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The Effect of Persian Gum Coating Enriched with Pomegranate Seed Oil on the Quality of Mexican Lime (*Citrus aurantifolia*)

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Abstract

The use of edible coatings has been considered as an effective solution to improve the shelf life and quality of fruits. In this research, increase in the shelf life of citrus fruits (*Citrus aurantifolia* cv. Mexican lime) coated with Persian gum and pomegranate seed oil was investigated. Different treatments of lemon fruit coated with Persian gum and pomegranate seed oil with concentrations (zero (control), 0.5% and 1% gum, combination of 0.5% and 1% gum and pomegranate seed oil, 0.05% and pomegranate seed oil 0.05 percent) were prepared and after 24 days of storage at ambient temperature (20 ± 2 °C and relative humidity of 50-60 percent) were statistically evaluated in the form of a completely random design with three replications. The results of this research showed that the treatments used had an effective role in controlling the weight loss of fruit during storage. Thus, the lowest percentage of weight loss was observed in the pomegranate seed oil treatment. Except pomegranate seed oil treatment, other treatments showed less TSS than the control. In most of the treatments, the content of phenol, flavonoid and antioxidant was at a higher level than the control. The average comparison results showed that the fruits coated with 1% gum (85.36 units/ml) showed significantly more peroxidase activity than the control (60.35 U/ml). Persian gum edible coating 1% and 0.5% as well as Persian gum 1% in combination with pomegranate seed oil significantly controlled the activity of polyphenol oxidase enzyme. The treated samples showed less yellowness (b*) than the control. In general, the best marketability was observed in fruits coated with 1% gum. Therefore, it is recommended to use this coating to preserve the freshness and quality of the Mexican lime fruit during storage in the environment.

Keywords: Antioxidant, Edible coatings, Mexican lime, Postharvest

Introduction

Lime (*Citrus aurantifolia* cv. Mexican lime) is an acidic citrus fruit of great economic importance, grown in tropical and subtropical regions, accounting for 5% of worldwide citrus production (Dunkersley *et al.*, 2018). According to data from the World Food and Agriculture Organization, the cultivation area of citrus fruits is 9.3 million hectares and yields an annual production of 132 million tons. Iran is ranked ninth in the world for lime production, while global lime production is estimated at approximately 21 million tons per year (FAO, 2019). The color of the fruit is a crucial determinant of the quality and marketability of citrus fruits (Ma *et al.*, 2023). Given the lime's economic importance, it's crucial to preserve its quality using environmentally-friendly techniques that benefit both people and the

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planet, especially during long-term storage (Champa *et al*., 2020).

Using edible coatings is a vital approach to reducing food waste and maintaining the quality of agricultural products (Ezati *et al*., 2022; Otoni *et al*., 2017; Galus *et al*., 2020; Hoffmann *et al*., 2019). Immersing fruits in an edible coating form a thin layer on the fruit, which can enhance tissue quality, preserve color and volatile compounds, and inhibit microbial growth by reducing respiration and transpiration (Mahfoudhi *et al*., 2014). Natural coatings with antimicrobial agents, antioxidants, nutrients, flavorings, enzymes, and colors have been explored in various foods (Mohammadi *et al*., 2016; Motallebi *et al*., 2017). To produce edible coatings, natural gums are a highly promising alternative due to their biocompatibility, cost-effectiveness, non-toxicity, and wide availability (Salehi, 2020; Yun *et al*., 2022). A study by Shahbazi and Shavisi (2020) found that gum-based coatings and films significantly decreased microbial proliferation in bananas during storage. Guar gum coating enhanced the preservation quality of green mangoes and delayed their ripening (Naeem *et al*., 2018). However, limited research has been conducted on Mexican lime during storage, despite its increasing socioeconomic significance over time. Thus, this research aims to assess the viability of using an edible coating derived from Farsi gum, either alone or in combination with pomegranate seed oil, to enhance the storage quality of lime.

Materials and Methods

Lime (*Citrus aurantifolia* cv. Mexican lime) fruit in the mature green stage (when the fruit is juicier) was harvested from a commercial orchard in Hormozgan province, Rodan City, River section at geographical coordinates (57°29°E and 27°59°N) according to technical criteria. Only fruits with uniform size, absence of defects and mechanical damage were chosen for the experiment. The fruits were washed and then treated with a one-minute wash of 0.05% sodium hypochlorite solution to disinfect them.

Preparation of Coatings

Preparation of coating using Persian gum (acquired from Rihan Gam Persian Company) involved gradually dissolving the gum in water and stirring for 30 minutes at room temperature. The resulting solution was stored in a refrigerator for 24 hours. Two concentrations of Persian gum coating, 0.5% and 1%, were prepared. To create different coating treatments, glycerol (0.5% w/v), pomegranate seed oil (0.05% w/v), and Tween 80 were sequentially added to the prepared gum solution at room temperature. The treatments included a control, gum 0.5%, gum 1%, gum 0.5% and 1% with pomegranate seed oil 0.05%, and pomegranate seed oil 0.05%. Lime fruits were coated by immersion in coating solution at room temperature for 5 minutes, and after the surface coating dried, they were stored in disposable plastic containers for 24 days at a temperature of 20±2 °C and a relative humidity of 50-60% (Khaledian *et al*., 2021). Finally, the weight loss factor, soluble solids, phenol content, antioxidant, total flavonoid, peroxidase and polyphenol oxidase activity, peel color (a*, b* and L*), and marketability of lime fruits were evaluated.

Weight Loss

The fruits were weighed using a digital scale with an accuracy of 0.1 grams, and the percentage of weight loss was calculated using following formula (Dong and Wang, 2018):

$$WL\ (\%) = (w_1 - w_2) \times 100 / w_1 \quad (1)$$

(WL: Weight loss percentage, W_1: Primary weight in grams, W_2: Secondary weight in grams).

