

Research Article

Effect of Sodium Alginate in Combination with *Zataria multiflora* Boiss. on Phenolic Compounds, Antioxidant Activity, and Browning Enzymes of Fresh In-Hull Pistachio (*Pistacia vera* L.)

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The color of fresh pistachio is used as a postharvest quality indicator. The present study was performed to investigate the chemical properties of fresh pistachios coated with different sodium alginate concentrations (1 and 1.5%), various amounts of Shirazi thyme essential oil (0.3 and 0.5%), and their combination during storage ($2 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH). Over the storage duration, chemical parameters were measured on days 13, 26, and 39. The results showed that although the application of sodium alginate in combination with thyme essential oil decreased polyphenol oxidase activity in comparison with other treatments, the highest total phenolics and phenylalanine ammonia lyase activity were found in pistachios coated with alginate (1%) + thyme essential oil (0.3% and 0.5%). In general, it was proven that treatments containing 1% alginate + 0.3% essential oil had the ability to maintain the quality of fresh pistachio fruit approximately over 39 days of storage.

1. Introduction

Pistachio nuts contain considerable amounts of essential minerals, phenolics, essential fatty acids, proteins, and vitamin A [1] and are known as healthy food. Pistachio is commonly used in the form of a dry product as nut, but the consumption of fresh pistachio has become more popular in the last decades [2].

Weight loss, lipid oxidation, browning, and microbial growth of fresh pistachio play important roles in its final appearance and quality during marketing [3]. Studies have shown that, after harvesting fresh pistachio, it immediately becomes brown and decayed that affect its final market acceptability [4]. Browning is one of the most important phenomena that occurs in the pistachio hull during processing and storage [3]. It is a common color change among

fresh vegetables and fruits and takes place because of the reaction of phenolic compounds, oxygen, and polyphenol oxidase (PPO) enzyme. Biochemical studies have shown that the oxidation of polyphenols (mainly the conversion of ortho-diphenols into quinones and semiquinones) is the reason for hull browning [4].

Edible coating of fresh products is applied to avoid quality deterioration of fresh fruits [5]. It can control gas exchange from the fruit surface, decrease respiration rate, and control water evaporation from fruits, thereby reducing moisture loss [6]. Furthermore, edible coating technology improves product quality along with its shelf life by changing the internal atmosphere and reducing microbial proliferation [7]. However, choosing suitable formulations for edible coatings considerably affects their effectiveness. Azarakhsh et al. [8] reported that respiration rate and weight loss were

decreased in alginate-coated pineapples compared with control fruits. Ribeiro et al. [9] showed that alginate was helpful in delaying color change, weight loss, and softening of coated plums. They concluded that edible coatings acted as barriers on the surface of fruit and reduced its permeability to gases (CO₂ and O₂) and water vapor. This decreased the rate of respiration and transpiration and delayed the ripening process.

The efficacy of edible coating could be improved by the addition of antibrowning and antimicrobial agents [10]. Nowadays, functional additives such as essential oils (EOs) are mainly added to edible coating formulations to enhance the appearance, integrity, microbial safety, and mechanical strength of food [11].

Direct surface application of antifungal and antibacterial compounds has slight benefits because active ingredients are neutralized at contact or are diffused into the bulk of fruit. Therefore, the addition of EOs into edible coating formulations is an efficient strategy to improve the functionality of coatings [12].

Shirazi thyme (*Zataria multiflora* Boiss.) has a high percentage of essential oil with antibacterial and antifungal activities. Application of *Zataria multiflora* essential oil in various antimicrobial edible coatings including alginate [13, 14], gum arabic [15], chitosan [16], and carboxymethyl cellulose [17] showed to increase total phenolic compounds and, as a result, antioxidant activity of films. The results showed that films containing essential oil could be potentially applied in active biodegradable packaging materials to inhibit or delay deterioration and oxidation and prevent microbial activity [17].

Considering limited studies on fresh pistachio storability and the potential of sodium alginate and *Z. multiflora* essential oil to prevent the activity of PPO and oxidation of phenolics, treatment with sodium alginate and *Z. multiflora* essential oil was applied to decrease browning. The present study aimed to investigate the possible potentials of various formulations of sodium alginate and *Z. multiflora* essential oil and their combination for suppressing the browning of fresh pistachio fruits.

