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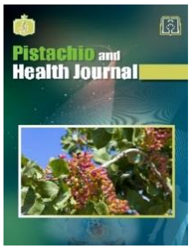

ORIGINAL ARTICLE

Effect of carboxymethyl cellulose edible coating enriched with *Zataria multiflora* essential oil on the quality and shelf-life of fresh pistachio (*Pistacia vera* L.) fruit

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Background: Fresh pistachio can be affected by physiological and biochemical changes both during harvest and after harvest, and its optimum duration of storage cannot therefore be prolonged.

Materials and methods: In order to maintain the quality of fresh pistachio fruits (*Pistacia vera L.* Ahmad-Aghaei), carboxymethyl cellulose at different concentrations accompanied by different concentrations of *Zataria multiflora* essential oil was used to produce an edible coating. The effects of these combinations were examined on the quality, shelf life, and sensory properties of the fresh fruitduring a storage period of 32 days (3±1°C, 85±5% RH).

Results: The results of sensory and instrumental tests during storage on days 8, 16, 24, and 32 showed that coated samples with 1.5% (w/v) carboxymethyl cellulose used in combination with *Zataria multiflora* essential oil had the longest shelf life compared to the other treatments. Among the treatments containing 1.5% (w/v) carboxymethyl cellulose, the lowest weight loss and the highest kernel carbohydrate, total soluble solid, phenolic compounds, and antioxidant activity were observed in the treatments containing 1.5% (w/v) carboxymethyl cellulose accompanied by 0.4% (w/v) and 0.2% (w/v) *Zataria multiflora* essential oil.

Conclusion: Application of 1.5% (w/v) carboxymethyl cellulose in combination with 0.4% (w/v) and 0.2% (w/v) *Zataria multiflora* essential oil effectively maintained the quality of fresh pistachio during the 32 days of storage.

Keywords: carboxymethyl cellulose; edible coating; essential oil; fresh pistachio; *Zataria multiflora*

1. Introduction

Pistachio is an agricultural product with high nutritional value. This fruit is a good source of vitamins A, B_1, B_2, B_6, and E, and minerals such as iron, phosphorus, selenium, zinc,and fatty acids. Fatty acids are precursors in thesynthesis of prostaglandins and ultimately prevent the accumulation of erythrocytes in the blood [1].

Recently, the application of natural and biodegradable coatings instead of artificial waxes has increased [2]. Edible coatings consist of a thin layer of nutrients that form directly on the fruit's surface. These coatings have the potential to create a selective barrier to moisture, CO_2, and oxygen. Edible coatings may control the internal atmosphere of the fruit, minimize the respiratory rate of the product, and delay the evaporation of water and moisture loss [3]. Edible coatings increase the shelf life of fresh fruits and vegetables by reducing metabolic processes and microbial growth [4].

Cellulose derivatives are linear polysaccharides composed of β-1 and 4 glucose units with methyl, hydroxypropyl, or carboxyl substitutions. Carboxymethyl cellulose is one of the most common cellulose derivatives used in the preparation of edible films [5]. It is linear, has a long chain, and is a water-soluble, anionic, and non-allergenic polysaccharide. This coating, in combination with antioxidant and antimicrobial agents, can effectively prevent the growth of fungi and microorganisms [5]. The respiration rate is one of the major factors contributing to the postharvest loss of fruit [6]. Azarakhsh *et al.* showed that the respiration rate of fresh-cut pineapple samples coated with an edible alginate coating was significantly lower than the uncoated samples during storage [6]. This reduced respiration rate is achieved by the coating creating an internal modified atmosphere which reduces the exchange of carbon dioxide and oxygen between the environment and the coated fruit [7]. Also, Asgar *et al.* observed that papayas coated with chitosan showed a lower respiration rate and ethylene production during storage [8]. Reductions in respiration rate and ethylene production as a result of edible coating has also been reported by many researchers reporting research on various fruits, such as grapes, strawberries, papayas, tomatoes, and mangos [9]. The application of this cellulose coating on pecan can limit the contact of oxygen with kernels and the exchange of gases by acting as a barrier. This results in a reduced oxidation of lipids in the kernel [10]. Edible coatings have the potential to carry active ingredients, such as anti-browning agents,

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coloring products, antimicrobials, and essential oils [11]. The results of Raeisi *et al.*'s study showed that edible carboxymethyl cellulose coating with the essential oil of *Zataria multiflora* and grape seed extract is very effective in reducing the undesirable chemical reactions in fish meat during storage [11]. According to the research conducted by Dhall Group, edible coatings should basically be resistant to water. They should not show destructive behaviors on O_2 or cause the accumulation of excessive amounts of CO_2 [2]. Edible coatings should have minimum permeability to water vapor, and their presence is expected to improve the fruit appearance and gloss, while preventing the fruit from becoming sticky. Edible coatings should be economical and optically transparent during the time of storage.

Therefore, this study aimed to evaluate the effects of different concentrations of carboxymethyl cellulose (CMC) edible coating in combination with different concentrations of *Zataria multiflora* essential oil on weight loss, total soluble solids (TSS), carbohydrate, phenolics, antioxidant activity, and sensory properties of fresh pistachio during 32 days of storage.

