

## Enhancing the Quality of Fresh Pistachio Fruit Using Sodium Alginate Enriched with Thyme Essential Oil

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### ABSTRACT

The combined coating-effects of Sodium Alginate (SA) and Thyme Essential Oil (TEO) was investigated at three levels of, respectively, (0.0, 1.0, and 1.5 %) and (0.0, 0.3, and 0.5%) on fresh pistachio during 6 weeks of cold ( $3\pm 1^\circ\text{C}$ ) storage. Physiochemical parameters (hull and hard-shell color, hull hardness, soluble sugar, chlorophyll, and soluble solids content) and microbial load (plate count agar) were measured every 2 weeks of storage. Similarly, the sensory attributes of pistachio were evaluated by trained panelists and Quantitative Descriptive Analysis (QDA) method from minimum (1) to maximum level (15) score. While weight loss of each pistachio sample increased during storage time, those treated with alginate had considerably less weight losses than other treatments. The pistachio treated with combination of SA (1%) and TEO(0.3 or 0.5%) showed significantly higher hull hardness,  $L^*$  values, soluble sugar, chlorophyll, and lower growth of microorganisms (mainly yeast and mold) than other treated and non-treated (control) samples. The highest sensory scores of 12.3 and 10.6 for hull color of pistachio were related to samples coated again with combinations of SA (1%) and TEO (0.3 or 0.5%), respectively, and were significantly higher than the score of control sample (6.4). Since the pistachio treated with the above-mentioned concentrations of SA and TEO had significantly higher physiochemical, microbiological, and sensory attributes than the other samples, their quality indicators were superior to other treatments (including control samples) and, most probably, they had longer shelf lives and potential to preserve their natural characteristics of fresh pistachio during storage.

**Keywords:** Cold storage, Physiochemical parameters, *Pistacia vera*, Weight loss.

### INTRODUCTION

*Pistacia vera* belongs to *Anacardiaceae* family and its flavor, taste and nutritional value (vitamins, minerals, and etc.) are among the characteristics that make pistachios better than other nuts (Shakerardekani, *et al.*, 2013). Fruit coating, not only improves the appearance of fruit, but also increases its shelf-life and quality. The main benefit of fresh fruit edible coating is usually the reduction of transpiration and respiration of the product (Flores-López, *et*

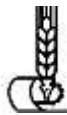
*al.*, 2016). Edible coatings have potential to control moisture, improve mechanical and textural properties, prevent flavor loss, and act as a carrier for various additives (Azarakhsh, *et al.*, 2014). Edible coatings increase the shelf-life of fresh fruits by reducing metabolic processes and microbial growth as well as providing protective barrier to reduce the rate of respiration and transpiration (Yousuf, *et al.*, 2018). Alginate is a gum produced as sodium alginic acid from marine seaweed called *Macrocystis pyrifera*. The most efficient and unique properties of alginates are their ability to

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react with multi-capacity metal ions to create strong gels or polymers. Sodium alginate with 2% concentration was effective in decreasing the respiration rate of corn and increasing the shelf-life of blue berries and maintaining its antioxidant properties (Duan, *et al.*, 2011). According to the report by Rojas-Graü, *et al.* (2008), alginate acts as a barrier to gas and is effective in creating a modified form of atmosphere to maintain the quality of apple. Alginate has been used to increase the shelf-life of plum (Yousuf, *et al.*, 2018) and strawberry (Díaz-Mula, *et al.*, 2012). Edible coating of pistachio kernel significantly reduced the oxidation of kernel and prolonged the shelf-life of the coated nuts (Javanmard, 2008). Combination of essential oils with edible coatings is an effective way to solve some post-harvest problems (Aloui, *et al.*, 2014). In a report by Guerreiro *et al.* (2015), alginate, in combination with eugenol and citral oils, was effective in increasing the shelf-life of *Arbutus unedo* (Guerreiro, *et al.*, 2015). Also, the use of lemon grass essential oils combined with alginate coating in pine fruit increased its shelf-life (Azarakhsh, *et al.*, 2014). Coatings from whey protein containing natural antimicrobial agents reduced the storage of edible cereals, such as weight loss, oxidative degradation and moisture absorption, resulting in an increase in shelf-life and health of edible kernel during the storage (Javanmard and Ramazan, 2009).

Shakerardekani *et al.* (2019) investigated the effect of using different concentrations of Arabic gum (0, 6 and 8% w/v) in combination with Shirazi thyme essential oil (0, 0.3 and 0.5% w/v), on the qualitative characteristics (microbial growth, water loss, carbohydrates, chlorophylls, phenolic compounds, and antioxidants) of fresh pistachio nuts during 36 storage days at a temperature of  $3\pm 1^{\circ}\text{C}$ . They reported that the growth of mold, yeast and aerobic bacteria on the fresh pistachios were significantly less than in other pistachios when using the 6% Arabic gum concentration enriched with the thyme

essential oil at 0.3 and 0.5%. Also, addition of Shirazi thyme essential oil to the formulation of Arabic gum (6%) reduced the weight loss of pistachio samples. Addition of essential oil to the Arabic gum coating preserved the carbohydrates, chlorophylls, phenolic compounds, and antioxidants in the fresh pistachios kernels (Shakerardekani, *et al.*, 2019). Radi, *et al.* (2018) reported that Micro-emulsion and nano-emulsion of orange peel essential oil in pectin based coatings reduced the quality loss. Also, higher antimicrobial effects were observed in orange slices coated with nano-emulsion.

Hosseini *et al.* (2019) reported that UV-C treatment caused a significant decrease in the weight loss of fresh pistachios compared to the control. The fruit treated with  $2.1\text{ kJ m}^{-2}$  of UV-C and the control packed in non-perforated polyethylene terephthalate were lighter, redder, and less yellow compared to  $4.5\text{ kJ m}^{-2}$  treated samples. Molamohammadi *et al.* (2020) reported that the highest sensory scores of color, texture, and overall acceptance were observed in the pistachio fruit treated with chitosan and salicylic acid. Also, these treatments alone or in combination reduced the growth of bacteria and fungi. The weight loss and the peroxide and free fatty acid values of treated fresh pistachios were lower than the controls at the end of the storage period.

In the current study, we aimed to reduce the microbial growth and improve the hull color, hard shell color, hull hardness, total chlorophyll content, soluble sugars content, total soluble solids and sensory properties of fresh pistachios using sodium alginate enriched with Shirazi thyme essential oil.

## MATERIALS AND METHODS

In this experiment, commercial Ahmad Ahaghi pistachio cultivars were harvested from pistachio trees in Pistachio Research Center (Rafsanjan, Kerman, Iran). After transferring the samples to the laboratory, fresh, healthy and uniform pistachios were separated from unripped, damaged and

early-split pistachios. The effects of different concentrations of sodium alginate (0.0, 1.0 and 1.5%) (Sigma-Aldrich, Germany) enriched with TEO (0.0, 0.3 and 0.5%) (Barij Essence Company, Iran), on the quality characteristics of fresh pistachio were investigated during 6 weeks of storage at  $3\pm 1^\circ\text{C}$  ( $80\pm 5\%$  relative humidity).