2. Materials and Methods

2.1. Materials. Fresh pistachio (*Pistacia vera* L.) fruits cv. 'Ahmad-Aghaei' were harvested manually on October 10 from 30-year-old pistachio trees. They were instantly transported to the laboratory of postharvest, and those without crack and with uniform size, color, and shape were separated from the clusters.

Food grade sodium alginate, glycerol, and sunflower oil were obtained from Sigma-Aldrich Chemicals (Germany). *Zataria multiflora* essential oil was purchased from Barij Essence Company (Kashan, Iran).

2.2. Edible Coating Solutions. Sodium alginate edible coating was prepared according to the method reported by Azarakhsh et al. [18]. To prepare 1 and 1.5% (w/v) edible coatings, 1 and 1.5 g sodium alginate powder (Merck, Germany) were

dispersed in 100 ml distilled water, respectively. The solutions were heated at 70°C, and stirring was carried out until the clear appearance of the mixture. Then, 1.5% (w/v) glycerol was added followed by 0.025% (w/v) sunflower oil. Then, *Zataria multiflora* Boiss. EO at concentrations 0% (control), 0.3%, and 0.5% (w/v) was added. Finally, these mixtures were homogenized for 5 min.

2.3. Edible Coating Treatments and Storage. Edible coatings were applied according to the method reported by Azarakhsh et al. [18]. The fruits were immersed in coating solutions for 3 min and then air-dried for 1 h at 25 ± 2°C. Then, they were immersed in calcium chloride (2% w/v) for 2 min to allow gel formation and air-dried at 25 ± 2°C. 200–250 g pistachio fruit was placed in each polypropylene container with dimensions 12 × 15.5 × 10.5 cm. Coated pistachios were stored at 2 ± 1°C and 85 ± 5% RH for 39 days. Fresh pistachio characteristics were determined every 13 days during storage.

2.4. Total Phenolics (TPs). TPs were measured by the Folin–Ciocalteu reagent [19]. 0.5 g flesh tissue of the pistachio hull was homogenized with 3 ml methanol (85%) and then centrifuged at 12,000 × g for 20 min at 4°C to remove any undesirable impurities. The supernatant was separated and used as the extract. 300 μL extract was mixed with 1200 μL 7% sodium carbonate and maintained at room temperature for 5 min. Then, 1500 μL 10% Folin–Ciocalteu reagent was added, and the mixture was shaken for 90 min in darkness at room temperature. Absorbance was read at 760 nm by using a spectrophotometer (Epoch Biotech, Germany). Gallic acid was used for drawing the standard curve. The concentration of the total phenolic content was expressed as mg gallic acid equivalents per 100 g FW.

2.5. DPPH Radical Scavenging Activity. Antioxidant activity (AA) was measured according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method described by Brand-Williams et al. [20]. 0.1 ml fruit hull extract, 1 ml DPPH (0.1 mM), and 1 ml Tris-HCl (pH 7.5) buffer were mixed using a vortex. The final mixture was incubated for 30 min in a dark room. Then, mixture absorbance was read at 517 nm by using a spectrophotometer (Epoch Biotech, Germany), and AA percentage was calculated using the following equation:

$$AA (\%) = \left[1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right] \times 100. \quad (1)$$

2.6. Enzyme Activities. 0.5 g frozen fresh fruit hull was powdered in a chilled pestle using liquid nitrogen and homogenized in 1 mL 100 mM phosphate buffer (pH 7) containing 0.5 mM EDTA and 6% (w/v) polyvinylpyrrolidone (PVPP). The mixture was centrifuged at 15,000 × g for 20 min at 4°C, and the supernatant was used for the measurement of enzyme activities.

2.7. Polyphenol Oxidase (PPO) Activity Assay. Polyphenol oxidase (PPO) activity was determined using the methods proposed by Koushesh Saba et al. [21]. Aliquots of the supernatant were added to two solutions containing catechol and pyrogallol at concentrations of 0.05 M and 0.02 M, respectively. Absorbance increase was monitored at 420 nm for 1 min at room temperature, and enzyme activity was expressed as unit per gram of fresh weight per minute.

