

Incorporation of *Zataria multiflora* Boiss essential oil into gum Arabic edible coating to maintain the quality properties of fresh in-hull pistachio (*Pistacia vera* L.)

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ABSTRACT

Edible coatings of gum Arabic (GA) (6.0 and 8.0 %) with Shirazi thyme (*Zataria multiflora*) (0.3 and 0.5 %) were assessed as potential postharvest treatments to protect the quality of fresh in-hull pistachio when stored at 85 ± 5 % RH and 2 ± 1 °C for up to 36 days. The results showed that 6 % GA combined with thyme at the concentrations of 0.3 and 0.5 % decreased color change and PPO activity of fresh pistachio in comparison with untreated control fruits. Treated fruit (6 % GA alone with the addition of 0.3 % thyme) also had higher PAL activity and total phenolics. Higher free fatty acid and peroxide value were also found in fruits coated with GA (8 %) containing thyme (0.5 %). Both 0.3 % thyme and 6 % GA solutions were found to be the most effective on saturated and unsaturated fatty acids of kernel.

1. Introduction

Pistachio (*Pistacia vera* L.) nuts are considered a healthy snack which contain high levels of essential minerals, phenolics, essential fatty acids, protein and vitamin A (Ozturk, Sagdic, Yalcin, Capar, & Asyali, 2016). The dried shelled edible part of the pistachio is kernel / nut which is commonly marketed around the world, but consumption of fresh pistachio is also increasing due to its unique taste, high level of phytochemical antioxidants and nutritional value (Ozturk et al., 2016; Sheikhi, Mirdehghan, & Ferguson, 2019). Production stages that span from harvest and marketing through to consumer purchase are characterized by postharvest loss. In developing countries, the rate of this loss is 20–50 %. However, these percentages depend largely on the kind of commodity, plant cultivar, environmental parameters and postharvest circumstances (Yahia, Fonseca, & Kitinoja, 2019). After harvesting, fresh pistachios begin to rapidly senesce with browning of hull and decay which leads to the loss of its market value (Gheysarbigi, Mirdehghan, Ghasemnezhad, & Nazoori, 2020). As endogenous polyphenols are oxidized, hull browning becomes evident. The mechanism

of hull browning involves ortho-diphenols conversion into semiquinones and quinones (Pristijono, Wills, & Golding, 2006). Fruits gradually become brown because PPO enzyme functions in fruit cells (Gheysarbigi et al., 2020). Postharvest browning is a common color reaction that occurs in fresh fruits and vegetables, generally due to the uncontrolled interaction of polyphenols, oxygen and polyphenol oxidase (PPO) enzyme (Pristijono et al., 2006). Browning reaction in fresh products often reduces visual quality and results in the loss of nutrients and flavor compounds and reduction of consumer acceptability (Luo & Barbosa-Cánovas, 1997). Indeed, postharvest browning of the external hull of fresh pistachio is a key quality parameter during processing and storage (Tavakolipour & Kalbasi-Ashtari, 2008). Fresh pistachios cannot be exported and little research has been performed on this topic (Kazemi, Hashemi-Moghaddam, Mohammadi Nafchi, & Ajodnifar, 2020). One solution to this problem is edible coating, which has been used in several fruits (Hashemi et al., 2020; Shakerardekani, Hashemi, Shahedi, & Mirzaalian Dastjerdi, 2021; Alali, Awad, Al-Qurashi, & Mohamed, 2018; Etemadipoor, Dastjerdi, Ramezani, & Shamili, 2019; Etemadipoor, Dastjerdi, Ramezani, & Ehteshami, 2020). Biopolymer coatings have

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attracted great attention due to their potential use in food industry and environmentally friendly nature (Zahid, Ali, Manickam, Siddiqui, & Maqbool, 2012). Application of edible coatings has been shown to be an important postharvest strategy to minimize quality deterioration during storage, including postharvest browning (Synowiecki & Al-Khateeb, 2003). Edible coatings create a barrier between the atmosphere and products which reduces water loss and limits gas exchange in-turn reducing respiration rate and moisture loss from the fruit (Duan, Wu, Strik, & Zhao, 2011). Edible coatings also maintain product quality by creating modified atmosphere around the product and can reduce microbial growth (Benitez, Achaerandio, Pujola, & Sepulcre, 2015). GA is a natural polysaccharide obtained from the stem and branch of Gum acacia (*Acacia senegal* L.) tree (Maqbool, Ali, Alderson, & Zahid, 2011). GA biopolymer consists of galactose, glucuronic acid, rhamnose, and arabinose (Anderson, Millar, & Weiping, 1991). GA has been used for centuries in beverages, food and cosmetics, but more recently, it has been applied in pharmaceutical products (Patel & Goyal, 2015). GA is a hydrocolloid that displays low-viscosity at high concentration and excellent water solubility compared to other gums (Ali, Maqbool, Ramachandran, & Alderson, 2010). This polysaccharide shows unique emulsifying properties (Maqbool, Ali, Alderson & Zahid, 2011). GA has been used in the development of edible coatings as it is a natural food additive (Motlagh, Ravines, Karamallah, & Ma, 2006). For example, a GA film has been shown to help maintain higher total polyphenol and total antioxidant capacity in guava fruit (Etemadipoor et al., 2020) and banana (Alali et al., 2018). GA coatings have also been shown to reduce the browning of mango fruit during storage at low storage temperatures (Khaliq, Mohamed, Ali, Ding, & Ghazali, 2015). The quality and shelf life of several horticultural commodities are reportedly affected by the positive effect of GA as an antioxidant. Similar research works have been performed on 'Kinnow' mandarin (Khorram, Ramezani, & Hosseini, 2017), Mexican lime (Atrash, Ramezani, Rahemi, Ghalamfarsa, & Yahia, 2018) and guava (Gurjar, Killadi, Lenka, & Shukla, 2018).