### Preparation of Sodium Alginate Solution

Sodium alginate powder (Merck, Germany) was dispersed in distilled water at  $70^\circ\text{C}$  until it changed to a clear solution. After cooling the solution, glycerol at concentration of 1.5% (Sigma Aldrich) was added to the solution as a plasticizer. In the next step, sunflower oil was added to the solution as an emulsion (Sigma Aldrich) at 0.025%. Finally, different concentrations of essential oil of TEO were added to the solutions. The volume of each solution was 1,000 mL and final formulation of each solution included sodium alginate, glycerol, sunflower oil and TEO. All formulations were homogenized (Ultra-Turax T25, Janke and Kunkle, IKA-Labortechnik, Breisgau, Germany) for 5 minutes at 24,500 rpm. After homogenizing the solution, small bubbles form in the solution, which may not form a uniform porous coating on the fruit. For this reason, the solution was stored in the refrigerator at  $4^\circ\text{C}$  for 24 hours to remove the bubbles inside (Azarakhsh, *et al.*, 2014).

### Fresh Pistachio Alginate Edible Coating

Fresh pistachios with a soft skin (hull) were used for testing. The pistachios were separated from the clusters and treated. The samples were immersed in sodium alginate coating for 3 minutes, then waited for 1 minute at the laboratory temperature until the gel layer on the surface of the fruit became firm and did not have a watery and soft state. In the next step, the coated fruits were immersed in 2% calcium chloride for 2 minutes to form a gel. Subsequently, the samples were dried at laboratory temperature and 400 to 450 g fresh pistachios were placed in each polypropylene container ( $12\times 15.5\times 10.5$  cm) (Figure 1). Then, pistachio samples were weighed after treatment and stored in a refrigerator at  $3\pm 1^\circ\text{C}$  and  $85\pm 5\%$  RH. The quality parameters and sensory evaluation was carried out in the zero week of experiment as well as at the end on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> weeks of storage.

### Weight Loss Measurement

In order to measure weight loss, fresh pistachios were weighed at the beginning and at the end of the storage period and their weight loss was calculated using Equation (1):

$$\begin{aligned} (\%) \text{Weight loss} &= \frac{(\text{Initial weight} - \text{Secondary weight})}{\text{Initial weight}} \times 100 \\ &\text{Pistachio Hull Color} \end{aligned} \quad (1)$$



**Figure 1.** Coated pistachio (left) and packed pistachio (right) in this research.



Pistachios fresh hull color was measured by determining the values of  $L^*$  (Lightness) and  $\Delta E$  (total color difference) at three points of the hull of the fruit with Chromameter of the Minolta CR-400. At each measurement step, eight fruits were randomly selected from each replicate. The following equation was used to calculate  $\Delta E$ :

$$\Delta E = [(L * t - L * 0)^2 + (a * t - a * 0)^2 + (b * t - b * 0)^2]^{1/2} \quad (2)$$

Where,  $L_{x_t}$ ,  $a_{x_t}$  and  $b_{x_t}$  represent the hull color index of the fruit at different times and  $L_{x_0}$ ,  $a_{x_0}$  and  $b_{x_0}$  are initial values.

### Hardness of Fresh Hull

To determine the hardness of fresh hull (N), a Penetrometer (FG-5020, Taiwan) was used.

### Soluble Sugars

In order to measure soluble sugars, 0.5 g of pistachio meal (kernel without oil) was weighed and mixed with 5 mL of 95% ethanol and the supernatant was separated. The extraction was repeated twice with 5 mL ethanol 70%. The solution was then centrifuged for 20 min at a Relative Centrifugal Force (RCF) of  $1,372 \times g$ . After separating the liquid phase from the solid, the liquid portion was used to measure the soluble sugars. Then, 0.1 mL of alcoholic extract was mixed with 3 mL of freshly prepared solution of 0.15 g of anthrone in 100 mL of sulfuric acid 72%. This solution was placed in a Bain-marie bath for ten-minute at a temperature of  $90^\circ\text{C}$  to allow for a color reaction. The absorbance of the samples was read by a spectrophotometer at 625 nm, then, the following formula was used to calculate the amount of soluble sugar ( $\text{g } 100 \text{ g}^{-1}$ ) (Irigoyen, *et al.*, 1992):

$$\text{Soluble sugars} = \left( \frac{\text{OD}_{625}}{1000} \right) \times \left( \frac{15 (\text{Extract volume})}{0.5 (\text{Sample weight})} \right) \times 100 \quad (3)$$

### Total Chlorophyll

The ground pistachio kernel (0.4 g) was homogenized with 5 mL and 80% (v/v) acetone. The solution was centrifuged at  $11,200 \times g$  for 20 minutes, and the supernatant was used for total chlorophyll measurement. Absorbance (A) was read at wave lengths of 645 and 663 nm (Lemoine, *et al.*, 2008). The following formula was used to calculate the amount of total chlorophyll.

$$\text{Total chlorophyll (mg/100g)} = \frac{((20.2 \times A_{645}) + (8.02 \times A_{663})) \times V}{10 \times 1000} \quad (4)$$

### Microbial Growth

The Plate Count Agar (PCA) medium was used for measuring the amount of aerobic mesophilic bacteria, and Dichloran Rose Bengal Chloramphenicol Agar (DRBC) for measuring the amount of mold and yeast. To measure the amount of pistachio contamination, 10 g of fresh pistachio samples were weighed and placed in sterilized plastics. Ninety mL of peptone water solution was added to the sample, and was then put inside the Pulsifier (Model PUL200, Microgen Bioproducts, United Kingdom) for 15 seconds to remove contamination from the pistachios. One mL of the resulting mixture was poured into a test tube containing 9 mL distilled water. This process was continued until the concentration of the solution reached  $10^6$ . Then, 1 mL of the  $10^6$  dilution was poured into each culture medium by the method of linear culture. The PCA culture medium containing the culture suspension was incubated at  $30^\circ\text{C}$  for 48 hours, and the bacteria with their colonies were counted. The mold and yeast in the DRBC medium

containing the suspension were counted after being incubated at 25°C for 5 days. Finally, the contamination was reported in CFU units (Ozturk, *et al.*, 2016).