2.8. Phenylalanine Ammonia Lyase (PAL) Activity Assay. For the measurement of phenylalanine ammonia lyase (PAL) activity, 0.1 mL supernatant was added to a mixture containing 0.5 mL 10 mM L-phenylalanine, 1 mL 50 mM phosphate buffer (pH 7), and 0.4 mL double-distilled water. After mixture incubation at 40°C for 1 h, the reaction was stopped by adding 0.5 mL 6 mM HCl. PAL activity was measured by the absorbance of the mixture at 290 nm based on the production of cinnamic acid. PAL enzyme activity was expressed as mg of cinnamic acid per gram of fresh weight per min [22].

2.9. Statistical Analysis. The experiment was set as factorial based on randomized complete block design with nine treatments and three replications. The source of variation included edible coatings, essential oils, and storage duration. Analysis of variance (ANOVA) was performed using SAS software (version 9.1). The mean values were compared according to Duncan's multiple range test at $P < 0.05$.

3. Results and Discussion

3.1. Total Phenolic Concentration and DPPH Radical Scavenging Activity. The effects of edible coating, essential oil, and essential oil-enriched edible coatings on the TP of the fresh hull are illustrated in Figure 1(a). Among different treatments, fruits covered with 1% sodium alginate + 0.3% thyme showed the highest TP content (57.01 mg/100 g fresh weight) after 39 days of storage. However, fruits treated with 0.5% thyme essential oil showed the lowest TP content (49.91 mg/100 g fresh weight) among all treatments (Figure 1(a)). As shown in Figure 1(b), initial DPPH radical scavenging activity of the fresh pistachio hull was 39.77%. During the storage, DPPH radical scavenging activities of all treated and control fruits were dramatically decreased and reached the lowest level after 13 days of storage. The DPPH radical scavenging level started to increase with increasing storage period up to 26 days and then decreased at the end of storage, but it was higher in all storage times in treated fresh pistachios than control ones (Figure 1(b)).

The reduction of antioxidant capacity in fruits could be attributed to fruit senescence and higher respiration rate due to the degradation and loss of some phenolic compounds. Hashemi et al. [13] showed that sodium alginate edible coating enriched with *Zataria multiflora* essential oil increased phenolic compounds in the kernels of coated fresh pistachio fruits. Other studies have shown that the application of *Zataria multiflora* Boiss. essential oil-enriched gum

arabic coated on fresh pistachio resulted in higher TP compared to control fruits [15]. Ali et al. stated decreased phenolic content is a natural part of senescence which can also be due to cell wall degradation when fruit is stored over long periods [23].

Hashemi et al. [24] reported that apricots coated with basil seed gum (BSG) + *Origanum vulgare* essential oil (2–6%) showed the highest antioxidant activity, while control and BSG-coated samples gave the lowest amount of antioxidants at the end of storage time. These effects could be the result of the oxygen barrier properties of edible coatings and the capacity of essential oil components to retain fruit quality factors along with the inhibition of enzyme activity that degrades antioxidant compounds [25]. Thus, the increase of antioxidant capacity is related to the increase in the TP content. Oms-Oliu et al. [26] observed that the increase of phenolic compounds increased the antioxidant capacity of 'Piel de Sapo' melon during 14 days of storage period at 4°C. Our results clearly revealed the increment of TP compounds of fruits coated with alginate + thyme oil. Similarly, thyme oil added to packaging materials increased the amount of TPs and flavonoids (catechin) in avocado fruits [27]. A similar trend was also stated by Ali et al. [28] for tomato fruits coated with gum arabic (5%) edible coatings. However, the increase of the total phenolic content might also increase due to the essential oil component in basil seed gum coating, which could delay the oxidation processes of phenolic compounds by scavenging free radicals and oxygen [29].

3.2. PPO and PAL Activity. As shown in Figures 2(a) and 2(b), the activity of enzymes associated with the browning of the fresh hull, i.e., PAL and PPO enzymes, was significantly affected by edible coating formulations. The activity of the PPO enzyme in fresh hulls was increased during storage so that the highest activity of PPO was observed in control (uncoated) pistachios and those coated with 1.5% sodium alginate + 0.5% essential oil after 39 days of storage, while the lowest levels were obtained in fruits treated with 1% sodium alginate solution + 0.3% thyme essential oil and 0.3% essential oil (Figure 2(a)).