Total Soluble Solids

The Brix degree of the fruit juice was measured using a digital refractometer (DBR95, Taiwan) (Barry *et al*., 2004).

Phenol Content

To measure the phenolic content of the fruit juice, 0.5 mL of lime juice was mixed with 3 milliliters of 85% methanol and kept in the refrigerator for 24 hours. Then, 150 µL of the methanolic extract and 750 µL of Folin's reagent (10%) were added to the mixture and left for 5 minutes. Next, 600 µL of 7.5% sodium carbonate solution was added to the mixture, and the sample was placed on a shaker in the dark for 2 hours and then the absorbance was read using a spectrophotometer at 750 nm (Ordonez *et al*., 2006).

Antioxidants activity

The antioxidant activity of the fruit juice was measured using the 2,2-dipheny-1-picrylhydrazyl (DPPH) free radical inhibition method. To do so, 30 µL of 85% methanol extract from the fruit and 1170 µL of DPPH (150 µmol) were combined and shaken for 1 minute. After keeping the samples in the dark for 40 minutes, the absorbance was read with a spectrophotometer at 515 nm and the antioxidant activity was calculated using the following formula (Bourtoom, 2008).

$$DPPH\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100 \quad (2)$$

Total Flavonoids

To measure flavonoids, 200 µL of methanolic extract was mixed with 600 µL of 85% methanol, 40 µL of 10% aluminum chloride, 40 µL of potassium acetate, and 1120 µL of distilled water and incubated at room temperature for 30 minutes. The absorbance was then measured using a spectrophotometer at 415 nm (Chang *et al*., 2002).

Peroxidase and Polyphenol Oxidase Activity

To prepare the extract, 500 µL of lime juice was mixed with a potassium buffer (pH: 7.4) containing 1 M EDTA and 1% PVP at 4 °C. The resulting mixture was centrifuged at 13,000 rpm at 4° C for 20 minutes, and the supernatant was used to investigate the activities of peroxidase and polyphenol oxidase.

The peroxidase enzyme activity of the fruit juice was measured using the method of Maehly and Chance (1954) with some modifications. In this method, 30 µL of the extracted sample was mixed with 1 mL of peroxidase solution containing 10 µmol of guaiacol, 5 µmol of hydrogen peroxide, and 50 µmol of potassium phosphate buffer, and the absorbance was read at 470 nm for 1 minute. The extinction coefficient used to calculate the enzyme unit was equivalent to 26.6 $mM^{-1}Cm^{-1}$, and the peroxidase enzyme activity was expressed in U/ mL FW.

For the measurement of polyphenol oxidase activity, pyrogallol was used as an enzyme precursor. The reaction mixture included 2.5 ml of potassium buffer (50 mM and pH 7), 200 µl of 0.02 M pyrogallol, and 100 µl of enzyme extract. The absorbance of the samples was measured at 420 nm after 3 minutes. The extinction coefficient used to calculate the enzyme unit was 6.2 $mM^{-1}Cm^{-1}$, and the polyphenol oxidase enzyme activity was expressed in U/ mL FW.

Peel color and Marketability

The peel color of the fruit was measured using a colorimeter (Minolta CR400, Japan) based on the color characteristics L*, a*, and b* (Zhou *et al.,* 2010). The evaluation of the marketability of fruits was done according to the appearance characteristics of the fruits and the scoring method. The grading criteria included aroma, color uniformity, dryness, rotting, and the appearance of the fruit, and scores from 1 to 5 were assigned to them. 1- poor, 2- average, 3- good, 4- very good, 5- excellent. Asghari and Aghdam, 2010).

Statistical Analysis

The experiment was conducted using a completely randomized factorial design with three repetitions, and each repetition included 9 fruits. The data were analyzed using SAS software version 9.4, and the means were compared using the LSD test at a significance level of 5%.

Results and Discussion

Fruit Weight Loss

The results of the analysis of variance showed that the treatments had a significant effect on preventing weight loss of lime fruit during storage (24 days) at a probability level of 1%. The pomegranate seed oil treatment (12.5%) had the greatest effect (Fig. 1). The reduction in fruit mass during storage can be attributed to the loss of water content resulting from alterations in surface transfer resistance towards water vapor, alongside variations in respiratory activity and the emergence of minute apertures that facilitate the exchange of atmospheric gases between the interior and exterior environments (Shahid and Abbasi, 2011). To prevent post-harvest weight loss, regulating the factors that trigger metabolic activity in fruit tissue is imperative. Coatings can be an efficient barrier against carbon dioxide, oxygen, and moisture, curtail respiration, and minimize water loss and oxidation reactions (Dong and Wang, 2018). Studies have shown that the use of gum Arabic coatings on guava (Murmu and Mishra, 2017) and banana (Maqbool *et al*., 2011) fruits at an ambient temperature effectively maintained fruit weight, which is consistent with our results.