### Sensory Evaluation

For sensory evaluation, parameters of changes in color of the fresh hull, hard shell color, aroma of fresh pistachio, undesirable odor, and juiciness of kernels texture were scored during the storage period. In sensory assessment, 8 female panelists with age ranges of 35 to 40 assisted. Fresh water was made available to panelists to drink between each step as a cleaner. Quantitative Descriptive Analysis (QDA) was used for sensory evaluation. The range for each quality parameter ranged from zero to 15 (Stone, *et al.*, 2012, Meilgaard, *et al.*, 1999). The score of the evaluators for the desired parameters such as fresh hull color, fresh pistachio aroma and juicy texture tended to be 15 points, but for undesirable parameters such as stained shell and unfavorable aroma and taste, it tended to zero (Shakerardekani, 2017, Stone, 2012).

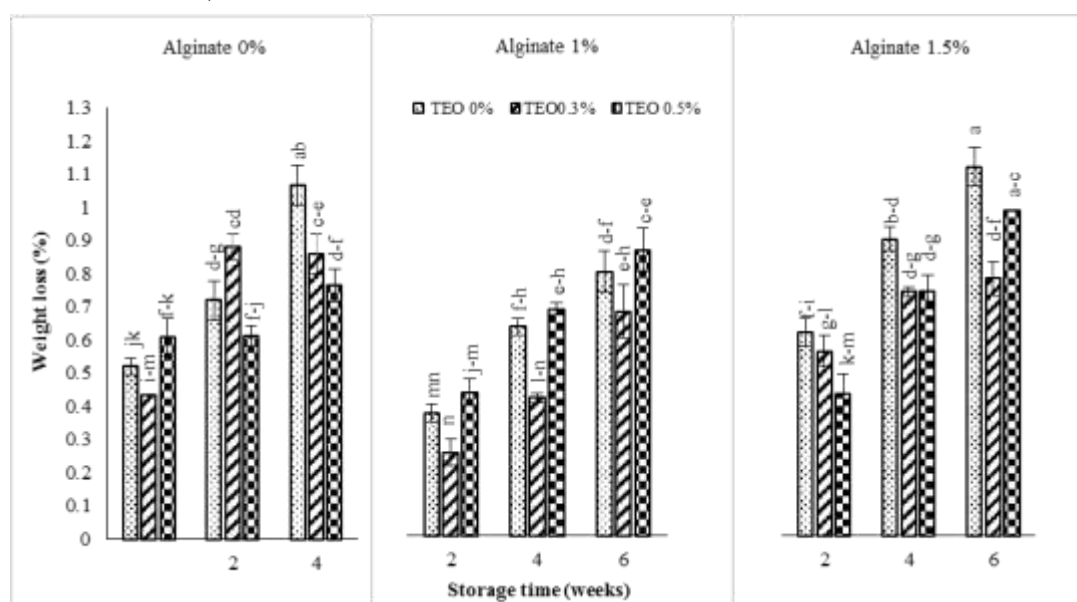
### Statistical Analysis

This experiment was performed on three factors from a factorial design based on a completely randomized design with three replications. The factors included edible coating, essential oil, and storage time. Statistical analysis of data was performed using SAS 9.2 software. Means of each trait were compared according to Duncan's multiple range tests at  $P \leq 0.05$ .

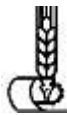
## RESULTS AND DISCUSSION

### Fresh Pistachio Weight Loss

Comparison of changes in mean weight loss of fresh pistachios in different storage times is shown in Figure 2. The results indicated that there was a significant difference between different treatments. On the 2<sup>nd</sup> week of storage, treatment with 1% sodium alginate combined with TEO 0.3% showed the lowest weight loss (0.25%), compared to control and other treatments. Also, in the same week, 1% sodium alginate



**Figure 2.** Effects of Alginate coatings enriched with TEO on weight loss percentage of fresh pistachios stored at  $3 \pm 1^\circ\text{C}$  and relative humidity of  $80 \pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P \leq 0.05$ ). (\*\*) The bars above the columns represents the Standard Error (SE).



treatment showed a lower weight loss (0.37%) than control samples. On the 4<sup>th</sup> week of storage, samples treated with sodium alginate 1% combined with TEO 0.3% showed the lowest weight loss (0.42%). Finally, on the 6<sup>th</sup> week of storage, the samples treated with 1% sodium alginate alone and in combination with TEO showed the least weight loss. Also, TEO treatments alone and 1.5 % sodium alginate treatment combined with 0.3 % essential oil showed significantly lower weight loss than control samples (1.07%). In all treatments, weight loss increased during storage time, but this increase was less in alginate treated pistachios (Figure 2).

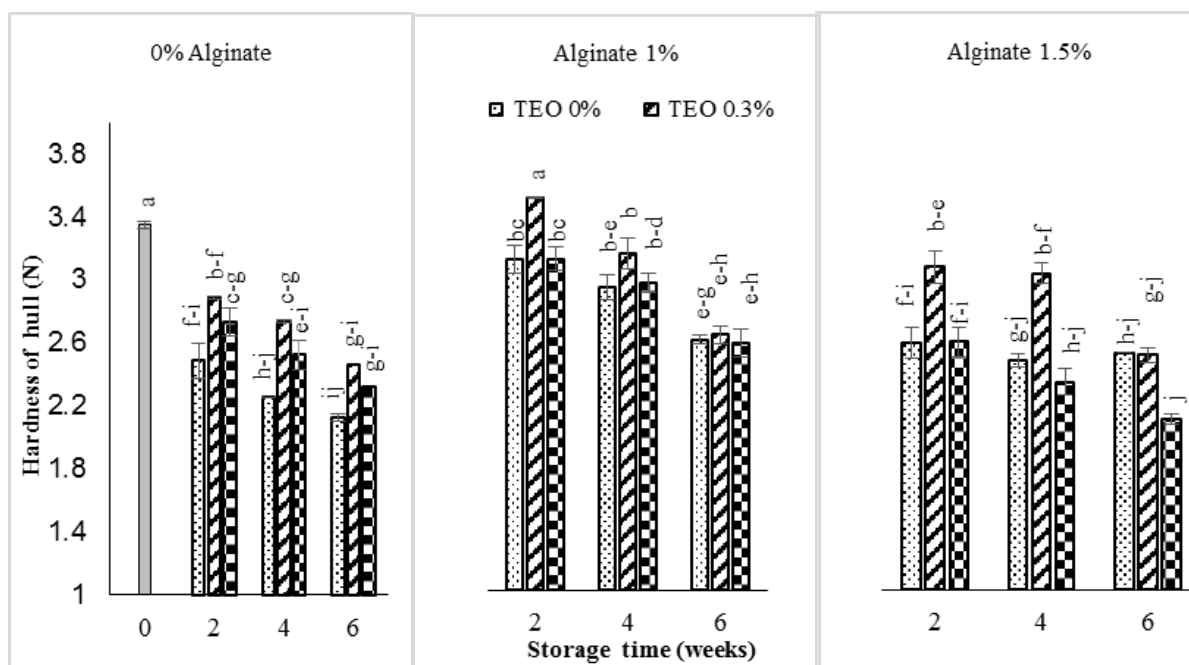
The edible coatings application can be regarded as a way to improve and increase shelf-life of the fruit (Tavassoli-Kafrani *et al.*, 2016). Various reports also indicated reduced weight of the fruits coated with polysaccharides (Yousuf *et al.*, 2018, Khaliq, *et al.*, 2015, Khaliq, *et al.*, 2016, Dong and Wang, 2017, Oregel-Zamudio *et al.*, 2017).