According to Figure 2(b), PAL enzyme activity in treated and control fruits was increased during the storage period. The lowest activity of the PAL enzyme was observed in control fruits. The fruit samples treated with 1% sodium alginate + 0.3% essential oil showed the highest level of PAL activity; however, it had no significant differences with other treatments except for 1.5% sodium alginate + 0.5% essential oil (Figure 2(b)).

PPO is a key enzyme in the texture browning of fruits and vegetables. Some assessments reported a significant increase in PPO activity during storage [30]. Enzymatic browning is often accompanied with damage to the cell membrane in texture [31]. When the integrity of the membrane is reduced, phenolic compounds are exposed to oxygen and oxidized by the catalytic activity of the released polyphenol oxidase [31]. Kader [32] reported that enzymatic browning can be reduced at low levels of oxygen. An

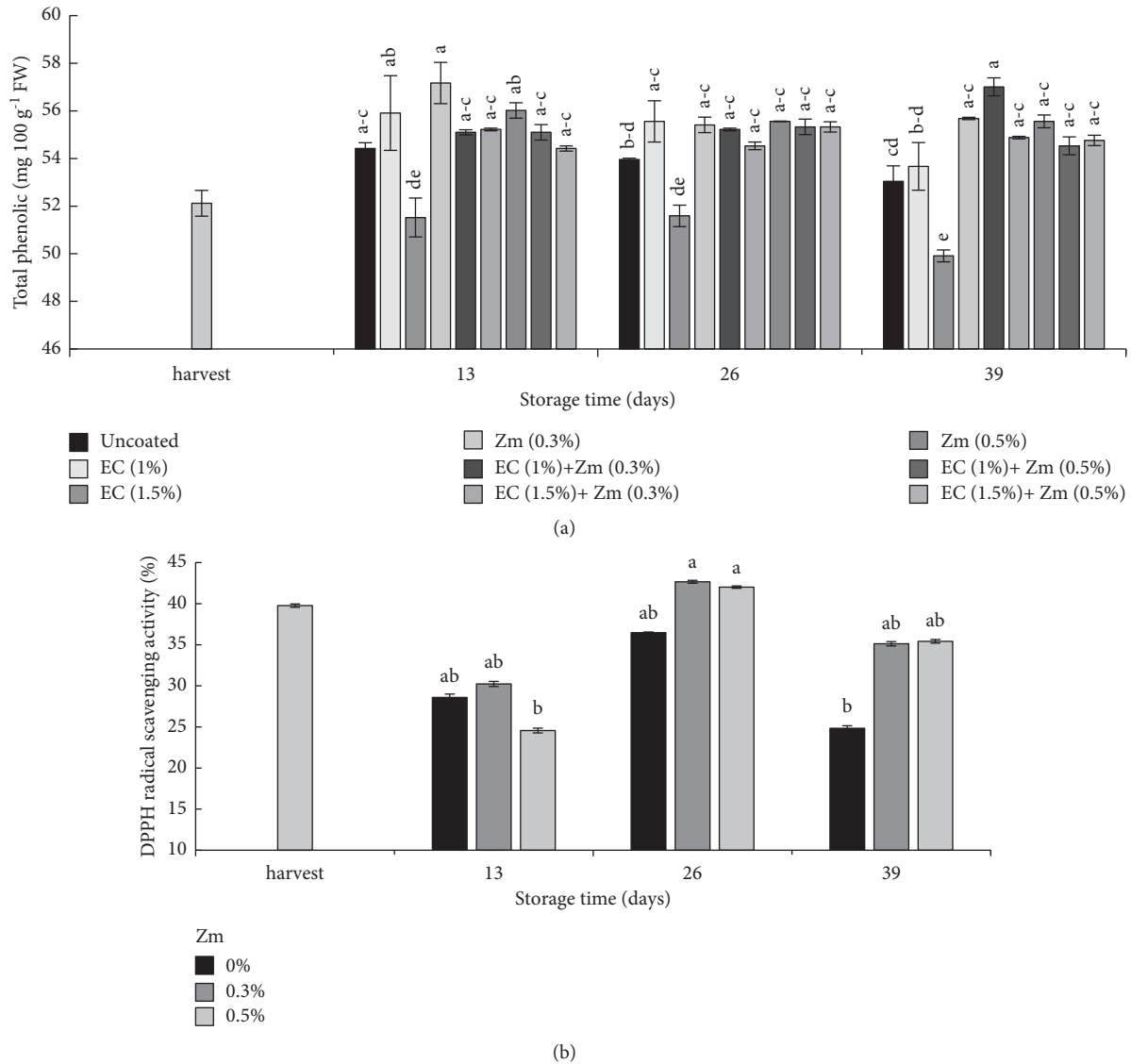


FIGURE 1: Total phenolics (a) of the fresh pistachio hull coated with sodium alginate + *Zataria multiflora* and DPPH radical scavenging activity (b) in the fresh pistachio hull treated with *Zataria multiflora* during storage at $3 \pm 1^\circ\text{C}$. Means ($n = 3$) with the same letters are not significantly different according to Duncan's test ($p < 0.05$). EC: alginate-based edible coating; Zm: *Zataria multiflora*.

