brand-Williams et al., 1995). The lime fruit extract (30 µL) was mixed with the DPPH solution (150 µL DPPH) that was made by dissolving 0.025 g DPPH in 100 mL of the 85 % methanol. The mixture was left in the dark

for 40 min and then the absorbance was measured at 517 nm using a UV spectrophotometer. The equation 2 was used to calculate the results and they are expressed as the inhibition percentage.

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

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

The activity of antioxidant enzymes

The modified method based on Aebi (1984) was used for measuring catalase (CAT) and peroxidase (POD) activity. Initially, a 2.0 g sample was homogenized in 20 mL of phosphate buffer (50 mM and pH 7) containing 1 mM EDTA and 4% (W/V) PVPP, while maintaining the temperature in an ice bath. Subsequently, the solution was subjected to centrifugation at 6000 $\times g$ for 10 min at 4 °C, and the resulting supernatant was collected for the CAT, POD and PPO activity assay.

For CAT assay, a reaction mixture containing 0.2 enzyme extract in 50 mM sodium phosphate buffer (pH 7.0) and 150 µL of 20 mM hydrogen peroxide (H_2O_2). The CAT enzyme in the solution catalyzes the breakdown of H_2O_2, leading to a reduction in absorbance at 240 nm. The results were expressed as U mg^{-1} FW.

For the assay POD, the reaction cuvette was prepared by adding 60 µL of 0.05 M guaiacol, 20 µL of the enzyme solution, and 20 µL of 0.05 M hydrogen peroxide. The changes in absorbance of the mixture were recorded at 15 s intervals over 2 min at a wavelength of 470 nm and the results were expressed as U mg^{-1} FW (Mohammadi et al., 2023).

The peroxidase (PPO) activity extraction and assay were performed following the protocol outlined by Serradell et al. (2000). The resulting reaction mixture consisted of 80 µL of 0.5 M catechol and 100 µL of 0.05 M phosphate buffer (pH 6.5). This mixture was incubated at 35 °C for 5 min, following which 20 µL of enzyme extract PPO was added. The increase in absorbance was read at 420 nm for 3 min and the results were expressed as U mg^{-1} FW.

Statistical analysis

Experiments were conducted factorially in the form of a completely randomized design (CRD) with two factors (coatings and time (0 and 24 days)) for this study. The experiment was done with three repetitions and each repetition included 10 fruits. The data were analyzed using analysis of variance (ANOVA), and the mean values were compared with the LSD test at ($P < 0.05$) significance level using the SAS software (version 9.1). Principal component analysis (PCA) and Pearson correlation were calculated using R v3.4.3 9 software (R Core Team, 2022).

RESULTS

Weight loss

Figure 1 describes the assessment of weight loss in both the control and coated samples throughout the storage period. Throughout the storage period at 20 ± 2 °C, the weight loss rate of each sample progressively increased and reached its peak at 24 days of storage. The coated samples demonstrated lower weight loss compared to the control samples, with the chitosan + Sp coating exhibited the minimum weight loss of 16.4% (Fig. 1).

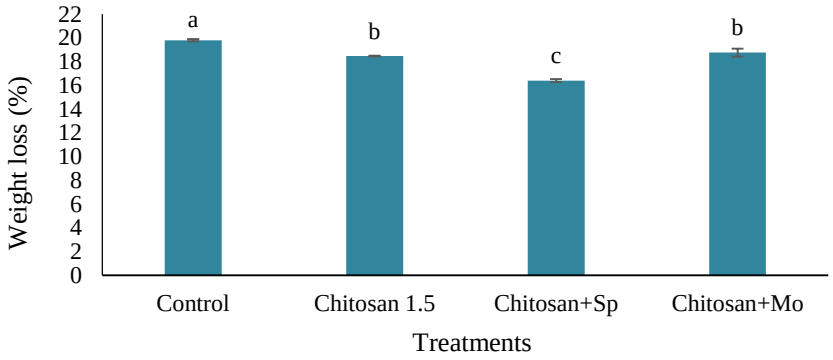


Fig. 1. Effect of treatment of chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa extract on a) weight loss of fruit juice of lime juice (*citrus aurantifolia*) stored at 20 ±2 °C. Vertical bars indicate the means' standard error (S.E.) (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

TSS and TA

The interaction effect of time and treatment on TSS and TA is shown in Fig. 1. During the storage period, the TSS content increased in all samples. However, after 24 days of storage, the fruit treated with chitosan +SP exhibited significantly lower (6.8 °Brix) TSS compared to the other treatments and the control group (7.9 °Brix) (Fig. 2a).

Fruit TA decreased over the storage period, but this decline was less pronounced in the treated fruits. Consequently, after 24 days of storage, a significant difference in TA was observed between the control (6.7%) and treatment groups (approximately 10.5%) (Fig. 2b).

Color parameters

The interaction effect of time and treatment on color parameters is shown in Fig. 3. Changes in the color parameters (L^*, a^*, and b^*) of lime fruit during storage were determined ($p < 0.05$). The characteristics of the color of the fruit at the time of harvest were L*=14.7, a*= 7.16 and b*=15.4. During storage, there was a significant increase in the L^* value of the fruit. However, after 24 days of storage, there was no significant difference found between the treated fruit and the control group (Fig. 3a).