The effectiveness of edible coatings is often improved by the addition of nutrients, antimicrobial and anti-browning agents, flavors and spices (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). Various essential oils (EOs) can be applied to manage postharvest diseases depending on their chemical structures and application strategy (Boubaker et al., 2016). For example, adding EOs to edible coatings has been shown to improve their functionality on fresh vegetables and fruits by enhancing the appearance, integrity, microbial safety, and mechanical strength of horticultural products (Murmu & Mishra, 2017). The benefits of EOs are their nontoxic nature, multi-purpose application, and wide consumer acceptance (Antunes & Cavaco, 2010). Mixing EOs with GA hinders fungi growth and protects fruit texture (Maqbool, Ali, Alderson & Zahid, 2011; Maqbool, Ali, Alderson, Mohamed et al., 2011). Shirazi thyme (*Zataria multiflora* Boiss.) grows in Iran, Pakistan and Afghanistan (Ali, Saleem, Ali, & Ahmad, 2000). Similar to other EOs, Shirazi thyme has some beneficial properties. For example, owing to its phenolic compounds, it has antimicrobial effects. Thymol, Carvacrol, and Eugenol are the main phenolic compounds of Shirazi thyme EO. Because of its antimicrobial and antioxidant properties, application of Shirazi thyme EO affects food preservation (Saei-Dehkordi, Tajik, Moradi, & Khalighi-Sigaroodi, 2010). Application of Shirazi thyme (*Zataria multiflora* Boiss.) EO in edible coatings such as alginate (Hashemi et al., 2020), chitosan (Moradi, Tajik, Rohani, Oromiehie, & Malekinejad, 2012) and carboxymethyl cellulose (Dashipour et al., 2015) have been shown to increase the total phenol content and antioxidant activities of coated fruits. EOs are rich in phenolic compounds which render their antioxidant and antimicrobial activity (Cardoso-Ugarte, López-Malo, & Sosa-Morales, 2016). The shelf life of fruits can be prolonged by adding EOs into edible coating formulations, while the benefit of this incorporation is that EOs are released gradually from edible coatings (Ouattara, Simard, Piette, Begin, & Holley, 2000).

Yahyaraeyat, Khosravi, Shahbazzadeh, & Khalaj, 2013 found that the incorporation of *Z. multiflora* into edible coatings has the potential

for further application in active biodegradable packaging materials. Indeed Dashipour et al. (2015) showed that carboxymethyl cellulose film containing *Zataria multiflora* EO inhibited and further delayed deterioration/oxidation and prevented *in vitro* microbial activity.

However, no research work has been reported on the combined application of GA edible coating and Shirazi thyme EO on fresh pistachios. As fresh pistachio fruit has poor storability, this study examined the effects of two formulations of GA by the addition of two concentrations of *Z. multiflora* EO on hull browning and other pistachio quality attributes during cold storage for up to 36 days.

2. Materials and methods

2.1. Materials

Fresh pistachio (*Pistacia vera* L.) fruit cv. 'Ahmad-Aghaei' was manually harvested on 10 October 2018 from healthy 30-year-old pistachio trees from an established orchard at Pistachio Research Center in Rafsanjan, Iran. Immediately after harvesting, fruits were transferred to the laboratory and fruits without cracks having uniform size, color and shape were separated from the clusters and used in this study.

GA (KB-120, Food Grade) and glycerol were purchased from Sigma-Aldrich Chemicals (Germany). *Zataria multiflora* EO was obtained from Barijessence Company (Kashan, Iran).

2.2. Preparation of the solutions of edible coating

GA edible coating was prepared according to the method of Ali et al. (2010). In summary, 6 and 8 g GA powder was dissolved in 0.1 L distilled water to prepare 6 and 8 % (w/v) edible coating solutions, respectively. The prepared solutions were heated to 40 °C for 60 min while stirring and 1.5 % w/v glycerol was added as a plasticizer. Solution pH was set to pH 5.6 using 1 N NaOH. 0.3 % and 0.5 % (w/v) Shirazi thyme EO was added to coating solutions based on the work of (Azaraksh, Osman, Ghazali, Tan, & Mohd Adzahan, 2014). Various edible coating formulations were prepared as follows: Control (fruit not coated); EO 0.3 %; EO 0.5 %; 6% GA; 8 % GA; 6 % GA + 0.3 % EO; 6 % GA + 0.5 % EO; 8 % GA + 0.3 % EO and 8 % GA + 0.5 % EO.

2.3. Edible coating treatments and storage

Edible coatings were applied to fresh pistachio fruit according to the method of Saberi et al. (2018). Fresh fruits were sprayed with a manual sprayer with each of the coating solutions and allowed to air-dry for 60 min at 25 ± 2 °C. Treatment units contained 200–250 g of pistachio fruit (approximately 60–70 fruits) which were placed in unsealed polypropylene containers (12 × 15.5 × 10.5 cm) after treatment and drying. To examine the effects of edible GA coating on storage life, uncoated fruits were also treated with 0 % (control), 0.3 % and 0.5 % (w/v) EO. Then, all pistachios were stored at 85 ± 5 % RH and 2 ± 1 °C for up to 36 days and quality determination was conducted every 12 days during cold storage.

2.4. Total phenolic content (TPC)

To estimate the TPC content of pistachio fruits, Folin-Ciocalteu method was applied (Ehteshami, Abdollahi, Ramezani, Rahimzadeh, & Dastjerdi, 2020). To do so, 500 mg pistachio hull was crushed and mixed with methanol (3 ml, 85 %) before being centrifuged at 12,000×g for 20 min at 4 °C. Then, 300 µL supernatant was mixed with 1200 µL sodium carbonate (7 %) and 1500 µL 10 % Folin-Ciocalteu reagent was added and shaken for 90 min at ambient temperature in dark. TPC was determined on a spectrophotometer (Biowave, WPA S2100, UK) and monitored at 760 nm where gallic acid was used as external standard.