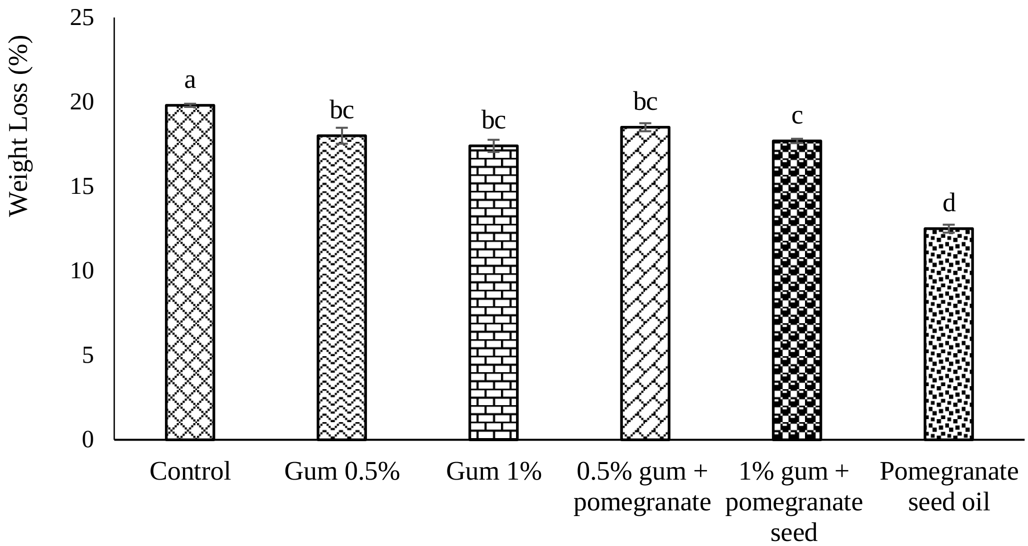


Fig. 1- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on the weight loss of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Total Soluble Solids (TSS)

The analysis of variance showed that the treatments had a significant effect on the dissolved solids at a 1% probability level. The gum treatment of 0.5% (6.5%) had the lowest soluble solids compared to the control (7.9%) (Fig. 2). The quantity of dissolved substances in fruit is a key marker of fruit maturation (Kazemi *et al*., 2011). The increase in soluble substances during storage is due to hydrolysis of starch to sugar (Wang *et al*., 2013). Storage leads to increased concentration of dry matter and destruction of the cell wall, resulting in a rise in soluble solids and a decrease in fruit juice content (Khorram *et al*., 2017; Dhall, 2013). Edible coatings can prevent the escalation of soluble solids in fruit by reducing respiration, altering the internal fruit atmosphere, and increasing carbon dioxide while decreasing oxygen and ethylene (Dong and Wang, 2018). During storage, organic acids degrade more rapidly than sugars, resulting in slight sweetness in the fruit (Razzaq *et al*., 2014). Blood oranges (Habibi and Ramezanian, 2017), oranges (Rasouli *et al*., 2019), and limes (Atrash *et al*., 2018) have shown an increase in the soluble solids.

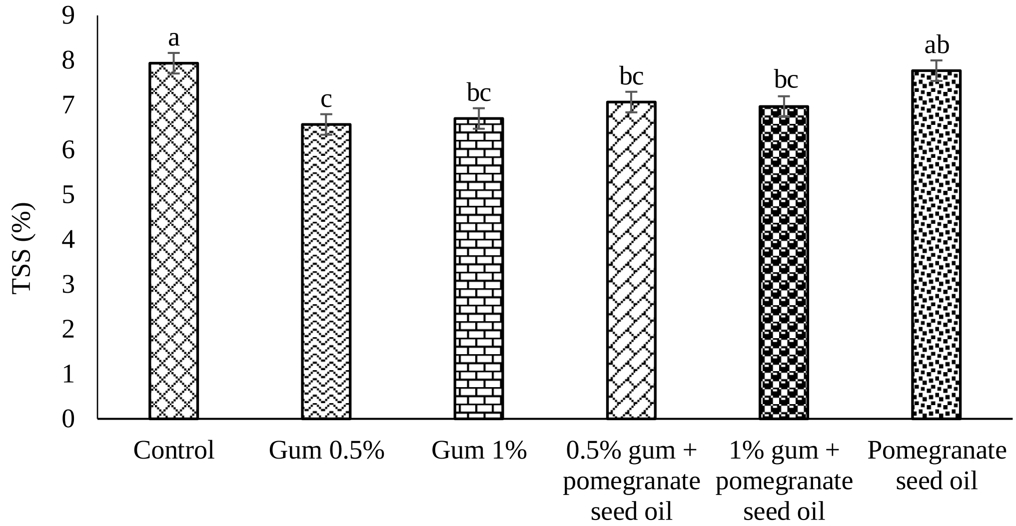


Fig. 2- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on soluble solids of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

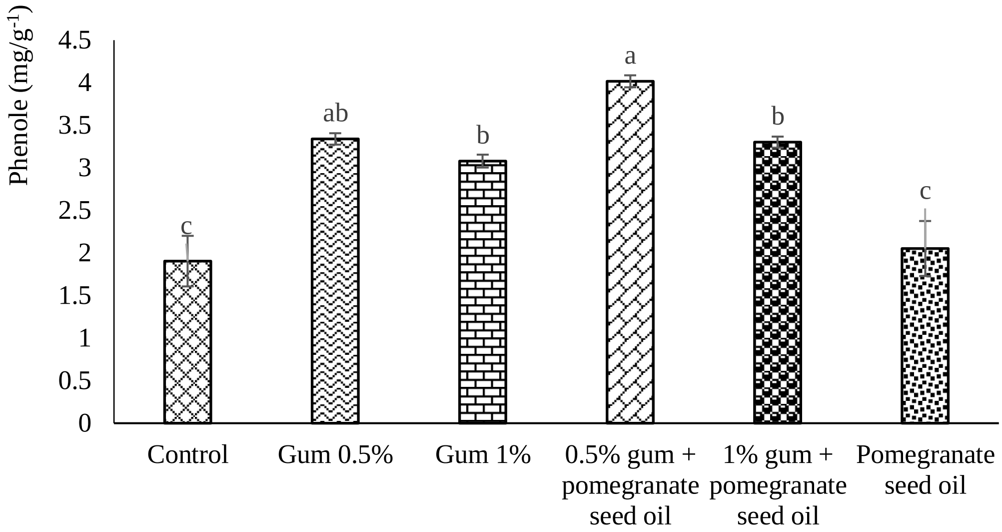
Phenol

The variance analysis of data showed that all treatments had a significant effect on lime phenol at a 1% probability level. The 0.5% gum treatment with pomegranate seed oil (4.01 mg/g) had significantly higher phenol content than the control (1.9 mg/g) (Fig. 3a). Previous research on tomato (Uckoo *et al*., 2015) and papaya (Addai *et al*., 2013) showed that the use of gum Arabic retained phenolic compounds in the plants, which is consistent with our results. Similarly, previous research on blood orange (Habibi and Ramezanian, 2017), orange (Rasouli *et al*., 2019; Khorram *et al*., 2017), and guava (Nair *et al*., 2018) were also observed the effects of using food coatings in maintaining phenol content.