The value of a^* increased gradually with increasing storage time. After 24 days of storage, the a^* value of control showed a significant increased rather than other samples. The minimum changes in the a^* was observed in the chitosan + Sp treatment, which also showed a significant difference compared to the other two treatments (Fig. 3b). The highest (most positive) level of a^* was observed in control (2.5) fruits. Similarly, the trend observed in the changes of the L^*, the b^* also gradually increased over time. There were no notable distinctions observed between the control and treatment groups concerning the b^* at final day (Fig. 3c).

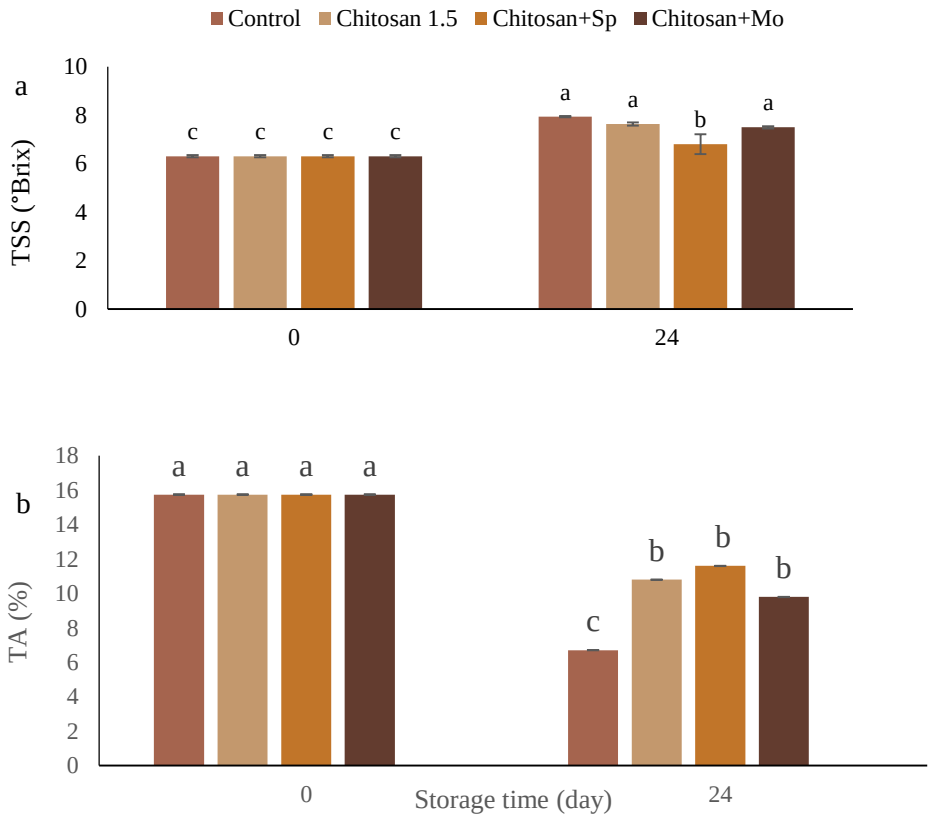


Fig. 2. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) TSS, and b) TA of lime juice (*citrus aurantifolia*) stored at 20 ±2 ˚C. Vertical bars indicate the means' standard error (S.E.) (n = 3). The means with the same LSD test letters in each column are not statistically significant ($P \leq 0.05$).