2.5. DPPH radical scavenging activity

Radical scavenging method using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was applied for measuring antioxidant activity (AA) according to (Ehteshami, Abdollahi, Ramezani, Dastjerdi, & Rahimzadeh, 2019). In summary 0.1 ml hull extract, 1 ml Tris-HCl (pH 7.5) buffer and 1 ml DPPH (0.1 mM) were mixed and incubated in dark for 30 min. Spectrophotometer (Biowave, WPA S2100, UK) at 517 nm was applied to measure AA% using Eq. (1):

$$AA (\%) = [1 - (A \text{ sample}/A \text{ control})] \times 100 \quad (1)$$

2.6. Polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activity

PAL and PPO were measured in an extract from 500 mg frozen powdered sample of fresh hull. Hull sample was added to a phosphate buffer (1 ml, pH 7, 100 mM) containing 0.5 mM EDTA and 6 % (W/V) polyvinylpyrrolidone (PVP) and centrifuged (15,000×g, 20 min, 4 °C). Enzyme activities were determined from a subsample of supernatant.

PAL activity was measured by adding 100 µL supernatant to phosphate buffer (1 mL, 50 mM, pH 7), L-phenylalanine (500 µL, 10 mM) and water (400 µL). After incubating the mixture for 1 h at 40 °C, reaction was stopped by adding HCl (500 µL, 6 mM). PAL activity level was determined by recording absorbance at 290 nm, where change in absorbance was in response to the produced cinnamic acid level. The activity of enzyme was presented as unit/g fresh weight per min (Nguyen, Ketsa, & van Doorn, 2003).

PPO activity was measured according to Koushesh Saba, Arzani, and Barzegar (2012) where the as-prepared supernatant aliquots were mixed with solutions containing 2.5 mL phosphate buffer (pH 7, 100 mM) and 0.2 mL pyrogallol (0.02 M). Absorbance increase was measured at 420 nm and 25 °C for 1 min and enzyme activity was presented as unit/g fresh weight per min (Koushesh Saba et al., 2012).

2.7. Color measurement

The measurement of pistachio hull color was performed according to (Shakerardekani et al., 2021) by Chroma Meter color meter (Konica, Minolta CR-400 Japan). Color coordinates, including L*, a*, and b* values were measured for 15 fruits in each replication. Total color difference (ΔE) caused by sample covering was measured by Eq. (2):

$$\text{Total color difference } (\Delta E) = \sqrt{(L_t - L_0)^2 + (a_t - a_0)^2 + (b_t - b_0)^2} \quad (2)$$

where L₀, a₀, and b₀ are the initial values hull color index of fruits and L_t, a_t and b_t are corresponding values at different times.

2.8. Free fatty acid (FFA)

Neutralized alcohol (100 ml) was used for extracting kernel oil from the fruit samples (3.5 g). Then, 1 ml phenolphthalein (1 %) (w/v) was added to the mixture and heated to 65 °C. The mixture was stirred thoroughly to ensure homogeneity. Then, 1 N NaOH was used for titration until the solution acquired a stable pink color. The amount of NaOH used in the titration was recorded (Hashemi et al., 2020) and FFA was determined according to Eq. (3).

$$\text{FFA as \% oleic acid} = [(ml \text{ NaOH} \times \text{NaOH normality} \times 28.2) / (\text{weight of sample (g)})] \times 100 \quad (3)$$

2.9. Peroxide value (PV)

A sample of kernel oil (5 g) was dissolved in 30 ml 3:2 solution of acetic acid:chloroform and thoroughly stirred to ensure complete dissolution of oil. After thoroughly mixing, 0.5 ml saturated potassium

iodide solution was added and the resulting mixture was left in dark for 1 min, where it was intermittently shaken. Following this incubation stage, distilled water (30 ml), 0.1 N sodium carbonate (Na₂CO₃) and 0.1 N sodium thiosulfate (Na₂S₂O₃) were added. The solution was then titrated up to a point where no yellow color remained. The total volume of Na₂S₂O₃ being added was recorded. An indicator of titration (a starch solution) was used at 0.5 ml 1 % (w/v). Na₂S₂O₃ (containing 0.1 N Na₂CO₃) was slowed was added until the titration ended which was indicated by the loss of the violet color of reaction mixture (Hashemi et al., 2020). The values of PV were determined according to Eq. (4).

$$PV = [(S-B) \times N \times 1000] / W \quad (4)$$

where S is the amount of Na₂S₂O₃ (ml) required for titrating the sample, B denotes the amount of Na₂S₂O₃ (ml) required for blank, N is the normality of standardized Na₂S₂O₃ solution and W is the weight of sample (g)

2.10. Saturated and unsaturated fatty acids

Saturated and unsaturated fatty acid levels were determined by gas chromatography (GC) using a TRACE 1300 Series GC (Thermo Scientific, Italy) with a capillary column (internal diameter of 0.25 mm and length of 60 m). Fatty acids were calculated by extracting oil using cold press method from pistachio fruits, as described by Holcapek, Jandera, Zderadicka, and Hurba (2003). Fatty acid methyl esters were prepared according to Arena, Campisi, Fallico, and Maccarone (2007). Saturated and unsaturated fatty acids were detected using a mass-ion detector (Neuringer, Anderson, & Connor, 1988).

2.11. Sensory analysis

The sensory evaluation of pistachios was performed according to Ozturk et al. (2016). A hedonic sensory scale with 1–15 points was used, where “1” denoted least acceptable pistachios and “15” denoted highly acceptable pistachios. Other consumer attributes included hull appearance and color, taste, flavor, odor, texture, and juiciness and overall acceptability was assessed at each removal time by 12 trained panelists. 1 h training sessions were held for panelists twice a week for four weeks, accounting for a total of 8 h training. In these sessions, they learned to examine fresh nuts in terms of various flavor and taste characteristics and their intensities. The numbers of men and women were equal and their age range was from 35 to 45.