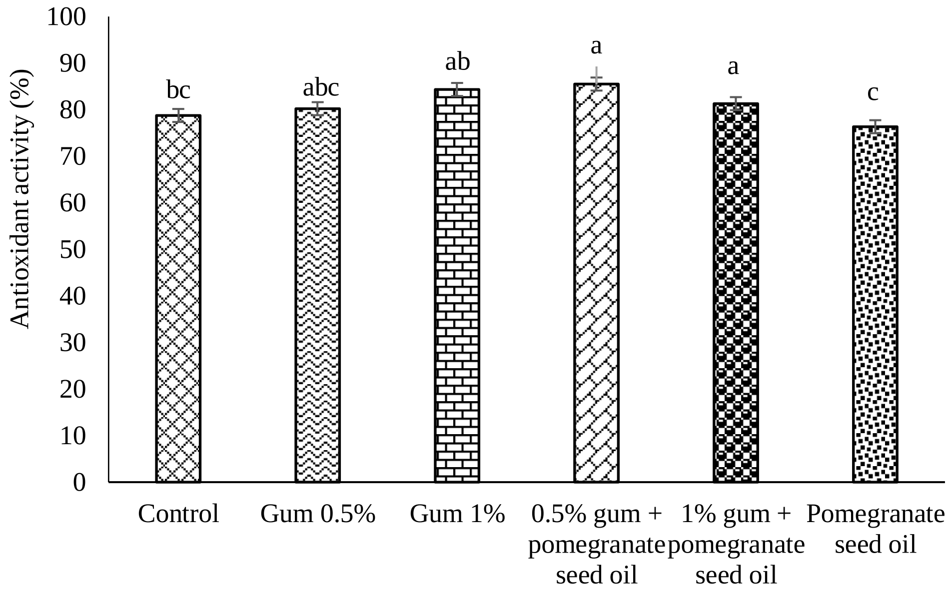
Antioxidants activity

The variance analysis of the data showed that the interaction effect of the treatments on the lime antioxidant activities was significant at a 1% probability level. The 0.5% and 1% lime gum treatments with 8.57% and 3.22% pomegranate seed oil had a higher content of antioxidants compared to the control (Fig. 3b). The increased antioxidant activity in these fruits can be attributed to their higher total phenol content, as there is a positive correlation between the quantity of phenol and antioxidant activity (Khaliq *et al*., 2016). Habibi and Ramezanian (2017) showed that antioxidant activity and phenol levels shift in parallel over the course of storage, as the generation of free radicals in fruits due to aging and stress prompts the deployment of antioxidants by fruit cells to counteract their deleterious effects. Thus, interventions that reduce respiration, alleviate stress, mitigate aging, and preserve the cellular concentration of antioxidants may be effective (Asghari and Aghdam, 2010). Nair *et al*. (2018) and Shah *et al*. (2015) demonstrated that the implementation of alginate coating on guava and tangerine, preserved antioxidant activity, which is consistent with the outcomes presented in this research.

a


Phenole (mg/g-1)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
c
ab
b
a
b
c
Control
Gum 0.5%
Gum 1%
0.5% gum + pomegranate seed oil
1% gum + pomegranate seed oil
Pomegranate seed oil

b


Antioxidant activity (%)
100
90
80
70
60
50
40
30
20
10
0
bc
abc
ab
a
a
c
Control
Gum 0.5%
Gum 1%
0.5% gum + pomegranate seed oil
1% gum + pomegranate seed oil
Pomegranate seed oil

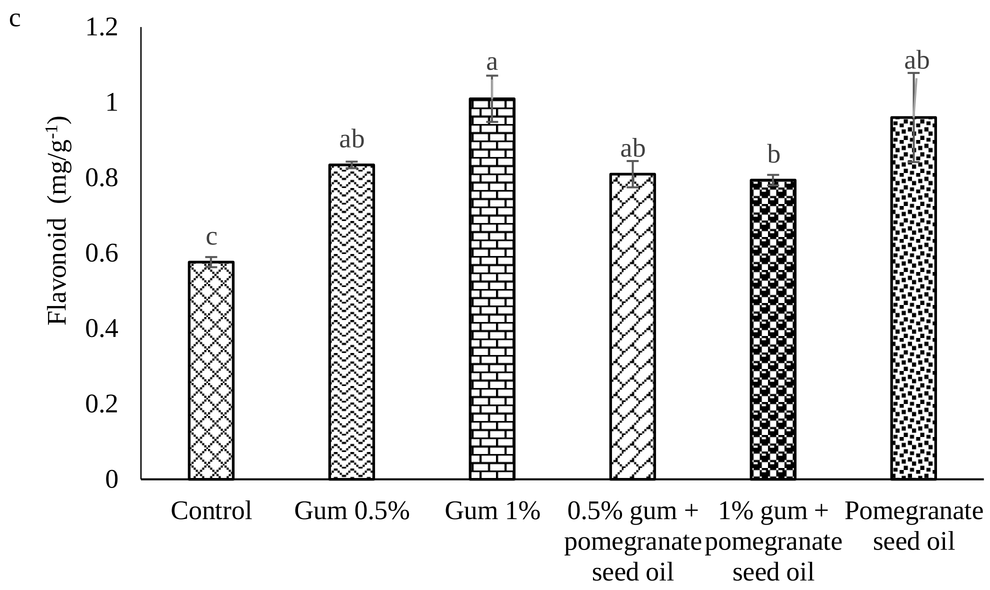


Fig. 3- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) phenol, b) antioxidant and c) flavonoid of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Flavonoid