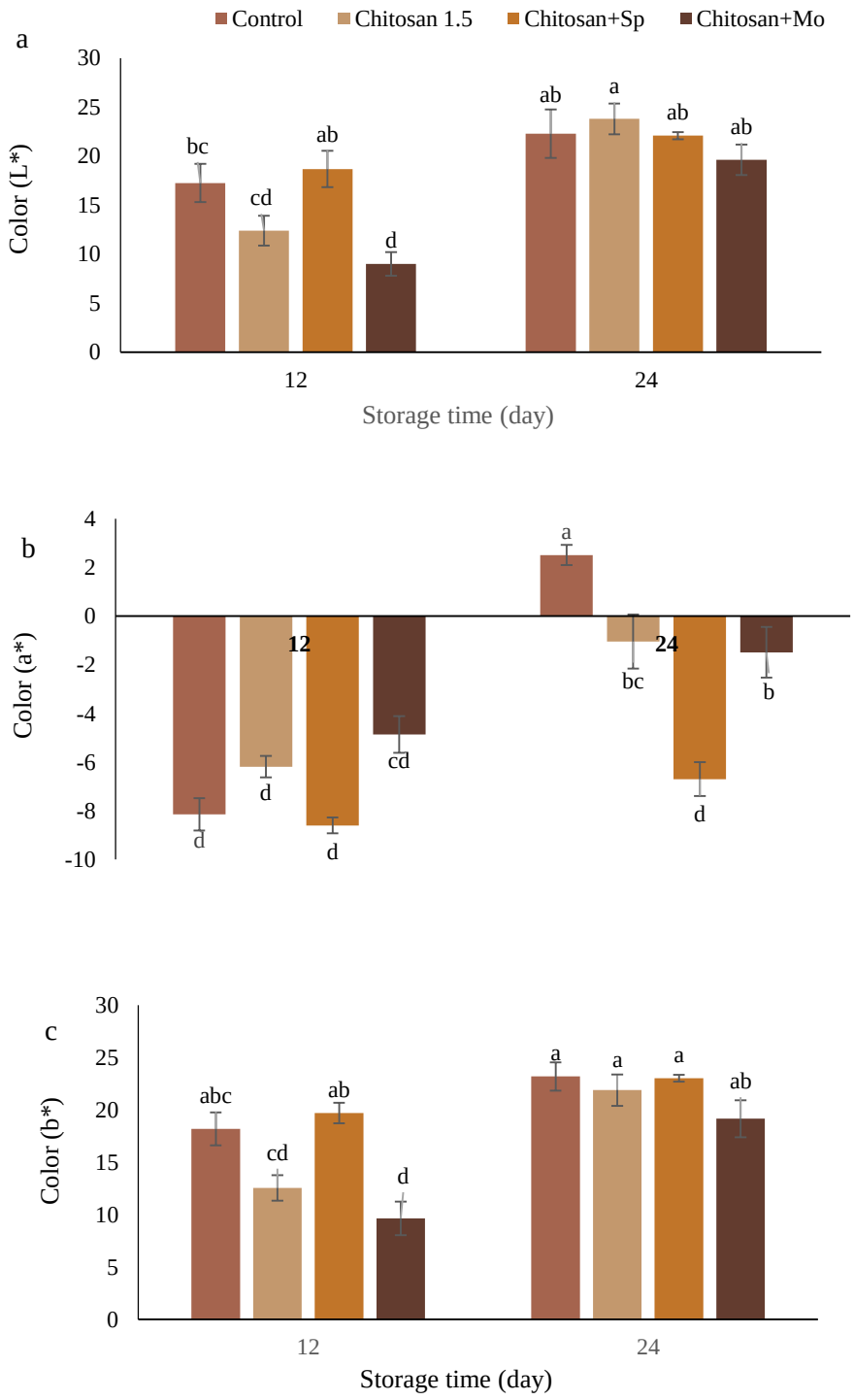


Fig. 3. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) *L**, b) *a**, and c) *b** of lime juice (*citrus aurantifolia*) stored at 20 ±2 ˚C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

TPC, TFC and RSA

The interaction effect of time and treatment on TPC, RSA and TFC is shown in Fig 4. As shown in Figure 4a, TPC content did not change significantly in the treated fruits, while a significant decrease was observed in the control fruits during storage.

The TFC in the fruits significantly decreased during storage, but the rate of reduction was higher in the control (reached 1.5 to 0.57 mg/g) group compared to the treated groups. A significant difference was observed between the control and treatment groups after 24 days of storage (Fig. 4b).

The trend in RSA reflected that of phenols, as a notable decrease was observed in the control group, while no significant changes were observed in the treated groups when compared to the initial day (Fig. 4c).