2.12. Statistical analysis

Experiments followed factorial design with randomized complete block design and three concentrations of GA (0, 6 and 8 %) and three concentrations of EO (0, 0.3 and 0.5 %) with three replications. The source of variation included edible coating, EO, and storage duration. SAS software (Ver, 9.1) was applied to perform analysis of variance. Means of each trait were compared to the findings of 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 and EO on TPC and DPPH radical scavenging activities of fresh pistachio hulls during cold storage are presented in Fig. 1A and B, respectively. TPC was increased after harvesting in 12 days assessment. After this time, the fruits treated with 6 % GA + 0.3 % EO before storage contained the highest total phenolic content (59 mg/100 g fresh weight). The addition of GA resulted in higher TPC after 36 days of storage than corresponding values obtained for untreated fruits. These fruits (6 % GA + 0.3 % EO) also presented

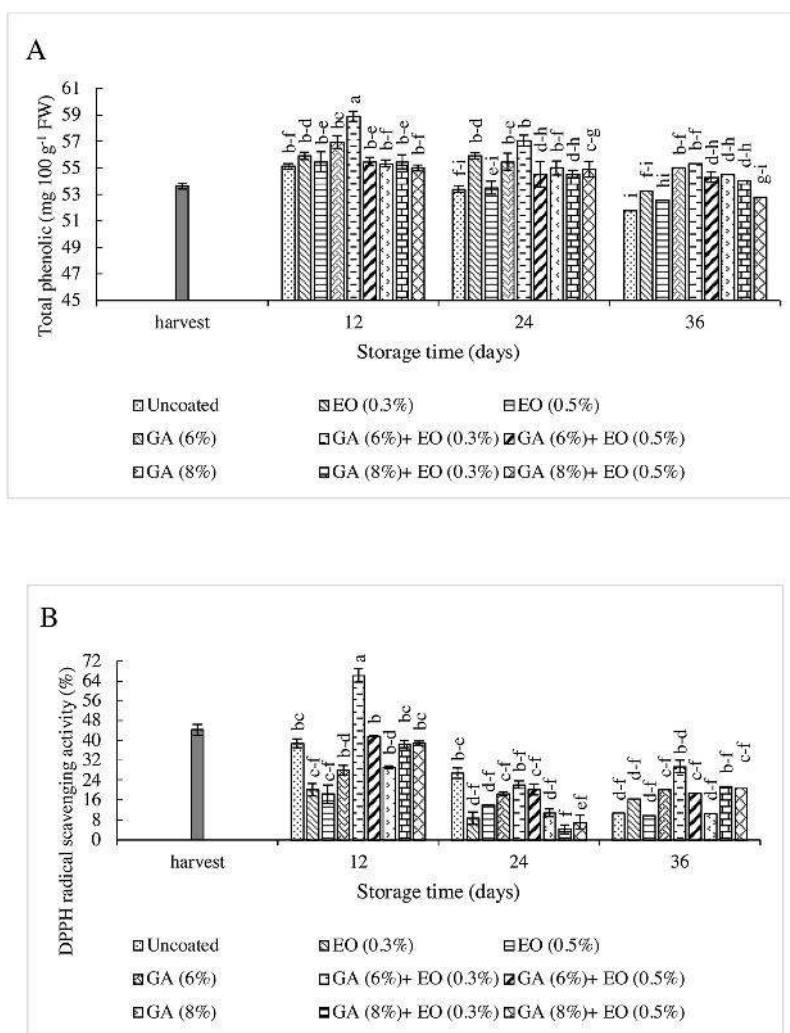


Fig. 1. Total phenol content (A) and DPPH radical scavenging activity (B) of fresh pistachio hull coated with gum arabic + *Zataria multiflora* during storage at 2 ± 1 °C. Means ($n = 3$) with the same letters are not significantly different according to Duncan test ($p > 0.05$). GA = gum arabic-based edible coating; EO = *Zataria multiflora*.

highest DPPH radical scavenging activity (Fig. 1B). However, the levels of DPPH were generally declined during storage across all treatments, while the levels of TPC tended to slightly decline during storage after initial increase in TPC after harvest. Decrease in phenolic content is a natural part of senescence which can also be due to cell wall degradation when fruit is stored over long periods (Ali et al., 2010). Nair, Saxena, and Kaur (2018) concluded that guava fruits coated with alginate and chitosan presented higher antioxidant activity than uncoated fruits. The application of GA treatment with thyme oil (0.25 or 0.5 %) decreased antioxidant activity loss during storage (Kawhena, Tsige, Opara, & Fawole, 2020). This could be due to secondary metabolite production by surface coating, resulting in the improvement of antioxidant activity (Wang & Gao, 2013). Also, modified internal atmospheres of coated fruits could be due to the decreased synthesis and metabolism of total flavonoids and phenolics (Elsabee & Abdou, 2013) which increases antioxidant capacity (Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, & Byrne, 2006).

The antioxidant contents of fresh fruits rely on various factors including analytical methods, cultivars, species, geographical origin, cultural practices, and environmental conditions (Chun et al., 2005). In this work, decrease in levels of DPPH complies with the earlier findings of Ali, Zahid, Manickam, Siddiqui, and Alderson (2014) and Ghazemzhad, Shiri, & Sanavi, 2010 who found that the reduction of total antioxidant activity in Dragon fruits and apricot were due to cell

structure breakdown during storage.

Other studies have shown that EOs such as basil seed gum (BSG) together with *Origanum vulgare* EO (2–6 %) resulted in increased antioxidant activity in coated apricot fruits (Hashemi, Khaneghah, Ghahfarrokhi, & Eş, 2017). Hashemi et al. (2020) reported that the application of *Zataria multiflora* Boiss EO-enriched sodium alginate coating on fresh pistachios resulted in higher TPC compared to control fruits. They also observed that sodium alginate combined with EO was able to maintain DPPH radical scavenging activity. Similarly, it has been reported that using GA in combination with EO can limit the activity of PPO and even other enzymes that degrade phenols by controlling the movement of oxygen to and from guava fruits (Etemadipoor et al., 2020). Ultimately, when degradative enzyme activities are reduced due to EO and the action of its components, antioxidant compounds are less affected and fruit quality is maintained (Sogvar, Saba, & Emamifar, 2016).