The analysis of variance showed that the effect of treatments on the amount of lime flavonoids was significant at a 1% probability level. The 1% gum treatment had a higher flavonoid content in lime fruit (1 mg/g) compared to the control (0.57) (Fig. 3c). Flavonoids and other phenolic compounds play a crucial role in the non-enzymatic antioxidant network, protecting against the harmful effects of free radicals and reducing oxidative stress (Shamloo *et al*., 2015). Lime contains a considerable of flavonoids, including naringenin, diosmin, and hesperidin, primarily present in its juice. Flavonoids, which are polyphenolic compounds, are released as part of the plant's defense mechanism, and hesperidin plays a significant role in this process (Uckoo *et al*., 2015).

Measurement of Peroxidase and Polyphenol Oxidase Activity

The analysis of variance showed that the activity of peroxidase, a polyphenol oxidizing enzyme in lime, was significant at a 5% probability level. The highest peroxidase activity was observed in the 1% gum treatment (85.3 units/ml), and the lowest was observed in the control treatment (60.3 units/ml) (Fig. 4a). No significant difference was observed between the other treatments. The lowest polyphenol oxidizing enzyme activity was observed in the 1% gum treatment (61.5 units/ml), while the highest was observed in the control treatment (101 units/ml) (Fig. 4b). Phenolic compounds are synthesized at an increased rate during the ripening process, but their levels decreased as the fruit approaches senescence. The contribution of phenolic compounds to fruit quality and phytochemical levels is significant (Shamloo *et al*., 2015), and their overall variation is influenced by genetics, temperature, and environmental conditions during post-harvest (Rasouli *et al*., 2019). Phenolic compounds in fruits directly correlate with their antioxidant capacity, and a decrease in phenolic content results in a decrease in antioxidant activity (Shiri *et al*., 2011). Edible coatings alter the fruit's atmosphere, increasing the concentration of carbon dioxide and reducing respiration and the rate of phenol oxidation reactions and polyphenol oxidase enzyme activity (Asghari and Aghdam, 2010). Polyphenol oxidase and peroxidase are crucial enzymes in plant defense mechanisms that contribute significantly to their ability to resist pathogenic attacks (Babu *et al.*, 2015). These defense mechanisms play a critical role in preserving fruit quality after harvesting (Zheng *et al.*, 2011). Peroxidase, the primary antioxidant enzyme in plants, impedes the accumulation of free radicals and works synergistically to inhibit their formation, promoting overall plant health (Kou *et al.*, 2014).