According to Rojas-Graü, *et al.* (2009),

application of alginate coating in combination with sunflower oil was effective in decreasing apple fresh cut moisture content during storage. Edible coating acts as an outer layer that reduces evaporation and transpiration. So far, beneficial effects of coatings on weight loss of a wide range of fruits including apricot (Ayrançi and Tunc, 2004), peach (Maftoonazad, *et al.*, 2008), strawberry (Díaz-Mula, *et al.*, 2012), and litchi (Lin, *et al.*, 2011) have been confirmed.

### Hardness of Pistachio Hull

The results of hardness evaluation of the fresh pistachio hull are shown in Figure 3. During the 2<sup>nd</sup> week of storage, 1% alginate treatment alone and combined with TEO 0.3 and 0.5%, as well as 1.5% alginate treatment combined with TEO 0.3% significantly increased the hardness of pistachio hull when compared to control (24.9 N) and other treatment samples. According to results, on the 4<sup>th</sup> week of storage, the fresh



**Figure 3.** Effects of Alginate coatings enriched with TEO on fresh hull hardness (N) of freshly stored pistachios at  $3\pm 1^{\circ}\text{C}$  and relative humidity of  $80\pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P\leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).

hull hardness decreased in all treatments, compared to the 2<sup>nd</sup> week of storage. The treatments mentioned on the 2<sup>nd</sup> and 4<sup>th</sup> week showed significantly higher hull hardness in comparison with the control (22.6 N) and other treatments. Finally, on the 6<sup>th</sup> week of storage, the treatments containing alginate 1% (26.0 N), alginate 1% combined with 0.3% essential oil (26.3 N), and alginate 1% combined with 0.5% essential oil (25.7 N) showed significantly higher hull hardness than control (21.3 N) and other treatment sample. Reducing the hardness of texture during storage is one of the major factors in reducing the quality and shelf life after harvesting the fresh product. During the storage period, breakage of the cell wall and the middle blade will reduce the hardness of the products (Li and Thomas, 2014).

The stiffness of fresh produce decreases during the post-harvest period. During storage, enzymes such as pectin-methyl esterase, polygalacturonase, and cellulose break down cell walls, thereby reducing product stiffness. Also, as the cell wall of the parenchymal skin cells decomposes, the stiffness decreases (Perkins-Veazie, 1995).

Decreased fruit firmness during the ripening period, along with a shortening of the pectin chain, is due to an increase in the activity of the enzymes pectin, methyl esterase, and polygalacturonase (Desai and Park, 2006). Many post-harvest treatments can be delayed to reduce stiffness, one of which is the use of edible coating. In a report, the use of chitosan coating reduced respiration rate and ethylene production of fruits during storage, thus increasing the shelf life of the product. This may be due to an increase in the concentration of internal carbon dioxide and a decrease in the concentration of internal oxygen. Also, a decrease in the activity of these enzymes may be due to a decrease in the concentration of oxygen due to the edible coating and, therefore, maintaining the firmness of the fruits (Vargas, *et al.*, 2006, Hernández-Muñoz, *et al.*, 2006). In the present study, more firmness in coated fruits

than in control may be related to lower activity of these enzymes due to less oxygen.

Fruit coating preserves integrity of the cell by reducing cell wall decomposition (Carvalho, *et al.*, 2016). The results of this study showed that covering fresh pistachios improves pistachio texture and reduces changes during storage, while reducing the stiffness of control samples may be due to increased activity of pectin, methyl esterase and polygalacturonase enzymes, resulting in reduced fruit firmness (Desai and Park, 2006, Siddig *et al.* 2012). The presence of sunflower oil in alginate formulations showed that sunflower oil can maintain moisture as well as product texture (Azarakhsh *et al.*, 2014).

Maintaining fruit hardness is one of the most important qualitative parameters of fruit during storage. The hardness of strawberry (*Arbutus unedo*) coated with alginates alone and combined with eugenol and citral essential oils was better than control (Guerreiro *et al.*, 2015). Fruit coating with sodium alginate retains the hardness of pineapple fresh cut (Azarakhsh *et al.*, 2014) and melon fresh cut (Raybaudi-Massilia *et al.*, 2008). Polysaccharide-based edible coatings combined with calcium chloride also maintains the firmness of fresh cut fruit (Moreira *et al.*, 2015). Calcium chloride can maintain the hardness of fruit texture by reacting with pectic acid in cell wall to form calcium pectate, which strengthens molecular bonds between the cell wall components (Chong *et al.*, 2015).

### Pistachio Hull Color

According to the results of interactions comparison between storage time and concentration of alginate edible coating (Figure 4), fruits covered with alginates 1 and 1.5% were lighter in the 2<sup>nd</sup> and 4<sup>th</sup> weeks, as compared to their color at the beginning. During the remaining storage weeks,  $L^*$  index decreased, but this reduction was not significant in alginate



concentrations of 1 and 1.5% in each of the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> storage weeks; however, significant differences were observed in the uncoated treatment (control) samples during the storage weeks. Also, during the last storage week (week 6), the highest and lowest  $L^*$  values of fresh fruits were observed in the fruits covered with alginate 1% (68.7 %) and control samples (42.4%), respectively. Besides, the effects of interaction between storage time and concentration of TEO (Figure 4) showed that, during the 2<sup>nd</sup> and 4<sup>th</sup> weeks of storage,  $L^*$  value increased compared to the week 0. There were no significant differences among the control treatments (without TEO), a concentration of 0.3 and 0.5% TEO on the 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> storage weeks. The treatments of 0.3% essential oil were lighter (60.9 and 62.0%) than 0.5% essential oil of thyme (51.3%). The essential oil had a negative effect on the fruit at a higher concentration, destroying the cells and making the color of the fruit duller.

Total color difference ( $\Delta E$ ) of uncoated samples increased significantly after 6 weeks of storage, while in the coated fruit, the slope showed a mild increase. The lowest total change (4.1) at the end of the experiment (week 6) was associated with fruit coated with alginate 1% combined with TEO 0.3% (Figure 5). Changes in color are one of the most important changes in fresh fruit during storage, which directly affect consumer's acceptance of the product. With respect to obtained results, fruit coating significantly reduced the fruits' weight, in comparison with uncoated fruit. Fruit coating with sodium alginate retains the color of plum and pineapple fruit cut (Azarakhsh et al., 2014, Valero et al., 2013) more than uncoated fruit, which may be due to delayed aging caused by protective effect of alginate coating. They also reported that, after adding lemongrass and vanillin oils to coating, color changes and color protection were more desirable, in comparison with control samples. In this study, coverage of enriched alginate with TEO showed a better color protection than 0.3%.