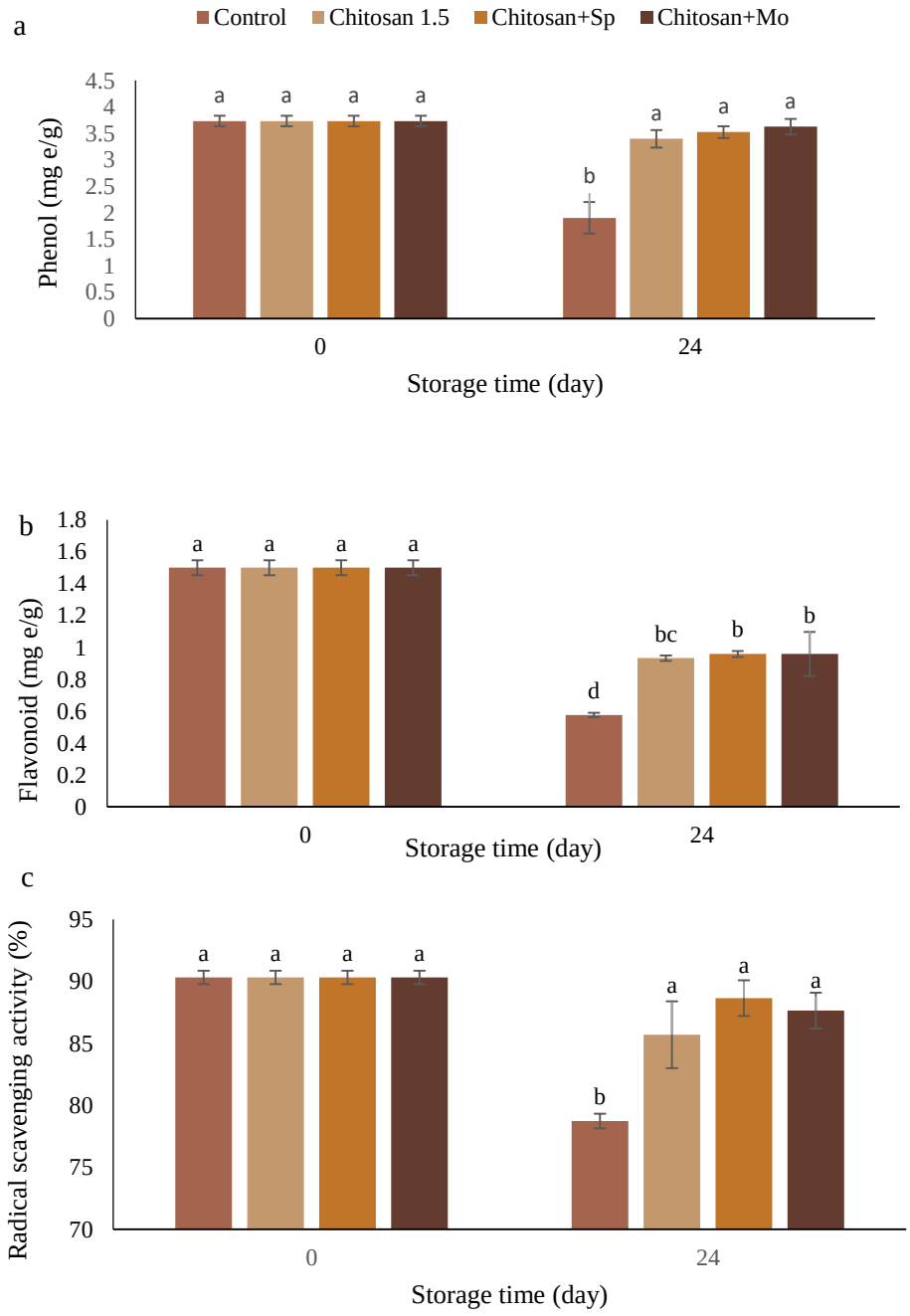


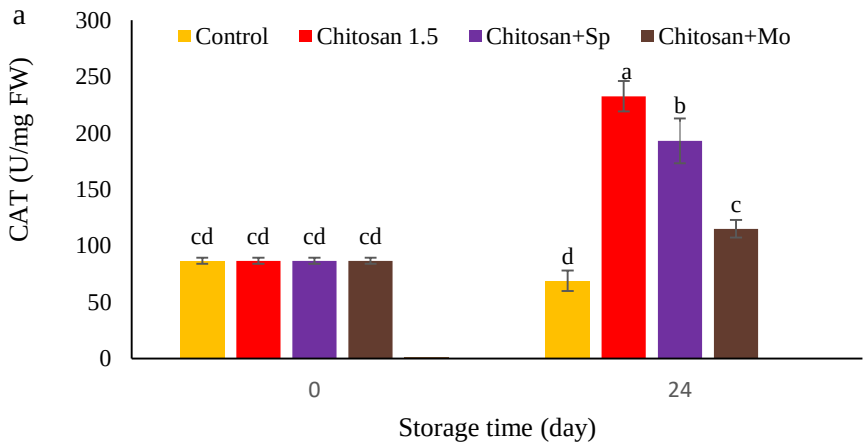
Fig. 4. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa leaf extract) on a) phenol, b) flavonoid, and c) radical scavenging activity of lime juice (*citrus aurantifolia*) stored at 20 ±2 ˚C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant ($P \leq 0.05$).

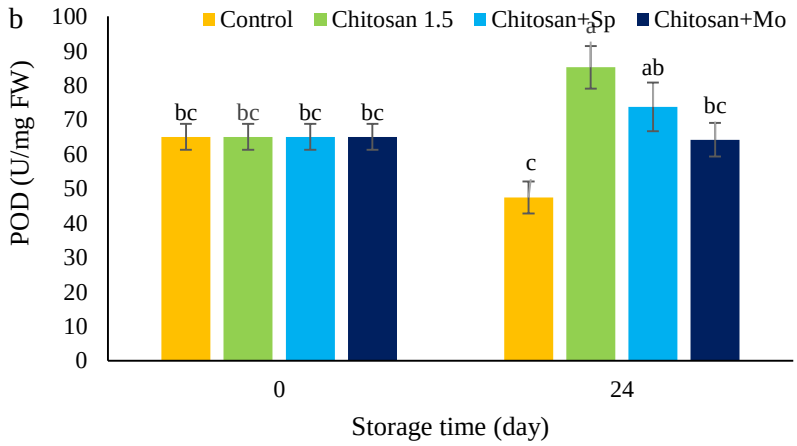
CAT, POD, and PPO

The interaction effect of time and treatment on CAT, POD, and PPO is shown in Figure 5. During storage, the activity of the CAT enzyme increased in the treated groups, while it remained relatively constant in the control group (Fig. 5a). The maximum enzyme activity was observed in the chitosan 1.5% treatment with a percentage increase of 168.6%.

The activity of the POD enzyme, unlike the treated samples that showed an increase during storage, decreased in the control samples. It changed from 65 U/mgFW to 47.5 U/mgFW value. Chitosan treatment exhibited the highest (85.2 U/mgFW) POD enzyme activity after 24 days of storage (Fig. 5b).

The activity of the PPO enzyme increased in most of the fruit, but the increase in enzyme activity was significantly advanced in the control group compared to the treated groups. After 24 days of storage, the enzyme activity in the control group was approximately 1.5 times higher than the enzyme activity in the chitosan 1.5%-treated group (Fig. 5c).





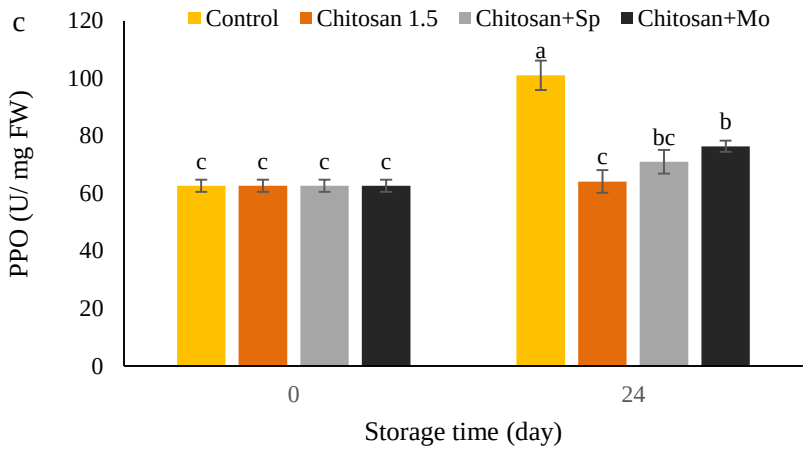


Fig. 5. Interaction effect of time and treatment (chitosan, chitosan 1.5, chitosan + spirulina algae, and chitosan + moringa extract) on a) CAT, b) POD, and c) PPO of lime juice (*Citrus aurantifolia*) stored at 20 ±2 ˚C. Vertical bars indicate the standard error (S.E.) of the means (n = 3). The means with the same LSD test letters in each column are not statistically significant (P ≤ 0.05).