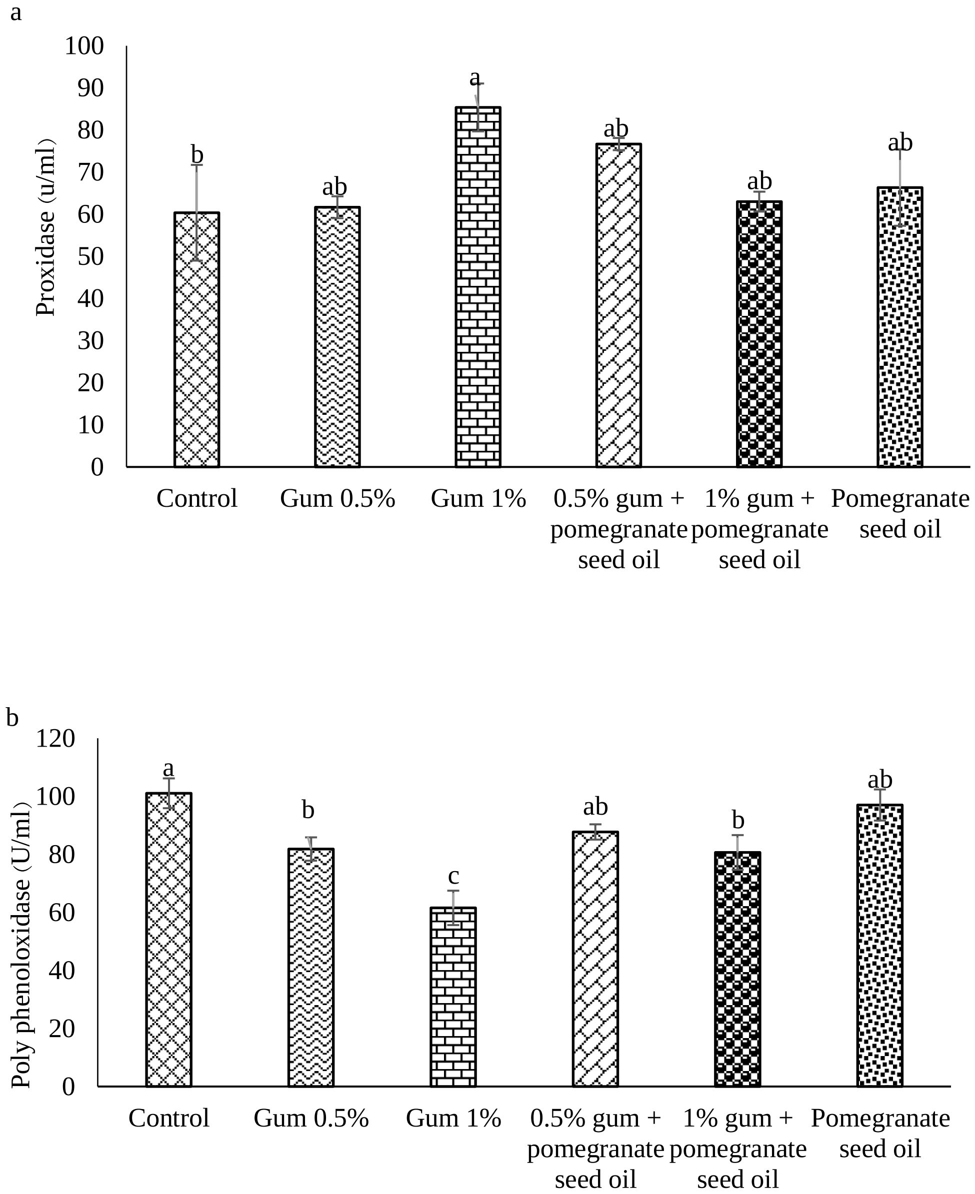
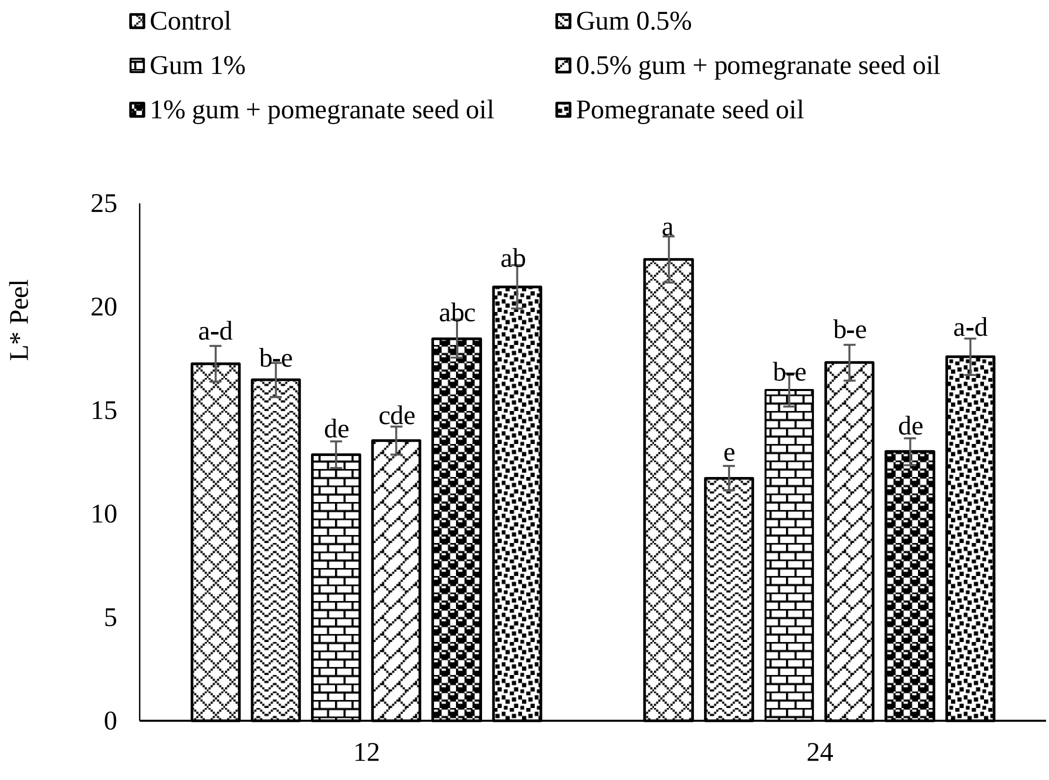


Fig. 4- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) peroxidase, and b) polyphenol oxidase of lime fruit stored at ambient temperature
The same letters have no significant difference with each other at the probability level of $p \leq 0.05$.

Peel Color Index

The analysis of variance showed that the lime peel color index was significant at a 1% probability level. Immersion of lime fruit in the treatments decreased the L* index of lime peel color during the storage period (24 days). The combined treatment of 1% gum and pomegranate seed oil decreased the L* index of lime fruit compared to the control (Fig. 5a). The a* index increased with the storage period, indicating that the fruit became yellower (more positive a* value) as it ripened. After 24 days of storage, the highest amount of a* index was positive in all treatments except for pomegranate seed oil (Fig. 5b). The treatment of 0.5% gum and the combination of 1% gum with pomegranate seed oil decreased the b* index compared to the control (Fig. 5c). The b* index (yellow color) of the fruit peel increased during the storage time due to the decrease in chlorophyll and the increase in carotenoid as the fruit ripened. This is consistent with the results of a study that used guar gum and chickpea starch coating to increase the shelf life of Valencia oranges (Saberi *et al*., 2018). The peel color of lime fruit is an important quality parameter that affects consumer choice, and the L*, a*, and b* indexes are used to evaluate fruit color (Nair *et al*., 2018). The polyphenol oxidase enzyme affects the fruit color by increasing the amount of brown color (L*) in the fruit (Nunes *et al*., 2005). The increase in a* during storage is the result of fruit ripening and aging and the speed of the non-enzymatic process of browning in the fruit (Etemadipoor *et al*., 2019). Visual appearance and freshness are key factors in determining the external quality of limes (Asencio *et al*., 2018).