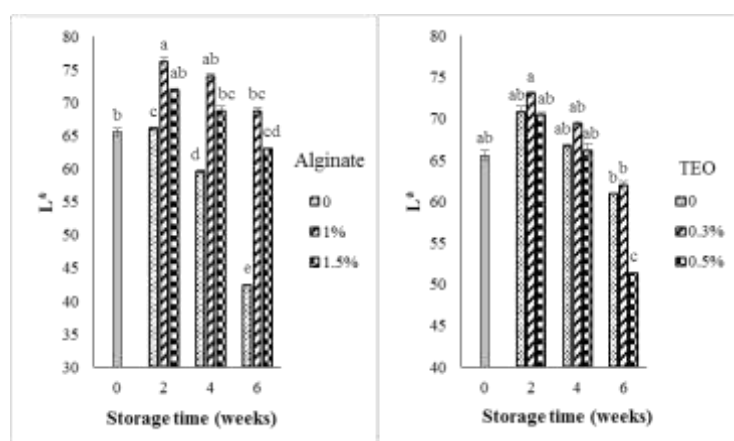
In one study, it was found that the use of essential oil-enriched food coatings on the edible fungus showed better  $L$ ,  $a$ , and  $b$  color indices, as well as fewer color changes. These results were directly related to the lower activity of polyphenol oxidase enzyme compared to control samples. The results show that the ability of coatings to act as a barrier to oxygen required for brown reactions is effective. Also, the presence of essential oils in the coating as an antimicrobial agent can prevent the growth of some bacteria, including *Pseudomonas tolaasii*, which cause brown spots on the surface of the fungus. Therefore, by reducing the activity of this enzyme by the coatings,  $L$  index increased. The use of chitosan coating containing salicylic acid on fresh pistachios was associated with a decrease in the activity of the polyphenol oxidase enzyme, which improved the color indices of  $L^*$ ,  $a^*$ , and  $b^*$  and better fresh hull. This coating acts as a barrier against gases and can reduce the browning reaction (Molamohammadi et al., 2020).

The reduction of  $L^*$ ,  $a^*$ , and  $b^*$  indicates an increase in the decomposition of anthocyanin pigments in the pistachio hull and browning reactions (Tomaino et al., 2010). The presence of oxygen can increase fruit browning reaction due to anthocyanins degradation and phenolic compounds oxidation by the enzymes (Kumari, et al., 2015).

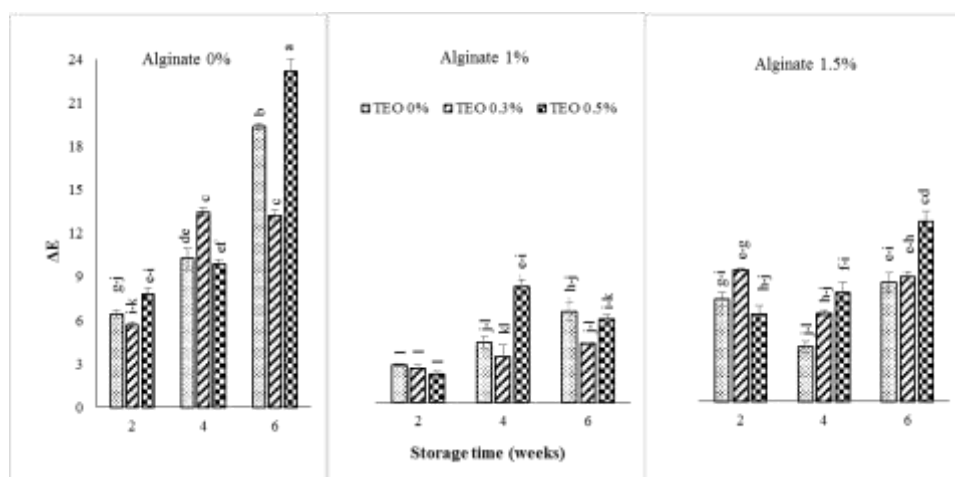
### Soluble Sugars

The effects of various concentrations of sodium alginate edible coating on the amount of kernel soluble sugars during storage weeks are shown in Figure 6. There was no significant difference between control treatments (without coatings) and coated treatments on the 2<sup>nd</sup> and 4<sup>th</sup> storage weeks. On the 6<sup>th</sup> week of storage, the difference between treatments and control samples was significant: 1% alginate with 9.02 g 100 g<sup>-1</sup> and 1.5% alginate with 9.00 g 100 g<sup>-1</sup> showed the highest soluble sugar,

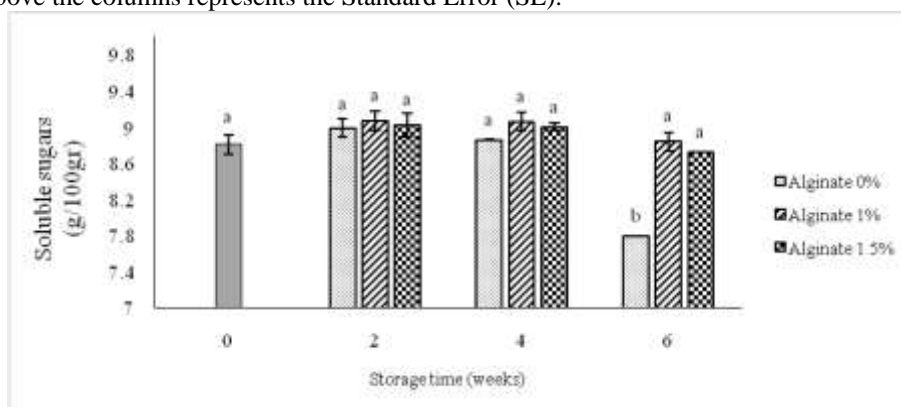




**Figure 4.** Effects of Alginate coatings (left) and TEO (right) on  $L^*$  fresh pistachios stored at  $3\pm 1^\circ\text{C}$  and  $80\pm 5\%$  RH. (\*) The columns with common alphabets have no significant difference ( $P \leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



**Figure 5.** Effects of Alginate coatings and TEO on  $\Delta E$  (total color variation) fresh pistachios stored at  $3\pm 1^\circ\text{C}$  temperature and  $80\pm 5\%$  RH. (\*) The columns with common alphabets have no significant difference ( $P \leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



**Figure 6.** Interaction effects of storage weeks and alginate coating on soluble sugars of fresh pistachios stored at  $3 \pm 1^\circ\text{C}$  and relative humidity  $80 \pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P \leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



compared to the control samples (8.72 g 100 g<sup>-1</sup>).

According to Chrysargyris, *et al.* (2016), the amount of soluble sugars in coated tomatoes was higher than control samples, which might be due to the creation of atmosphere controlled by fruit over the edible coating that ultimately reduced breathing and transpiration.