Correlation and principal component analysis (PCA)

The heat map obtained from Hierarchical Cluster Analysis (HCA) showed that the different treatments and measured parameters were divided into two groups (Fig. 6). The group included: (I): DDPH, POD, TPC, TFC, TSS, pH, and CAT and (II): L^*, WL, b^*, a^*, and PPO. On the other hand, clustering into two groups that include: (IV): Chitosan 1.5-T2, Chitosan+Sp-T2, Control-T2 and Chitosan +Mo-T2 (V): Chitosan+Sp-T1, Control-T1, Chitosan 1.5-T1, and Chitosan +Mo-T1 (T1 and T2 represent the initial and subsequent time of storage, respectively) (Fig. 6a). The results showed that with the passage of time, chitosan + Sp and chitosan + Mo showed the highest positive correlation with L^*. In the observed control, it showed a positive correlation with L^* and it showed more darkness (darkness) of lime color. It showed a positive correlation of weight loss with the treatments of chitosan +SP over time (24 days). At the end of the experiment, the control showed a positive correlation with b^*, which can be said that the lime fruit in the control tended to be green to yellow in comparison to the treatments. Chitosan 1.5 showed a positive correlation with PPO at 24 days of storage time in fruit. The results showed a positive correlation in the treatment of chitosan 1.5 at the end of the experiment with the CAT enzyme, which indicates an increase in the activity of the CAT enzyme.

All 12 physiological and biochemical traits were loaded into two principal components (PC1 and PC2), which explained 1.87% of the total variances (Fig. 6a). While a lower proportion of variance (9.7%) was represented by PC2 (Fig. 6b). The most similarity in treatments was observed in CAT, DPPH, POD, PPO, b^* and L^* treatments. In the next group, TFC parameter, pH, TPC and TSS were placed in the same group in terms of similarity. Weight loss and a^* showed more similarity in another group.