3.2. PPO and PAL activity

The activities of PPO and PAL enzymes in fresh pistachio hull are presented in Fig. 2A and B, respectively, and show that enzyme activity was significantly affected by GA coating and EO formulation. PPO activity in fresh hulls was increased in both treated and untreated fruits during storage, where the highest PPO activity was observed in

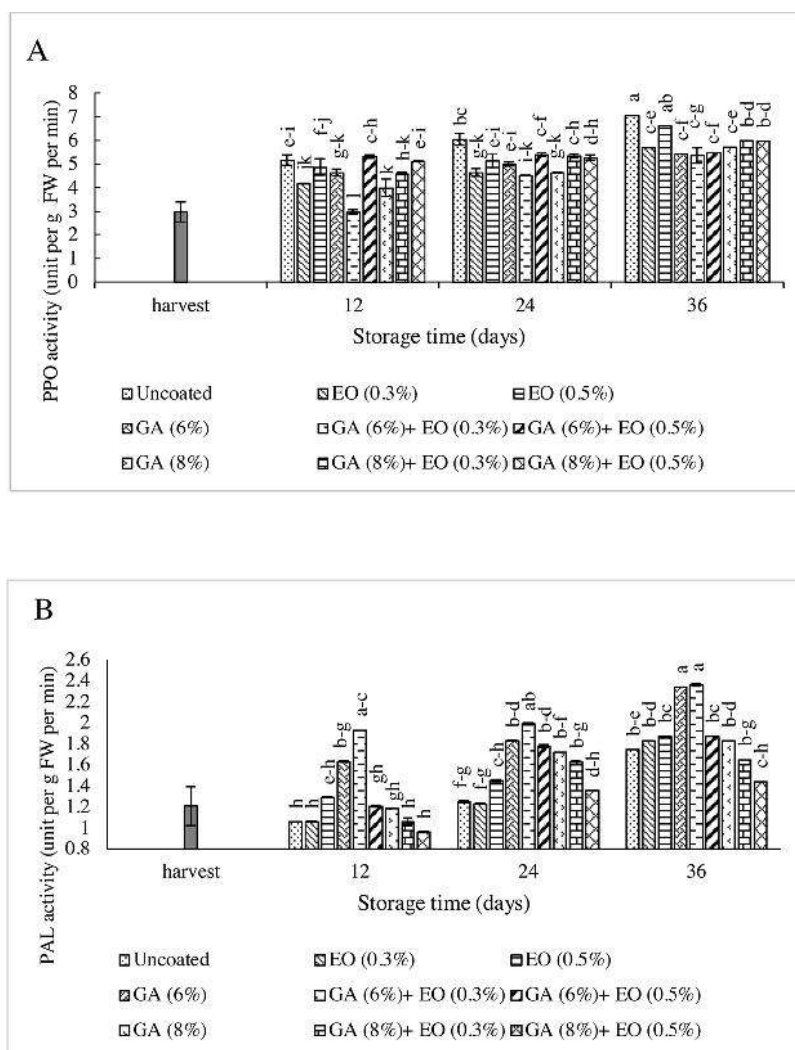


Fig. 2. PPO (A) and PAL activity (B) of fresh pistachio hull coated with gum arabic + *Zataria multiflora* during at 2 ± 1 °C. Means ($n = 3$) with the same letters are not significantly different according to Duncan test ($p > 0.05$). GA = gum arabic-based edible coating; EO = *Zataria multiflora*.

untreated pistachio fruit after 36 days of storage, while the lowest PPO levels were observed in GA (without and with EO) and 0.3 % EO treated fruits compared to control (Fig. 2A).

PPO is among vital enzymes in enzymatic browning in several fruits. Many studies have reported severe PPO activity increase during storage (Gheysarbigi et al., 2020). As enzymatic browning is often associated with the loss of membrane integrity which results in phenolic compounds being exposed to oxygen and becoming oxidized by PPO (Tano, Oul, Doyon, Lencki, & Arul, 2007). In this experiment, PPO activity increase was witnessed in fresh pistachios during storage which would have led to higher potential oxidization of polyphenols leading to lower TPC. This is shown in Fig. 1A and was also reported by Duan et al. (2007) who also showed increased PPO activity in longan fruits with lower TPC. A previous study examining the effects of GA and sodium casein in combination with cinnamon and lemongrass extracts on guava fruit also showed that these treatments were effective in reducing PPO activity (Murmu & Mishra, 2017). PAL is the primary enzyme involved in the biosynthesis and accumulation of phenols and is often correlated with the increase of the activity of PAL enzyme (Ojeda, Sgroppo, & Zaritzky, 2014). In this experiment, PAL enzyme activity of both treated and untreated fruits was increased during storage, particularly after 12 days of storage (Fig. 2B). Fruit samples treated with 6 % GA and 6 % GA + 0.3 % EO showed the highest level of PAL activity after 36 days of storage (Fig. 2B). This may be because EOs play a decisive role in secondary

plant metabolites and stimulate the biosynthesis of phenolic compounds and anthocyanins by increasing the activity of this enzyme (Jin et al., 2012).

3.3. Hull color

The lightness / darkness of pistachio hull was measured with L^* value using Minolta color meter and showed that hull color was darkened during storage in all treatments except for treatment with 6 % GA without EO, 6 % GA + 0.3 % EO and 8 % GA + 0.3 % EO after 24 days of storage which were higher than that observed after 12 days of storage (Fig. 3A). After 36 storing for days, the lightness of untreated control hull was the lowest, meaning a darker color which is undesirable. All other GA and EO treatment combinations had higher L^* values than untreated pistachios indicating that all treatments had some beneficial effects on hull color.

The results of color index a^* , which describes the green-red component of color spectrum, with red and green in positive and negative directions, respectively, are presented in Fig. 3B and show a general decrease in a^* value during storage which represents a more green color. Molamohammadi, Pakkish, Akhavan, and Saffari (2020) and Sheikh et al. (2019) similarly showed that a^* value was reduced in fresh pistachio during storage.