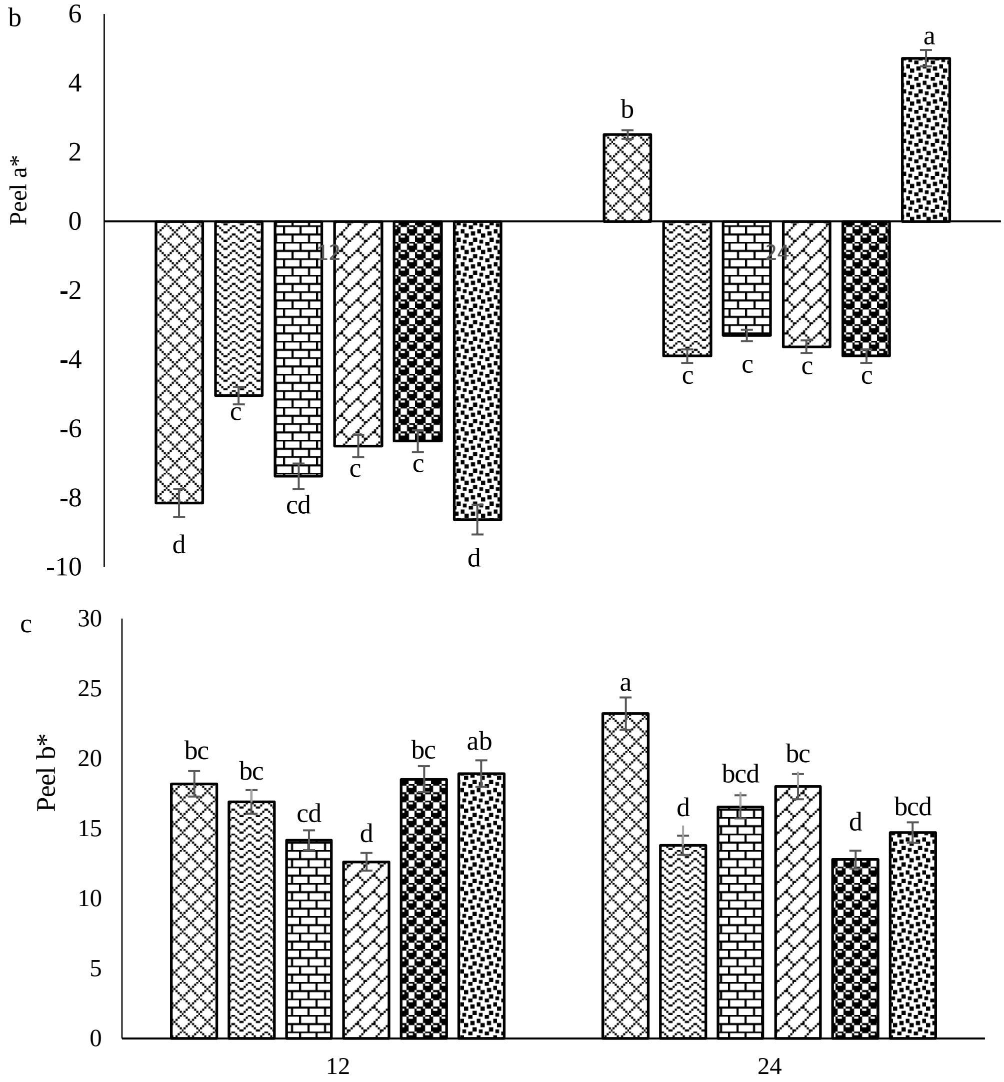


Fig. 5- The effect of Persian gum edible coatings, Persian gum in combination with pomegranate seed oil and pomegranate seed oil on a) L *, b) a * and c) b * lime fruit stored at ambient temperature

The same letters have no significant difference with each other at the probability level of p≤0.05.

Marketability

Appearance is the most crucial factor in determining the evaluation and marketability of a product. The 1% gum treatment was the most marketable among the various treatments. Although pomegranate seed oil effectively controlled fruit weight loss, it was not marketable due to the effect on browning of the fruit peel. The control fruits exhibited the lowest level of marketability. The quality of fruits, such as color uniformity, firmness, and absence of decay, is crucial in maintaining quality and facilitating export to distant markets. The impact of treatments on 24 days of lime fruit storage can beobserved in Fig. 6.