Kernel quality indices included the weight, moisture content, oil quality and composition. Sugar composition is necessary for good flavor and taste. Protein, oil, sugar, and other components of nut kernel influence its industrial use. The main sugar in pistachio kernel is sucrose, followed by glucose, fructose and inositol and total sugars reached a peak of 43.3% of dry weight early in the development of the kernel, but then decreased to 7% at nut maturity (Kazankaya *et al.*, 2008).

### Total chlorophyll content

Table 1 shows changes in the total chlorophyll content during 6 weeks of storage. Significant differences were observed in the total chlorophyll content between coated and uncoated fruits during 2 weeks of storage. The control and uncoated fruits, immersed in oil, showed a higher total chlorophyll content than other treatments during 2 weeks of storage. Coated fruits with 1% alginate combined with 0.3 and 0.5% TEO and coated fruits with 1.5% alginate enriched with 0.3% TEO had higher chlorophyll contents than the samples of other treatments after 4 and 6 weeks of cold storage (Table 1).

The alginate, enriched with essential oil, has antioxidant properties and is able to preserve phenolic compounds and total chlorophyll content. The modified atmosphere created by the Aloe vera gel coating material delayed the ethylene production rate, chlorophyll degradation, and as a result, color change of fruits (Misir, *et al.*, 2014).

**Table 1.** Effect of Alginate coatings enriched with TEO on the total chlorophyll (mg 100 g<sup>-1</sup>) content of fresh pistachio stored at 3±1°C, 80±5% RH.

Storage week	Control	A0TEO1*	A0TEO2	A1TEO0	A1TEO1	A1TEO2	A2TEO0	A2TEO1	A2TEO2
Total chlorophyll	1.11±0.01 ab	1.07±0.01 b	1.26±0.01 a	0.39±0.04 c-e	0.531±0.005 cd	0.43±0.00 f-h	0.40±0.05 f-h	0.42±0.01 c-g	0.40±0.22 f-h
	0.23±0.01 e-g	0.45±0.04 e-h	0.44±0.05 h	0.67±0.03 c-f	0.608±0.036 c	0.52±0.04 c-g	0.54±0.04 f-h	0.69±0.03 c	0.46±0.00 c-g
	0.38±0.02 gh	0.48±0.00 d-g	0.38±0.01 f-h	0.61±0.02 eh	0.571±0.05 c-g	0.50±0.00 d-g	0.39±0.04 f-h	0.55±0.03 e-h	0.39±0.01 f-h

\* A: Alginate edible coating (0:0, 1:1.0, 2: 1.5%); TEO: Thyme Essential Oil (0:0, 1:0.3, 2:0.5%).<sup>a-h</sup> Values with similar letters are not significantly different (P<0.05). Data represent mean values±SE.

Vishwasrao and Ananthanarayan (2016) reported that guava coated with Hydroxyl Propyl Methyl Cellulose (HPMC) show a slow decrease in total chlorophyll content in comparison with the control during storage. Also, Nair *et al.* (2018) reported that guava coated with chitosan and alginate coating enriched with pomegranate peel extract showed antioxidant activity and total phenolic compounds higher in comparison control samples. Joshi *et al.* (2017) mentioned that the chlorophyll content in papaya fruits coated with gum ghatti (*Anogeissus latifolia*), enriched with clove oil, decreased gradually during the ripening stage and the use of coating can delay the degradation of chlorophyll.

### Microbial Evaluation

The results presented in Table 2 show the effect of treatments on microbial activity in PCA culture media. Samples treated with 1% alginate with TEO (0.3 and 0.5%), respectively, had a value of 1.308 and 1.544 (log CFU g<sup>-1</sup>), which exhibited significantly

less amounts of contamination compared to the control (3.89 log CFU g<sup>-1</sup>) and other treatments.

The comparison of mean values in Table 3 shows that during the storage time, the activity of mold and yeast increased. On the second week of storage, all treatments showed less growth of mold and yeast compared with samples of the control and samples treated with 0.3% TEO. On week 4 of the storage period, fruits covered with 1% alginate and 1% alginate enriched with TEO of 0.3 and 0.5% concentrations, as well as 1.5% alginate enriched with TEO of 0.3 and 0.5% showed significantly less growths of mold and yeast. At the end of storage (week 6), the combination of 1% alginate with TEO at 0.3 and 0.5% concentrations, as well as 1.5% alginate in combination with 0.5% TEO, significantly reduced the growth of mold and yeast on the DRBC medium in comparison with the control (4.17 log CFU g<sup>-1</sup>). Food microorganisms are one of the main causes of deterioration among fresh fruits (Antunes and Cavaco, 2010). Edible coatings can protect the fruit from pathogens by creating a coating on the surface of the

**Table 2.** Interaction effects of alginate coating enriched with Thyme Essential Oil (TEO) on aerobic mesophilic bacteria (log CFU g<sup>-1</sup>) of fresh pistachios stored at 3±1°C and relative humidity of 80±5%.

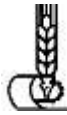
TEO (%)	Alginate (0%)	Alginate (1%)	Alginate (1.5 %)
0%	3.9 ± 0.0 a <sup>a</sup>	3.2 ± 0.1 a	3.0 ± 0.1 a
0.3%	3.7 ± 0.1 a	1.3 ± 0.0 b	2.7 ± 0.1 ab
0.5%	3.5 ± 0.1 a	1.5 ± 0.0 b	3.2 ± 0.1 a

<sup>a</sup> a-b Values with similar letters are not significantly different (P < 0.05). Data represent mean values±SE.

**Table 3.** Interaction effects of alginate coating enriched with Thyme Essential Oil (TEO) on mold and yeast growth (log CFU g<sup>-1</sup>) of fresh pistachios stored at 3±1°C and relative humidity of 80±5%.