Fig. 3C shows the total color difference (ΔE) of fresh pistachio hulls

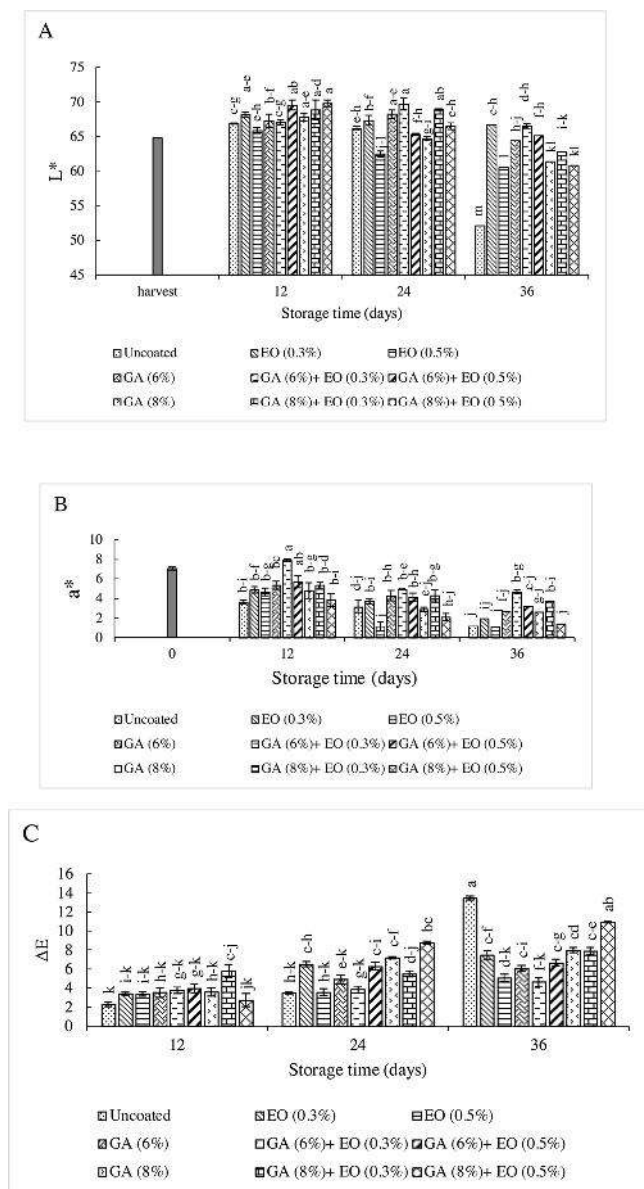


Fig. 3. L* value (A), a* (B) and Total color difference (ΔE) (C) of fresh pistachio (hull) coated with gum arabic + *Zataria multiflora* during storage at 2 ± 1 °C. Means (n = 3) with the same letters are not significantly different according to Duncan test ($p > 0.05$). GA = gum arabic-based edible coating; EO = *Zataria multiflora*.

in uncoated and coated samples at different time intervals during 36 days of cold storage. Although, ΔE level was increased during storage, the pistachios coated with 8 % GA in combination with 0.5 % EO and untreated control fruits showed significantly higher total color variation after 36 days of storage, in comparison with other treatments. No significant difference was detected among other treatments and the lowest color difference was observed in GA 6 % + 0.3 % EO coated pistachios.

GA is thought to hinder gas transfer and reduce surface browning. Similarly, GA treatment of tomato reduced color change associated with fruit ripening possibly due to reduced O₂ and increased CO₂ by GA coating (Ali et al., 2010). Ali, Maqbool, Alderson, and Zahid (2012) indicated GA effectiveness as an edible coating on L*, a* and b* values and it was found to have a significant effect on the reduction of respiration rate and delaying ripening process in papaya and banana fruits.

3.4. Free fatty acid (FFA) and peroxide value (PV)

FFA and PV levels in GA-treated pistachio fruits during storage are presented in Table 1 and show that the levels of both FFA and PV were increased in all samples over 36 days of storage (Table 1). No difference was observed in the FFA and PV levels of EO treatments (data not shown). There was also no difference in the FFA and PV contents among various treatments in untreated control fruits after 12 and 24 days of storage. However, after 36 days of storage, the fruits coated with 8 % GA had higher FFA values than those with 6 % GA. PV is extensively applied as a measure of primary lipid oxidation which indicates total peroxide amounts produced in oils and fats during oxidation (Tibolla et al., 2020). Peroxide value specifies the formation of hydro peroxides (primary oxidation products) (Ozturk et al., 2016). Elevated peroxide values indicate the occurrence of lipid oxidation (Park, Stan, Daeschel, & Zhao, 2005). Ozturk et al. (2016) showed peroxide values increase in fresh pistachio nuts during storage, where the oxidation of fats increases peroxide values during storage. Hydroperoxides are immediate results of lipid oxidation and thus the level of oxidation is determined initially by measurable amounts of hydroperoxide throughout storage. In the current study, peroxide value in each examined sample was significantly increased over time which was consistent with (Hamasali & Rasul, 2020). Acid value is an indicator of oil quality (Shahidi & Zhong, 2005). When oil quality is considered, FFA is shown to be a good indicator throughout production and storage (Smith, Geeson, & Stow, 1987). Oils with higher FFA contents have poor quality (Yee & Tiu, 2019). As the amounts of peroxide and FFA increase during storage, the progress of oxidation reactions can be determined (Ozturk et al., 2016). The barrier properties of many edible coatings often act as protectants against the oxidation of lipids (Bonilla, Atarés, Vargas, & Chiralt, 2012). Therefore, less-oxygenated or oxygen-free packaging results in less FFA and PV values in fresh pistachios than conventional packaging (Ozturk et al., 2016).

Application of other coatings such as chitosan has also been shown to result in reduced lipid oxidation in fresh walnuts during storage (Sabaghi, Maghsoudlou, Khomeiri, & Ziaifar, 2015). While Larrauri et al. (2016) showed that a carboxy methyl cellulose coating decreased peroxide values in treated almonds. Hashemi et al. (2020) also reported that the application of alginate edible coating reduced kernel PV value in fresh pistachio nuts. GA applied as edible coating on fresh pistachios

Table 1

Effects of gum Arabic edible coating on free fatty acid and peroxid value of fresh pistachio storage at 2 ± 1 °C, 85 ± 5 % RH.