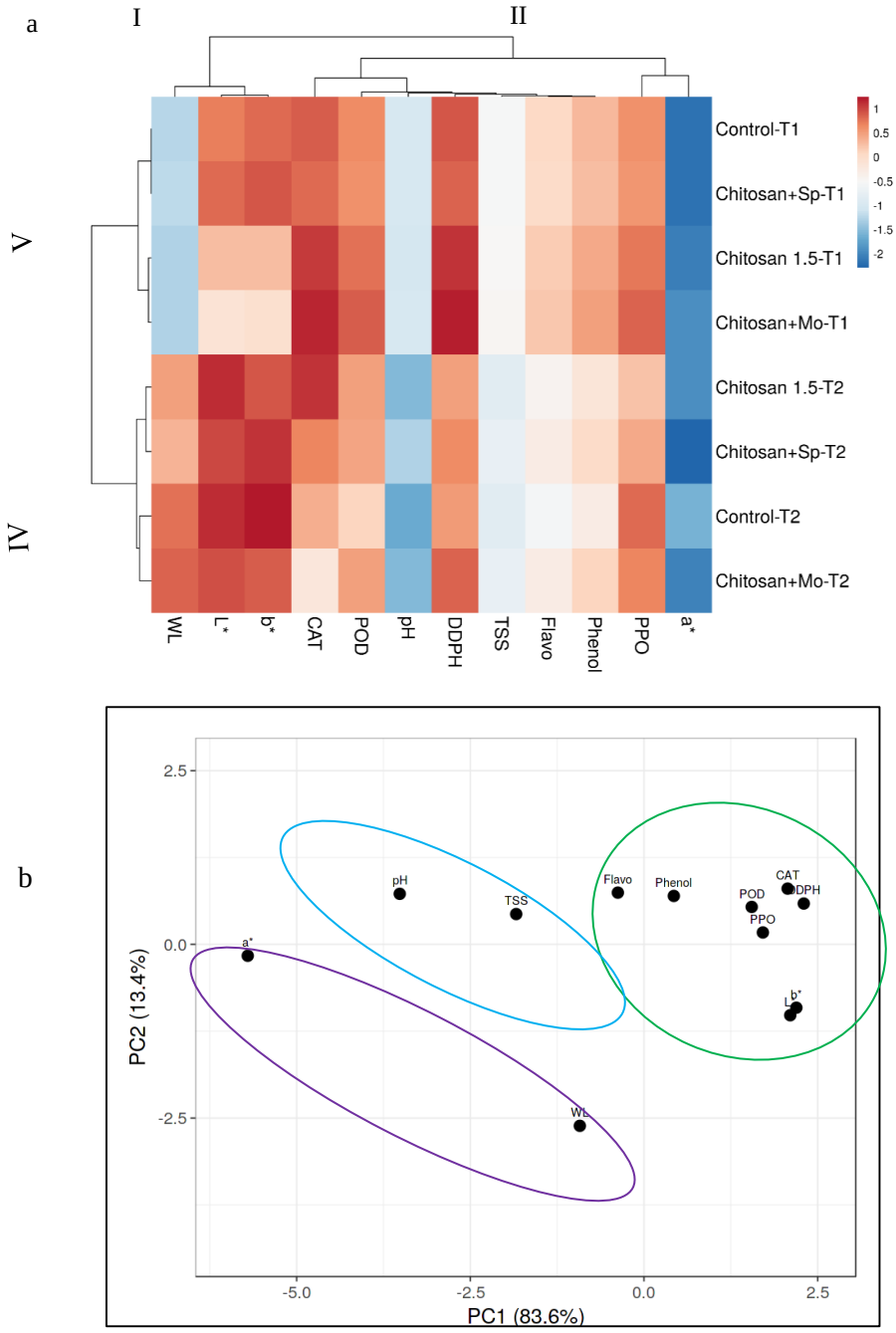


Fig. 6. a) Hierarchical cluster analysis (HCA) of cover treatments and variable trait relationships in lime fruit over time. Heat map of Pearson's correlation coefficient (r) values of variable traits, where the color scale that indicates r coefficient values (r=0.5 to 2) indicates positive (red) and negative (blue) correlations. (b) Dendrogram clustering of coating treatments in lime fruit and dose times 0 and 24 in all treatments except for fruit color. b) Principal component analysis (PCA) of variable trait relationships in lime fruit. PCA loading diagrams of the examined variable traits, and the circles show the highest correlation of the variables. PCA loading graphs of variable traits were examined. The tested variables include Flavo: Flavonoid, pH, Phenol, TSS: The Soluble Sugar, Color: L^*, a^*, b^*, WL: Weight Loss, PPO: Polyphenol Oxidase, POD: Peroxidase, CAT: Catalase, DDPH: Antioxidant activity.

DISCUSSION

The weight loss in fresh fruits and vegetables holds significant importance with respect to economic losses (Iftikhar et al., 2022). Weight loss during the storage of fresh fruits is a prevalent issue that significantly impacts their quality and shelf life. Weight loss in fruits is primarily attributed to moisture loss or dehydration, which can occur over time due to respiration, transpiration, and other physiological processes. During the storage of fruits, the loss of water content can lead to noticeable changes such as shrinkage, wilting, softening, and the development of a dull appearance (Kritzinger, 2019). Furthermore, weight loss can also lead to a decline in flavor and nutritional value, further compromising the overall quality of the fruits. Therefore, managing and minimizing weight loss is crucial to maintaining the freshness, appearance, and taste of fruits throughout their storage period (Artés et al., 2006). Edible coatings, which create a semi-permeable layer around fruit, can act as a barrier to water vapor, reducing transpiration and slowing down moisture loss from the fruit. This can help maintain the fruit's turgidity and prevent wilting or shrinkage, ultimately reducing weight loss (Sapper & Chiralt, 2018). In addition, edible coatings modulate the exchange of gases (such as oxygen and carbon dioxide) between the fruit and its external environment. This can help to slow down the respiration rate of the fruit, which in turn reduces weight loss associated with metabolic processes. Chitosan coating has shown a significant effect on weight loss of different fruits during storage, such as pear (Iftikhar et al., 2022) and apple (Zeb et al., 2020). The results exhibited that chitosan coating could decrease weight loss by up to 65% compared to the uncoated control (Parvin et al., 2023). In addition, the coated samples showed a lower weight loss than the control samples, with the chitosan + Sp coating showing a minimum weight loss of 16.4%. It has been reported that *Spirulina platensis* is rich in carbohydrates, mineral salts, high protein content, vitamins, antioxidants, and unsaturated fatty acids, which allow the formation of mechanical and interactive structural chains on the surface of the coated fruit, regulating transpiration and fruit mass loss (Santos et al., 2023). The incorporation of spirulina into films and coatings can improve their tensile strength and mechanical properties due to solid intermolecular interactions with the polymer matrix (Nakamoto et al., 2023). The inclusion of moringa in an edible carboxymethyl cellulose coating has also been documented to have a positive impact on the preservation of guava fruit. This was achieved by mitigating the loss of fruit moisture and reducing the rate of fruit respiration, thus enhancing the overall quality of the fruit during storage (Tesfay & Magwaza, 2017).

The TSS of fruits is known to influence their sweetness, which is an important factor in determining fruit quality and is extremely correlated with maturity in most fruits. In the current study the TSS values showed an increase in both coated and uncoated fruits, with uncoated fruits exhibited higher values (Thakur et al., 2019). The TSS content of citrus fruits tends to increase during storage periods due to various breakdown processes (Sun et al., 2019). However, the application of direct coatings has been observed to inhibit the synthesis and utilization of substances within the fruits, leading to decreased respiration rates and a subsequent decline in TSS concentration (Kou et al., 2014). By creating a modified atmosphere around the fruit, edible coatings can influence the physiological and biochemical processes that affect TSS. There have been varying reports on the effect of edible coatings on the TSS of different fruits. It has been reported that a polysaccharide-based edible coating did not significantly affect the TSS levels of juice in various citrus fruits, including mandarins, oranges, and grapefruit (Arnon et al., 2014). After 24 days of storage, the fruit treated with chitosan + Sp showed significantly lower TSS compared to the control group. Chitosan-based coatings have the potential to control fruit ripening, particularly when combined with

Spirulina. Bioactive substances present in spirulina, including vitamins, minerals, and phytohormones, may have an impact on the processes involved in fruit ripening (Hadiyanto et al., 2019). The fruit's shelf life might be prolonged and overripening could be delayed by the chitosan + Sp treatment by reducing ethylene production, the respiration rate of fruits and other ripening-related activities (Rastegar & Atrash, 2021) .