Treatment <sup>a</sup> /Storage time (Week)	0	2	4	6
Control	1.0 ± 0.0cd	3.0 ± 0.4ab	3.5 ± 0.1 ab	4.2 ± 0.0 a
A0TEO1	1.0 ± 0.0cd	3.0 ± 0.3 ab	3.1 ± 0.1 ab	3.1 ± 0.1 ab
A0TEO2	1.0 ± 0.0cd	1.2 ± 0.1 cd	3.1 ± 0.1 ab	3.8 ± 0.2 ab
A1TEO0	1.0 ± 0.0cd	1.2 ± 0.1 cd	1.2 ± 0.01 cd	3.7 ± 0.0 ab
A1TEO1	1.0 ± 0.0cd	1.0 ± 0.1 cd	1.1 ± 0.1 cd	2.2 ± 0.1 bc
A1TEO2	1.0 ± 0.0cd	1.0 ± 0.1 cd	1.0 ± 0.1 cd	2.3 ± 0.2 bc
A2TEO0	1.0 ± 0.0cd	1.2 ± 0.1cd	3.2 ± 0.2 ab	3.7 ± 0.2 ab
A2TEO1	1.0 ± 0.0cd	1.0 ± 0.1cd	2.1 ± 0.1cd	3.8 ± 0.1 ab
A2TEO2	1.0 ± 0.0cd	1.0 ± 0.1 cd	1.0 ± 0.1 cd	2.2 ± 0.1 bc

<sup>a</sup> A: Alginate edible coating (0:0, 1:1.0, 2:1.5%); TEO: Thyme Essential Oil (0: 0, 1:0.3, 2:0.5%). a-d Values with similar letters are not significantly different (P < 0.05). Data represent mean values±SE.



fruit, and since some herbal essential oils have antimicrobial effects, the inclusion of herbal essential oils in edible coatings improves this quality (Raybaudi-Massilia *et al.*, 2008). Fruit coating with sodium alginate in combination with lemongrass essential oils reduced the growth of mold, yeast, and bacteria in slices of the apple fruit (Rojas-Graü *et al.*, 2007) and pineapple slices (Azarakhsh *et al.*, 2014). Guerreiro *et al.* (2015) stated that the use of alginate coating in combination with eugenol and citral oil reduced microbial growth during fruit storage (*Arbutus unedo* L.). Furthermore, Rojas-Graü *et al.* (2007) and Azarakhsh *et al.* (2014) found that the alginate edible coating cannot reduce microbial growth effectively when used alone, but it controlled microbial contamination when herbal essential oils were added. The 1% alginate treatment was more effective than the 1.5% concentration, which may be due to the higher carbohydrate content of alginate at 1.5%. Higher carbohydrate content provides more suitability for the growth of microorganisms. Also, as Buchanan and Shepherd (1981) reported, thyme oil can reduce bacterial growth due to the presence of thymol and

carvacrol, which are very effective antimicrobial components in essential oils.

### Sensory Evaluation

The effects of alginate and TEO on the hull color changes of treated pistachios (Table 4) showed that the best scoring by panelists was related to pistachios coated with 1% alginate in combination with TEO 0.3 and 0.5% (12.3 and 10.6), which was significantly higher than control samples (6.4). Among the rest of the treatments and the control samples, there was no significant difference, but the treatments showed less color variation than control samples.

Comparison of the effects of storage time on the hard shell color (darkness) of treated pistachios showed that, during storage, the amount of hard shell darkness increased and there was a significant difference between storage times (Figure 7). Also, the least darkness of hard shell (2.4) was observed by the application of 1% alginate coating enriched with 0.5% thyme essential oil on the surface of fresh pistachios. Edible coating of 1.5% of alginate did not show any significant difference with control and the

**Table 4.** Effects of interaction of Alginate coatings with TEO on sensory properties of pistachios at  $3\pm 1^\circ\text{C}$  and relative humidity  $80\pm 5\%$ .

Sensory properties	TEO (%)	Edible coating		
		Alginate (0%)	Alginate (1%)	Alginate (1.5%)
Hull color	0.0	6.4 ± 1.2 c <sup>a</sup>	9.6 ± 0.4 abc	9.8 ± 0.2 abc
	0.3	8.5 ± 0.2 abc	12.3 ± 0.5 a	8.8 ± 0.0 abc
	0.5	8.1 ± 0.8 bc	10.6 ± 0.4 ab	7.7 ± 0.1 bc
Hard shell color (Darkness)	0.0	3.2 ± 0.3 a	3.6 ± 0.4 a	3.8 ± 0.2 a
	0.3	2.7 ± 0.1 a	2.5 ± 0.4 a	3.7 ± 0.2 a
	0.5	2.4 ± 0.0 a	3.3 ± 0.4 a	3.4 ± 0.3 a
Fresh pistachio aroma	0.0	8.3 ± 0.7 a	8.8 ± 0.6 a	9.5 ± 0.4 a
	0.3	9.3 ± 0.7 a	9.9 ± 0.5 a	9.5 ± 0.1 a
	0.5	9.3 ± 0.3 a	9.5 ± 0.2 a	8.8 ± 0.4 a
Unfavorable Odor	0.0	2.6 ± 0.4 ab	1.4 ± 0.2 bc	1.7 ± 0.4 bc
	0.3	1.3 ± 0.1 bc	1.0 ± 0.4 c	3.3 ± 0.4 a
	0.5	3.3 ± 0.3 a	1.1 ± 0.1 c	3.4 ± 0.3 a
Texture (Juiciness)	0.0	7.3 ± 0.2 a	8.0 ± 0.2 a	8.1 ± 0.2 a
	0.3	7.8 ± 0.3 a	8.3 ± 0.4 a	8.3 ± 0.4 a
	0.5	7.5 ± 0.1 a	7.9 ± 0.0 a	7.4 ± 0.0 a

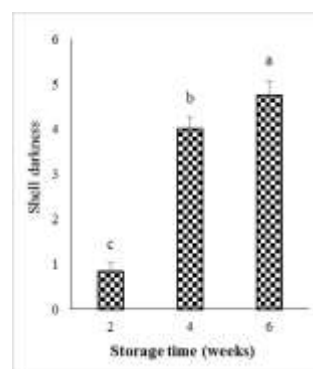
<sup>a</sup> a-c Values with similar letters are not significantly different ( $P < 0.05$ ).

highest hard shell darkness (3.8 %) (Table 4).

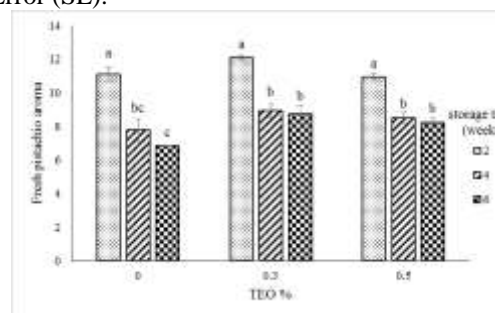
The results of the effects of different concentrations of TEO on fresh pistachios aroma (Figure 8) indicate that fresh pistachio aroma decreases during the storage weeks, but the slope of this decrease is steeper in treated samples without essence (control). There was no significant difference between control treatment and 0.3 and 0.5% concentration of TEO on the 2<sup>nd</sup> and 4<sup>th</sup> storage weeks. However, during the 6<sup>th</sup> storage week, essential oil treatments of 0.3% and 0.5% showed a new score of 8.7 and 8.2 compared with control samples (6.8), respectively. The juiciness score of the texture decreased during the storage but the reduction was less in pistachios coated with 1% alginates in uncoated fruit. According to Figure 9 and Table 4, on the 2<sup>nd</sup> week of storage, although there was no significant difference between different concentrations of alginate coating, the highest moisture content score (9.0) was observed at the concentration of 1% alginate. Also, on the 4<sup>th</sup> and 6<sup>th</sup> weeks of storage, 1% alginate treatment with 8.5 and 7.6 moisture content score was significantly higher than the control score (7.0 and 6.5).