increase of PPO activity was observed in pistachio during storage, and as a result, the oxidization of phenolic compounds occurring during this process led to lower amounts of polyphenol compounds [33]. In contrast, higher phenolic contents of samples coated with 1% alginate + 0.3% thyme might be attributed to lower PPO enzyme activities. It seemed that alginate in solutions acted as a barrier for oxygen needed for PPO activity. In addition, de Sousa [34] reported that mushrooms coated with alginate enriched with eugenol essential oil and cinnamic acid reduced polyphenol oxidase activity which resulted in lower enzymatic browning, and alginate coating formed a protective barrier on the surface of samples, reducing the supply of O_2 which can help reduce PPO activity. Previous studies have shown that the application of Arabic gum and sodium casein in combination with cinnamon and lemongrass essential oils on

guava [11] was effective in reducing the activity of the PPO enzyme. A previous study examining the effects of gum arabic in combination with *Zataria multiflora* essential oil on fresh pistachio fruits also showed that these treatments were effective in reducing PPO activity [15]. Regarding the PAL enzyme, several studies have shown that the accumulation of phenols and anthocyanins was correlated with the increase of the activity of this enzyme in some fruits. This is the first key enzyme involved in the biosynthesis of phenols in fruits and vegetables [35]. In addition, herbal essences have not only antimicrobial properties but also the ability to increase TP compounds [36]. This may be because essential oils play positive roles in secondary plant metabolites and stimulate the biosynthesis of phenolic compounds and anthocyanins by inducing an increase in the activity of this enzyme [37]. In addition, Chiabrando and Giacalone