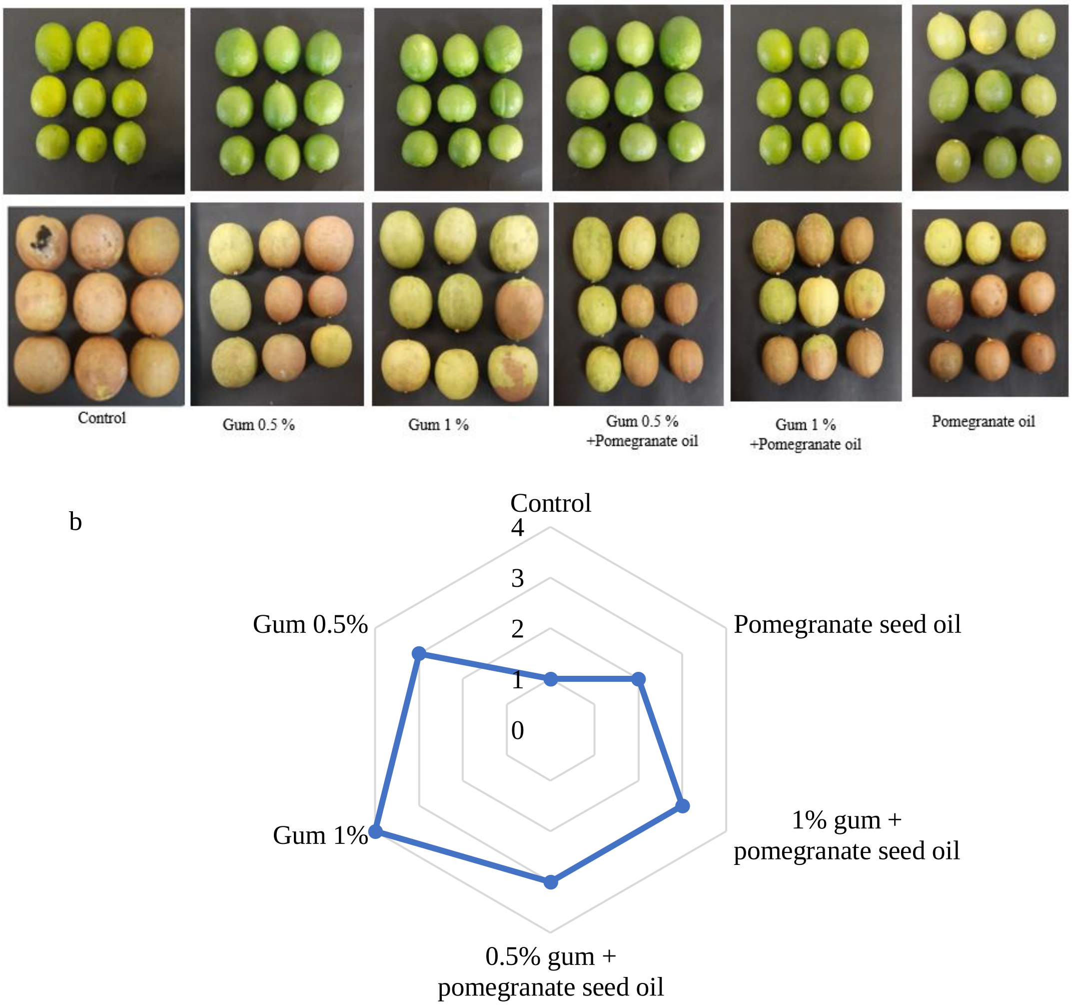


Fig. 6- a) Treated fruits after 24 days of storage b) Comparison of treatment combinations (control, 0.5% gum, 1% gum, 0.5% gum with pomegranate seed oil, 1% gum with pomegranate seed oil, pomegranate seed oil) on the marketability of Mexican lime

Conclusion

Persian gum and pomegranate seed oil coatings can retain the peel color, marketability, fruit phenol content, and soluble solids of Mexican lime at ambient temperature. These coatings are recommended as a substitute for chemical compounds to ensure storage quality. The addition of 1% gum in conjunction with pomegranate seed oil resulted in a significant increase in peroxidase and flavonoid levels compared to the control group. The 0.5% gum treatment, which incorporated pomegranate seed oil, showed a decrease in the concentration of soluble solids and polyphenol oxidase compared to the control group. As gum-based coatings and films for consumption are still in the developmental phase, future investigations should focus on creating prototypes. It is predicted that researchers will be able to overcome fundamental obstacles and develop appropriate skills to enhance edible films and coatings for food products.

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اثر پوشش خوراکی صمغ فارسی غنی‌شده با روغن دانه انار بر کیفیت لیموترش مکزیکن لایم (*Citrus aurantifolia*)

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چکیده

استفاده از پوشش‌ها به عنوان راهکاری موثر برای بهبود عمر میوه و کیفیت آنها مورد توجه قرار گرفته است. در این تحقیق، افزایش عمر ماندگاری میوه‌های لیمو ترش مکزیکن لایم (*Citrus aurantifolia* cv. Mexican lime) با پوشش صمغ فارسی و روغن دانه انار بررسی شد. ترکیبات مختلف لیموترش با پوشش صمغ فارسی و روغن دانه انار با غلظت‌های مختلف (صفر (کنترل)، ۰/۵ درصد و ۱ درصد صمغ فارسی، ترکیب ۰/۵ درصد و ۱ درصد صمغ فارسی با روغن دانه انار، و روغن دانه انار به تنهایی ۰/۰۵ درصد) تهیه شده و پس از ۲۴ روز نگهداری در دمای محیطی (۲۰ ± ۲ درجه سانتی‌گراد و رطوبت نسبی ۵۰-۶۰ درصد) با تکرار سه بار به صورت طرح کاملاً تصادفی مورد ارزیابی قرار گرفتند. نتایج این تحقیق نشان داد که تیمارهای استفاده شده نقش مؤثری در کنترل افت وزن میوه در طول نگهداری داشتند. بنابراین، کمترین درصد افت وزن در تیمار روغن دانه انار مشاهده شد. به جز تیمار روغن دانه انار، سایر تیمارها مقدار کمتری از میزان محلول جامد قابل اندازه‌گیری (TSS) نسبت به گروه کنترل نشان دادند. در بیشتر تیمارها، محتوای فنل، فلاونوئید و آنتی‌اکسیدان در سطح بالاتری نسبت به گروه کنترل بود. نتایج مقایسه میانگین نشان داد که میوه‌های پوشش داده شده با ۱ درصد صمغ فارسی (۸۵/۳۶ واحد/میلی‌لیتر) فعالیت پراکسیداز را به طور قابل توجهی بیشتر از گروه کنترل (۶۰/۳۵ واحد/میلی‌لیتر) نشان دادند. پوشش صمغ فارسی ۱ درصد و ۰/۵درصد به همراه روغن دانه انار به طور قابل توجهی فعالیت آنزیم پلی فنول اکسیداز را کنترل کردند. نمونه‌های تیمار شده میزان زردی (b*) کمتری نسبت به گروه کنترل نشان دادند. به طور کلی، بهترین قابلیت بازارپذیری در میوه‌های پوشش داده شده با صمغ فارسی ۱ درصد مشاهده شد. بنابراین استفاده از این پوشش برای حفظ تازگی و کیفیت میوه لیمو ترش مکزیکی در طول نگهداری در محیط توصیه می‌شود.

واژه‌های کلیدی: آنتی‌اکسیدان، پس از برداشت، پوشش خوراکی، مکزیکن لایم

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