According to Table 4, the fruit coated with 1% alginate with 0.3% and 0.5% concentration of TEO showed the lowest unfavorable odor (1.0 and 1.1, respectively). The results indicate that although treated samples showed no statistically significant difference with control samples (score of 2.6), the fruit coated with 1.5 % alginate enriched with TEO 0.3 and 0.5% obtained the highest unpleasant odor score by panelists with 3.3 and 3.4, respectively.

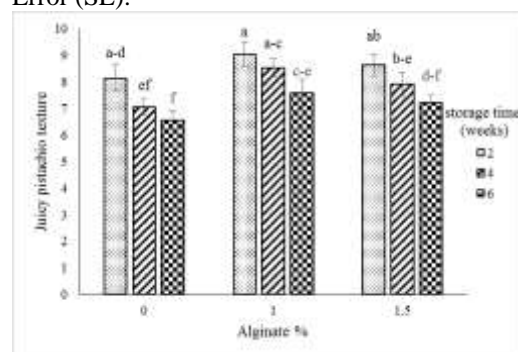
It is an important issue for consumers to accept fruits treated with edible coatings because these coatings can change sensory properties of the fruit. Sensory evaluation is of great importance because treatments and storage time can change the quality of the fruit, as well (Rojas-Graü, *et al.*, 2009). There is no doubt that alginate edible coating combined with TEO improves storage time and assessment of panelists on



**Figure 7.** Effects of storage time on the hard shell darkness of fresh pistachios stored at  $3\pm 1^{\circ}\text{C}$  and relative humidity  $80\pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P\leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



**Figure 8.** Effects of interaction of storage time and TEO on the aroma of fresh pistachios at  $3\pm 1^{\circ}\text{C}$  and relative humidity  $80\pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P\leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



**Figure 9.** Effects of interaction of storage time and TEO on the juicy texture of fresh pistachios at  $3\pm 1^{\circ}\text{C}$  and relative humidity  $80\pm 5\%$ . (\*) The columns with common alphabets have no significant difference ( $P\leq 0.05$ ). (\*\*) The bar above the columns represents the Standard Error (SE).



freshly treated pistachio fruit compared to control fruit. Similar results were observed for coating of alginates combined with lemongrass essential oil used on the pineapple fresh cut by Azarakhsh, *et al.* (2014). In this study, TEO (0.3%) in the alginate coating improved storage life and did not change the sensory properties. The fruit coated with 1.5% alginate enriched with TEO 0.3% and 0.5% obtained the highest unpleasant odor by panelists with 3.3 and 3.4, respectively. According to the results of other researchers, there is no significant difference in sensory properties of fruit coated by adding low-level essential oil to alginate, while in high concentrations, TEO reduces sensory properties. Du *et al.* (1997) showed that, with the use of edible coatings, the amount of internal carbon dioxide increased but the amount of oxygen decreased, and resulted in preserving the quality of fruit during storage.

### CONCLUSIONS

The results of this study indicated that, during the storage period, the quality properties (weight loss, hull and hard shell color, hull hardness, soluble sugar, total chlorophyll, total soluble solids and microbial growth) and sensory evaluation properties of fresh pistachios changed. In all treatments, weight loss increased during storage time, but this increase was less in alginate treated pistachios. The results showed that edible coating of fresh pistachios improved pistachio texture and reduced changes during storage. On the 6<sup>th</sup> week of storage, the 1% alginate and 1.5 % alginate showed the highest soluble sugar, compared to the control samples. Also, the combination of 1% alginate with TEO at 0.3 and 0.5% concentrations, as well as 1.5% alginate in combination with 0.5% TEO, significantly reduced the growth of mold and yeast in comparison with the control. The best hull color score and chlorophyll contents were found in pistachios coated

with 1 % alginate in combination with TEO 0.3 and 0.5%.

It can be concluded that, during storage, the use of 1% alginate coating enriched with 0.3 and 0.5% TEO can extend the shelf life and preserve the natural characteristics of fresh pistachios compared to the control and alginate treatment without TEO.

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## افزایش کیفیت میوه تازه پسته با استفاده از سدیم آلزینات غنی شده با اسانس آویشن شیرازی

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### چکیده

اثرات پوشش ترکیبی آلزینات سدیم (SA) و اسانس آویشن (TEO) به ترتیب در سه سطح (۰/۰، ۱/۰ و ۱/۵٪) و (۰/۰، ۰/۳ و ۰/۵٪) طی ۶ هفته انبارداری سرد (۳ ± ۱ درجه سانتیگراد) پسته تازه بررسی شد. پارامترهای فیزیکوشیمیایی (رنگ پوست نرم رویی و پوست استخوانی، سختی پوست نرم رویی، قند محلول، کلروفیل و مواد جامد محلول) و بار میکروبی (پلیت کانت آگار) هر دو هفته یک بار اندازه گیری شد. به طور مشابه، ویژگیهای حسی پسته توسط ارزیابی های آموزش دیده و روش آنالیز توصیفی کمی (QDA) از نمره حداقل (۱) تا حداکثر (۱۵) ارزیابی شد. در حالی که کاهش وزن هر نمونه پسته در مدت زمان انبارداری افزایش یافته است، اما تیمارهای پوشش داده شده با آلزینات نسبت به سایر تیمارها،



کاهش وزن قابل توجه کمتری داشتند. پسته پوشش داده شده با ترکیبی از SA (۱ درصد) و TEO (۰/۳ یا ۰/۵ درصد) میزان سختی پوست، مقادیر  $L^*$ ، قند محلول، کلروفیل و رشد پایین تر میکروارگانیسم ها (عمدتا مخمر و کپک) را نسبت به سایر تیمارها و نمونه پوشش داده نشده (شاهد) نشان داد. بالاترین نمرات ارزیابی حسی ۱۲/۳ و ۱۰/۶ برای رنگ پوست نرم رویی پسته به ترتیب مربوط به نمونه های پوشش داده شده با ترکیبات SA (۱ درصد) و TEO (۰/۳ یا ۰/۵ درصد) بود و این نمرات حسی در مقایسه با نمره نمونه شاهد (۶/۴) به طور قابل توجه بالاتر بود. از آنجا که پسته پوشش داده شده با غلظت های یاد شده SA و TEO از لحاظ خواص فیزیکوشیمیایی، میکروبیولوژیکی و حسی به طور قابل توجهی بالاتر از سایر نمونه ها بود، شاخص های کیفیت آنها نسبت به سایر تیمارها (از جمله نمونه های شاهد) برتر بوده و به احتمال زیاد عمر ماندگاری طولانی تر داشته و پتانسیل بیشتری برای حفظ خصوصیات طبیعی پسته تازه طی انبارداری دارند.