Parameters	Gum arabic concentration (%)	Storage time					
		First day	12 days	24 days	36 days		
Free fatty acid as % oleic acid	0	0.61 ± 0.04	0.64 ± 0.03b [†]	0.66 ± 0.05b	0.74 ± 0.02ab		
		6	0.61 ± 0.04	0.58 ± 0.03b	0.64 ± 0.02b	0.65 ± 0.04b	
			8	0.61 ± 0.04	0.6 ± 0.04b	0.6 ± 0.03b	0.82 ± 0.03a
	Peroxide value (meqO ₂ /kg oil)	0	0.11 ± 0.02	0.41 ± 0.04b	0.66 ± 0.07b	0.82 ± 0.02b	
			6	0.11 ± 0.02	0.11 ± 0.03b	0.27 ± 0.06b	0.41 ± 0.03b
				8	0.11 ± 0.02	0.12 ± 0.08b	0.74 ± 0.03b

[†] Values with similar letters are not significantly different ($p < 0.05$). Data represent mean values ± S.E.

could act as a barrier against carbon dioxide, oxygen, solute movement and moisture, limiting water loss and respiration, as well as the oxidation rates of fruits (Ali et al., 2010; Ali, Cheong, & Zahid, 2014). All these opportunities are due to the modified atmosphere produced by edible coatings, which partially sealed the pores on fruit skin thus altering gas exchange and transfer rates (Lima et al., 2010). The current study indicated the excellent film forming ability of GA in preserving fruit quality, reduce in oxidation of FFA and peroxide value.

3.5. Saturated and unsaturated fatty acids

Oxidative rancidity is a manifestation of deterioration in many food products. The primary targets of oxidation are unsaturated fatty acids (Hashemi et al., 2020). In this experiment, the levels of fatty acids varied as saturated and unsaturated forms in pistachio kernels after 36 days of storage (Fig. 4). The highest palmitic acid amount was witnessed in fruits treated with 0.3 % EO and 6 % GA + 0.5 % EO. GA and EO affected

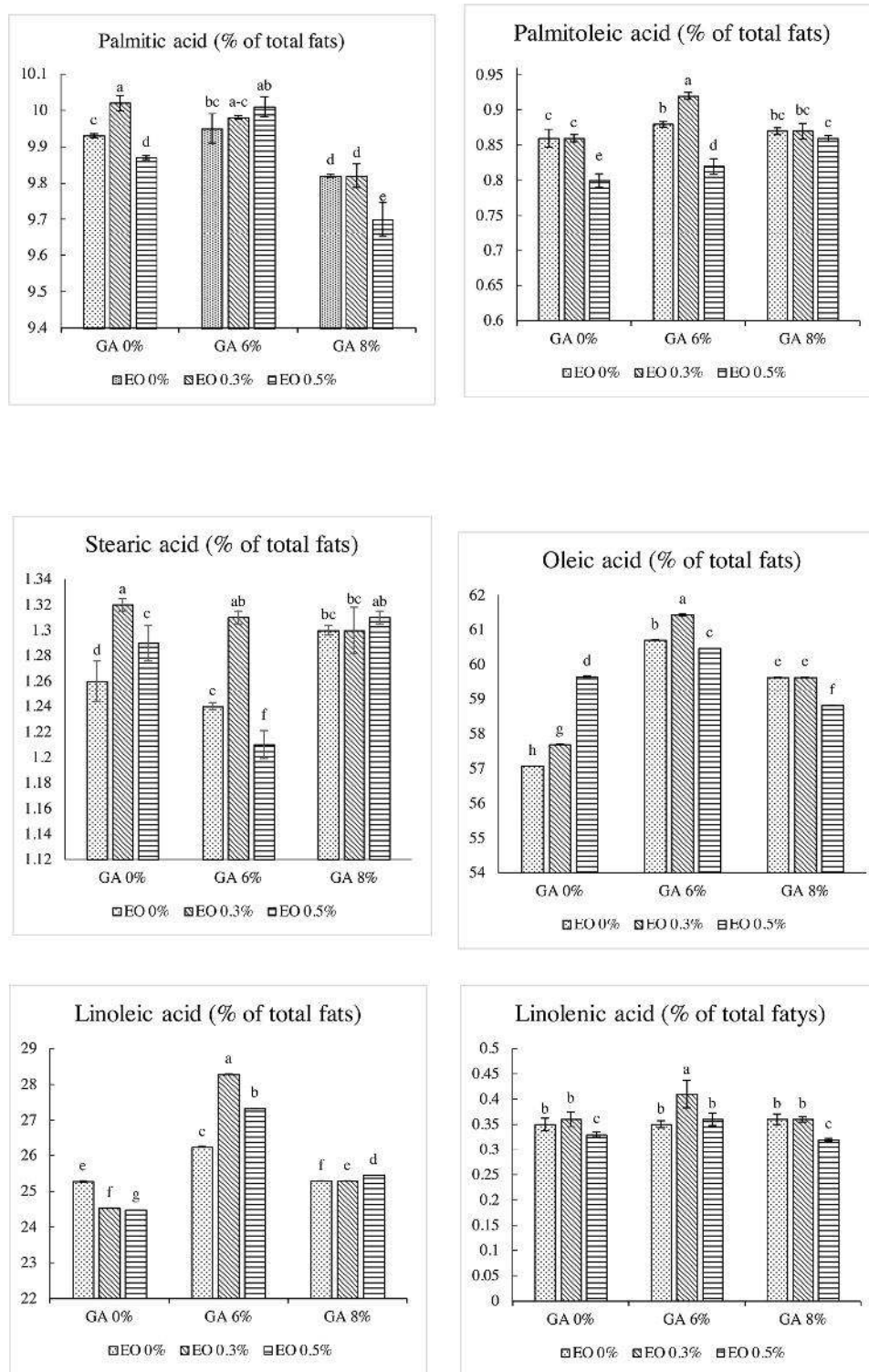


Fig. 4. Effect of gum arabic edible coating enriched with *Z. multiflora* essential oil on saturated fatty acids and unsaturated fatty acids of fresh pistachios stored at 2 ± 1 °C, 85 ± 5% RH. Values with similar letters are not significantly different (p < 0.05). Data represent mean values ± S.E. EO = *Zataria multiflora* Boiss.

the percentage of stearic acid and its highest amount occurred in 0.3 % EO, 6 % GA + 0.3 % EO and 8 % GA + 0.5 % EO treated products.