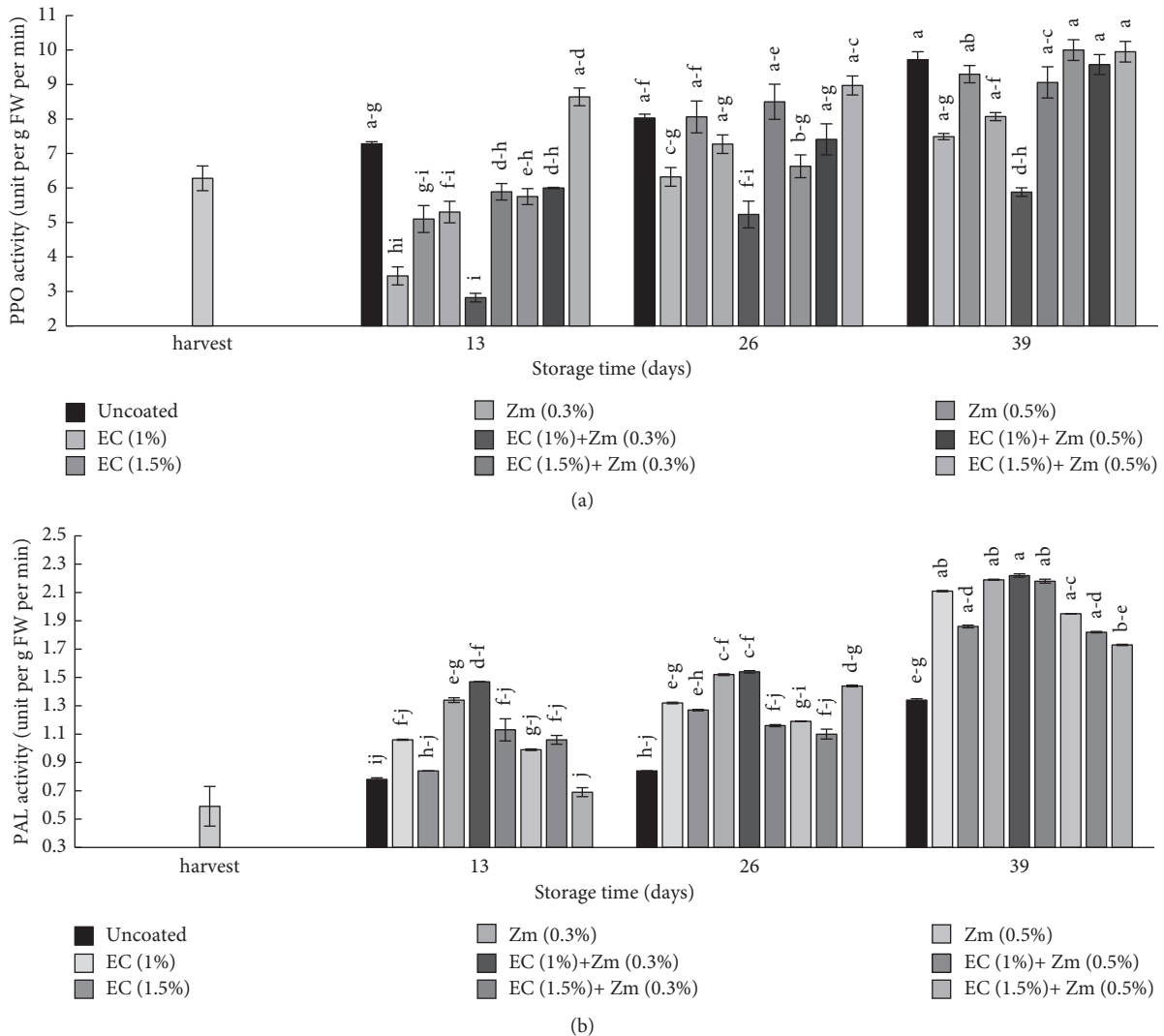


FIGURE 2: PPO (a) and PAL activity (b) of the fresh pistachio hull coated with sodium alginate + *Zataria multiflora* at $3 \pm 1^\circ\text{C}$. Means ($n=3$) with the same letters are not significantly different according to Duncan's test ($p > 0.05$). EC: alginate-based edible coating; Zm: *Zataria multiflora*.

[34] reported that fresh-cut apple coated with alginate enriched with cinnamon and rosemary essential oils reduced polyphenol oxidase activity which resulted in lower enzymatic browning and alginate coating formed a protective barrier on the surface of samples, reducing the supply of O_2 which can help reducing PPO activity.* should replace the previous text.

4. Conclusion

One of the most important issues associated with the storage of fresh pistachios is their short storage life. Fresh pistachios lose their quality shortly after harvest because the pistachio hull is very perishable and is rapidly spoiled and browned. Application of edible coatings is an important protection method. However, this method alone is not enough. Certain functional and bioactive compounds can be incorporated into edible coatings, thus enhancing the safety of coated products and bringing benefits to the health of consumers. Thus, edible

coatings based on the incorporation of active ingredients, especially essential oils, are considered as one of the approaches with the greatest interest for managing the quality of fresh products. Such a strategy can inhibit enzymatic or biochemical damage during postharvest storage. This study showed that the application of alginate coating enriched with *Zataria multiflora* could reduce the browning of fresh pistachios during storage. Based on our findings, control (uncoated) fruits showed the most intense hull browning followed by samples treated with 0.5% essential oil, but it should be noted that careful selection of thyme concentration was required in coating formulations to improve the storability of fresh in-hull pistachio fruits. Also, the lowest PPO enzyme activity was observed in the samples coated with 1% sodium alginate + 0.3% *Zataria multiflora* essential oil. In addition, a higher TP content in the hull was also obtained in fruits coated with 1% sodium alginate + 0.3% *Zataria multiflora* essential oil which was selected as the best formulation for increasing the storage life of fresh pistachio.

Data Availability

The data belong to a project, and the authors do not have the permission to publish and/or share the data and provide appropriate attribution.

Conflicts of Interest

The authors declare no conflicts of interest.

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