Citrus fruits are known to undergo color changes during storage, and the peel color is one of the parameters that can be affected. The citrus fruit color changes because of the breakdown of chlorophyll and the accumulation of carotenoids pigments in the outer layer of the fruit (Keawmanee et al., 2022). Some of the studies found that chitosan coating delayed color changes in fruit skin and pulp during storage. Coatings can help to extend the shelf life and color preservation, improve the visual quality, prevent shriveling and wilting, and maintain biochemical properties of fruits (Firdous et al., 2022). Similar to current study Krishna and Rao (2014) reported a lower b^* value in guava fruits coated with chitosan (1 and 2%) in comparison to fruits treated with acetic acid and those that were left untreated. Coatings can help prevent chlorophyll breakdown by lowering O_2 and raising CO_2 in the storage atmosphere. This has been shown to be beneficial in previous studies (Olawuyi et al., 2019). Contrary to the results of the present research, a study on mangoes found that applying a composite of 10% Arabic gum and 1% chitosan as an edible coating did not significantly affect the color of the fruit skin or pulp (Handojo et al., 2022). A study on blueberries found that chitosan coating plus silicon dioxide nanoparticles and nisin helped to control the color parameters of the fruit during storage (Eldib et al., 2020). Overall, the effect of chitosan coating on the color parameters of fruit during storage seems to depend on the specific fruit and the type of coating used. However, in general, chitosan coating appears to delay color changes and help maintain the quality of fruit during storage. The combined treatment of chitosan with Spirulina improved fruit color during storage in this study. Fruit color generally improves during storage by combining chitosan and spirulina treatment. This is achieved by lowering oxidative stress, stabilizing pigments, preventing microbial development, preserving the water status, and postponing senescence processes. Together, these systems preserve the aesthetic appeal and freshness of the fruit throughout the storage period (Santos et al., 2023). One of the effects of chitosan edible coating on fruits and vegetables is the modulation of their phenolic and flavonoid compounds. Phenolics and flavonoids are secondary metabolites that have antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. They also contribute to the color, flavor, and nutritional value of fruits and vegetables (Esmaeili, 2024). Chitosan edible coating can reduce the oxygen permeability and water loss of fruits and vegetables, thus preventing oxidative stress and enzymatic browning that degrade phenolics and flavonoids (Sarengaowa et al., 2022). Edible coatings can be designed to have selective permeability, allowing certain gases, such as oxygen and carbon dioxide, to pass through while limiting the entry of other gases. This controlled gas exchange can help regulate the enzymatic activity involved in phenol metabolism, thus impacting the phenolic content of the fruit (García-Betanzos et al., 2017). In addition, Chitosan edible coating can induce the expression of genes related to flavonoid 3-hydroxylase (F_3H), phenylalanine ammonia-lyase (PAL), which increase the synthesis of phenolics and flavonoids in fruits and vegetables (Adiletta et al., 2021).

It has been reported that phenolic compound and the antioxidant activity in stored figs were significantly improved by the chitosan-based coating (Adiletta et al., 2019). Chitosan-based coating with rosemary essential oil increased the total phenolic content, antioxidant activity, color retention, and sensory quality of strawberries during storage (Quintana et al., 2021).

Both phenols and flavonoids contribute to the antioxidant capacity of fruits and vegetables, and their consumption has been linked to various health benefits. Phenolic compounds found in fruits and vegetables serve as crucial antioxidants that play a vital role in eliminating free radicals and safeguarding cells from damage (Toor & Savage, 2005). During the ripening process, flavonoids can undergo conversion into secondary phenolic compounds. Additionally, certain enzymes may act on these compounds as substrates specifically during the ripening stage. In both situations, there is a tendency for the concentration of these compounds within the fruit to decline (Howard et al., 2003). It has been suggested that the edible coating plays a role in inhibiting oxygen penetration, which leads to a significant delay in the breakdown of flavonoids (Ruzaina et al., 2017). The edible coating may cause the coated fruit to have a higher level of phenolic compounds. This is because the edible coating lowers the activity of polyphenol oxidase, which leads to less breakdown of phenolic compounds (Kerch, 2015). The observed reduction in flavonoid content in control samples could plausibly be attributed to their higher respiration rate, thereby leading to the breakdown of total phenolics. The fruits treated in this study showed higher phenol, flavonoid, and antioxidant content than the control. It has been reported that leaf extracts of moringa plants possess antimicrobial and antioxidant activities due to a high concentration of phenolics, vitamins, and carotenoids (Saucedo-Pompa et al., 2018). In addition, it has been reported that spirulina has antioxidant properties, which can help in the development of antioxidant coatings for various applications (Nakamoto et al., 2023). Moringa and spirulina, which are rich in antioxidants, flavonoids, and phenols, increase the protective ability of coatings by supplying more antioxidants that scavenge free radicals and stop the oxidation of phenolic components (Saucedo-Pompa et al., 2018; Budak & Sarıkaya, 2022).