Results showed that 6% GA coating alone and in combination with 0.3 % EO had high percentage of palmitoleic acid in pistachio kernels. 6 % GA alone and in combination with 0.3% and 0.5% EO led to high oleic acid contents (60.71%, 60.47 % and 61.44%, respectively) compared with control fruits (57.07 %). Pistachios coated with 6% GA + 0.3 % EO gave the highest percentages of linolenic and linoleic acids among other treatments. Linolenic and linoleic acids are primary constituents of fats that are susceptible to oxidation. However, the predominant presence of oleic acid in fats usually makes them less susceptible to oxidation (Hashemi et al., 2020). Fats require oxygen for auto-oxidation. Thus, protective measures can best be applied when the atmosphere surrounding a food product is deprived of oxygen (Hashemi et al., 2020). These mechanisms describe how edible coatings protect fresh pistachio nuts from oxidation, thereby preventing fatty acids from degradation. Similarly, Dang, Singh, and Swinny (2008) carried out research on carnauba gum as coating material on mango fruits and showed that coated fruit pulp maintained high levels of fatty acids through storage. Hashemi et al. (2020) also reported that enriching alginate edible coating with EO can make an efficient treatment on fresh pistachio nuts while protecting saturated and unsaturated fatty acids in kernels.

3.6. Sensory properties

The sensory assessments of different GA and EO treatments were conducted after 36 days storage and are presented in Fig. 5. The results showed that all treatments affected fresh pistachio sensory properties. The best sensory attributes such as hull color, shell color, taste, odor and juiciness were observed in fruits coated with 6 % GA + 0.3 % EO in comparison with uncoated and treated fruits. Treatment with 6% GA along with 0.3% and 0.5 % EO positively affected the storage quality of fresh pistachio fruits. Furthermore, at the end of the experiments, maximum overall acceptance scores were observed in 6% GA + 0.3% treatment. Coating treatments showed positive influences on the storage life of fresh pistachios where at the end of storage period, treated fruits were still organoleptically acceptable. Current results showed that fresh pistachios with GA-based edible coating containing EO were not negatively perceived by consumers. Molamohammadi et al. (2020) also reported that fresh pistachios coated with chitosan edible coatings showed higher sensory acceptability compared to untreated control fruits. Moslehi, Mohammadi Nafchi, Moslehi, and Jafarzadeh (2021) studied the effect of the application of methylcellulose coating on the flavor, aroma, color, and total acceptability of pistachios. The results indicated favorable influences of coatings on sensory evaluation. The addition of suitable concentrations of EO into GA coating maintained sensory quality compared to untreated fruit. However, many studies have shown no significant differences in the sensory properties of different fruits by adding EO to edible coatings (Azarakhsh et al., 2014; Rojas-Grau, Tapia, Rodriguez, Carmona, & Martin-Belloso, 2007). However, the results in this study complied with those of Guerreiro, Gago, Faleiro, Miguel, and Antunes (2015), who showed that the application of edible coatings combined with citral and eugenol EOs had positive effects on the sensorial quality of strawberry fruits. The results in Boghori, Latifi, Ebrahimi, Mohamadi Kartalaei, & Dehghan, 2020 revealed that peanut coated with whey protein concentrate-nanocapsulated Shirazi thyme EO acquired the highest overall acceptability. Also, coatings preserved the taste and color of samples and prevented peanuts from oxidation. The findings of current study complied with those of Maghsoudlou, Maghsoudlou, Khomeiri, and Ghorbani (2012) on pistachio nuts.

4. Conclusion

Since the main case of this study was an edible fruit, the safety of the selected edible EOs and coatings for human consumption was essential. Shirazi thyme EO was considered as a safe material with public and

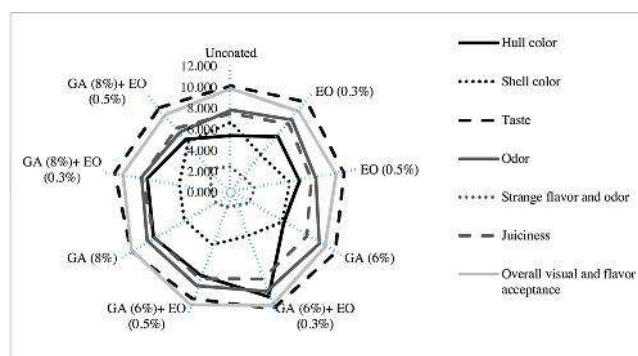


Fig. 5. Sensory properties of fresh pistachio coated with gum arabic + *Zataria multiflora* during storage at 2 ± 1 °C. Means ($n = 3$) with the same letters are not significantly different according to Duncan test ($P > 0.05$). GA = gum arabic-based edible coating; EO = *Zataria multiflora*.

pharmaceutical applications around the world (Ali et al., 2015).

In this paper, the effect of different concentrations of GA and EO alone and in combination was studied during 36 storage days at 85 ± 5 % RH and 2 ± 1 °C. Depending on concentration, GA and EO can show a positive effect on products. The coating of GA enriched with EO on fresh pistachios had considerable effects on the quality and appearance of fruits. The results of this study on the effects of GA and EO as edible coatings on fresh pistachio showed that total color difference (ΔE) was reduced by the addition of EO into GA coating. Low concentrations of GA most effectively decreased peroxide value and FFA. The lowest PPO enzyme activity and highest TPC were also observed in samples coated with 6 % GA + 0.3 % EO and this combination was identified as the best formulation in increasing fresh pistachio storage life; however, more work is required to further optimize these results. Application of GA enriched with EO presented good potential in agricultural products without safety concerns regarding residual materials. It could also be used against pests and to protect fresh fruit quality. The results of this research supported the effectiveness of Shirazi thyme EO in GA as a coating for protecting fresh pistachios from browning and preserving their quality. Additional survey is required for the determination of the effectiveness of these formulations in their potential applications at larger scale.

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Author statement

All of the authors read the articles. They committed that this article did not publish or send in any other journals.

Declaration of Competing Interest

Authors have no declarations of interest.

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