The results of this research showed that during storage, the maximum activity of CAT and POD enzymes was observed in 1.5% chitosan treatment. Postharvest oxidative stress is a condition that can happen during the storage of fruit, which means that there is an imbalance between the production and elimination of reactive oxygen species (ROS) within the fruit tissues. ROS, such as H_2O_2, O^{2-}, and hydroxyl radicals, are generated as natural byproducts of cellular metabolism and can cause damage to cellular components if their levels are not properly regulated. The degree of protection against oxidative injury in fruit cells is closely related to the activity levels of antioxidant enzymes, particularly CAT and POD (Meitha et al., 2020). Numerous studies have provided evidence that the application of chitosan coating on fruit helps maintain the balance of intracellular oxidation metabolism. This is achieved through the efficient clearance of cytotoxic compounds via enzymatic antioxidants. Additionally, non-enzymatic antioxidants including glutathione (GSH), phenols, ascorbic acid (AA), anthocyanins, and flavonoids also contribute to this balance. By employing these antioxidants, chitosan-coated fruit effectively mitigates oxidative injury produced by reactive oxygen species, confirming the preservation of fruit quality, and extending its shelf life (Adiletta et al., 2021). Research has indicated that the application of chitosan coating on fig fruit leads to an increase in the activity of important antioxidant enzymes, including SOD, POD, and glutathione reductase (GR). By enhancing the activity of these antioxidant enzymes, chitosan coating contributes to the preservation of fig fruit quality during storage (Adiletta et al., 2019). The utilization of chitosan enriched with arginine nanoparticles in plum fruit has shown beneficial outcomes in maintaining fruit quality. Specifically, the application of this formulation has been observed to result in a decrease in the activity of the polyphenol oxidase (PPO) enzyme within the fruit, contributing to the preservation of its quality (Mahmoudi et al., 2022). It has been reported that the application of chitosan coating in pear fruit has been found to effectively restrict the activity of PPO enzyme. This restriction of PPO

activity helps prevent browning and consequently extends the storage life of the pear fruit (Adhikary et al., 2022).

In general, the coating of chitosan + Sp showed the best appearance (color and freshness) of the fruit. It has been reported that spirulina contains chlorogenic acid, which can bind and permeabilize the cell membrane of microorganisms, leading to the loss of membrane potential and inhibition of bacterial growth (Nakamoto et al., 2023). Spirulina is recognized for its abundance of bioactive compounds including proteins, vitamins, minerals, and antioxidants. These compounds play a significant role in the potential benefits of spirulina for the postharvest quality of fruits. By incorporating spirulina, several positive effects were observed, including increase of soluble solids, enhanced flesh firmness, increased ascorbic acid content, and a reduction in weight loss (Nakamoto et al., 2023).

CONCLUSION

In conclusion, the findings of this research demonstrate that the application of chitosan-based coatings, particularly when combined with spirulina algae, has demonstrated to be an effective method for preserving the storage quality and extending the shelf life of Mexican lime fruit stored at 20 ± 2 °C (Fig. 7). The coated fruit exhibited less weight loss (16.4%), improved color stability, maintained phenol and flavonoid content, and higher antioxidant activity compared to the control group. Furthermore, the treatments led to a decrease in PPO enzyme activity (36.6% rather than control) and an increase in the activity of POD (1.8-fold) and CAT (3-fold) enzymes, suggesting a positive impact on fruit freshness and quality. These results highlight the potential of chitosan coatings, in combination with natural extracts such as spirulina algae, as a viable solution to address the challenge of limited shelf life in Mexican lime fruits. Studying the impact of different combinations of chitosan-based coatings and natural extracts on the shelf life and health benefits of Mexican lime fruit to enhance consumer experience should be considered. Additionally, further research and optimization of coating formulations and application techniques may provide even more significant benefits in terms of fruit preservation and waste reduction in the future.

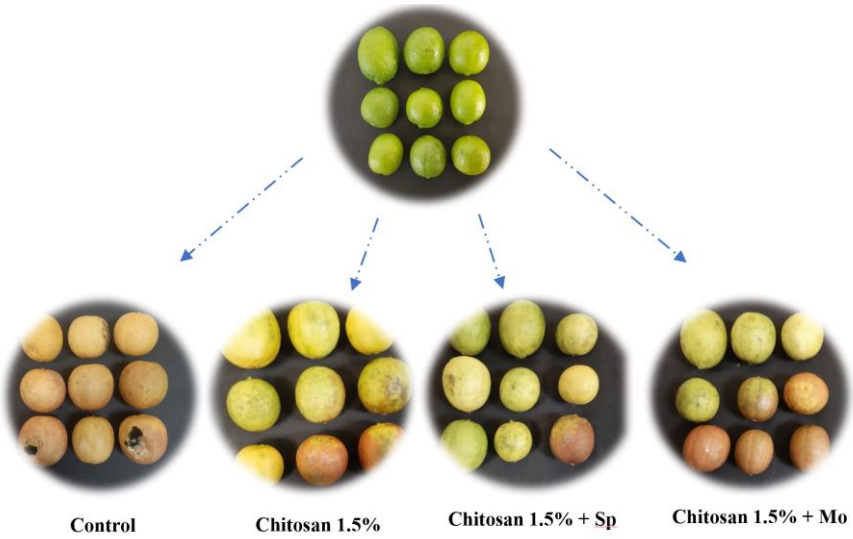


Fig .7 The effect of different treatments on maintaining the quality of Mexican lime fruit during 24 days of storage at 20 ± 2 ˚C.

CRediT authorship contribution statement

Soheila Aghaei Dargiri: Writing – original draft, Formal analysis, Data curation. Somayeh Rastegar: Review & editing, Validation, Methodology, Investigation, Project administration, Supervision. Mahbobeh Mohammadi: Performed experiment, Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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