2. Materials and Methods

2.1 Fruit

This study was conducted on an important commercial pistachio cultivar called Ahmad-Aghaei. The samples were prepared from a pistachio orchard located in Pistachio Research Center in the city of Rafsanjan.

2.2. Coatings

Carboxymethyl cellulose as an edible coating and glycerol as a plasticizer in the edible coating were supplied from Sigma-Aldrich (Steinheim, Germany). The Essential oil of *Zataria multiflora*, was purchased from Barij Essence (Esfahan, Iran).

2.3. Preparation of samples and treatments

The fresh Ahmad-Aghaei pistachios were harvested at maturity and transferred to the laboratory, and pistachio fruits were then isolated from the cluster in order to treat.

The coating solution was prepared following the method used in Arnon *et al.* [12]. Briefly, a solution ofan appropriate amount of carboxymethyl cellulose powder in distilled water was prepared by heating at 80°C and stirring to form a clear solution. Then, 1% (w/v) glycerol was added to the solution as a plasticizer. Different concentrations of *Zataria multiflora* essential oil (0.0, 0.2, and 0.4% (w/v)) were added to the mixture. Finally, all formulations were homogenized for 10 min.

2.4. Treating and storage of fruits

Fresh Pistachios were immersed in coating solutions for 3 min and were then air-dried at room temperature for 1 min. They were then sorted to 200-gr packs in each polypropylene dish and stored at 3±1°C and 85±5% RH for 32 days. Fruits without coating and essential oil were also placed at the same condition as control. Weight loss, total soluble solids, carbohydrate, phenol, antioxidant activity , and hedonic test were evaluated after 0, 8,16,24, and 32 days of storage.

2.5. Parameters assay

2.5.1. Weight loss

Fruit weight loss percentage was measured using the following equation (Eq. 1):

$$\%\ \text{Weight loss} = \frac{initial\ weight - second\ weight}{initial\ weight} \times 100 \quad (1)$$

2.5.2. Total phenolic compounds and total antioxidant activity

To estimate the phenolic value of the pistachio, the method reported by Singleton *et al.* [13] was used. Briefly, 0.5 gr of the kernel was homogenized with 3 mL of 85% (v/v) methanol, and the resulting mixture was then centrifuged at 10,000 rpm for 15 min. Then, 150 µL of the supernatant was transferred to a test tube where 75 µL of Folin-Ciocalteu phenol reagent was added. After an incubation period of 5 min, 600 µl of 7% (w/v) Na_2CO_3 was added to the solution, which was then mixed well and kept in dark for 1.5 h. The samples were vortexed. Their absorbance was then measured at 760 nm.

Antioxidant activities were measured using the method presented by the Brand-Williams Group [14]. To do this, an extraction procedure similar to the one applied for phenolic value was conducted. After the extraction, 250 µL of the supernatant was mixed with 250 µL of distilled water and centrifuged for 10 min. Then, 75 µL of the combined solution was mixed with 2.925 ml DPPH 85% (w/v), and the absorbance of each solution was recorded at 517 nm. After incubation in dark for 30 min, the absorbance was again measured at the same wavelength.

2.5.3. Total soluble solids (TSS) and arbohydrate

The TSS value was measured as an average of 10 fruits in each replicate and assessed by a digital refractometer (ATAGO,PAL-1model Japan).To determine carbohydrate content, 0.5 g of pistachio ash (without oil) was homogenized with 5 ml of 95% (v/v) ethanol. The extraction was repeated twice using 5 ml of 70% (v/v) ethanol, and the obtained mixture was then centrifuged at 3,500 rpm for 20 min. After that, 100 µL of the alcoholic extract was mixed with 3 ml of freshly prepared anthrone (150 mg of anthrone in 100 ml of sulfuric acid 72% (v/v)). The solution was placed in a water bath at 90°C for 10 min. Then, absorption rate was measured at 625 nm [15].

2.5.4. Sensory analysis

For sensory analysis, eight trained panelists were selected from the Pistachio Research Center staff. The sensory evaluation form had a score scale from 0 to 15, the lowest to the highest admission. The panelists assessed the parameters hull color, pistachio color, taste, flavor, strange flavor and odor, juiciness texture, and overall visual and flavor acceptance based on the evaluation forms.

2.6. Statistical analysis

This experiment was performed with three factors in a factorial design based on a completely random design. Each value is the average of three replications. Sources of variation were CMC edible coating, *Zataria multiflora* essential oil, and the duration of storage. The experimental data were subjected to analysis of variance (ANOVA) by using the SAS 9.1 statistical software.

3. Results

3.1. Weight loss

As depicted in Fig.1, the weight loss of all coated fruits increased after 32 days of storage. The highest percentages of weight loss were observed in the control (8.28%) and the fruits coated by 0.2% (w/v)*Zataria mulrifolra* essential oil (9.06%) and the lowest was associated with 1.5% (w/v) CMC. In 1.5% (w/v)CMC edible coating fruits, the weight loss gradually decreased with increasing the *Zataria mulrifolra* essential oil concentration, and finally 1.5% (w/v) CMC treatment in combination with 0.4% (w/v) *Zataria mulrifolra* essential oil showed the least weight loss (4.43%) after 32 days of storage.

3.2. TSS and carbohydrate content

The highest and the lowest TSS% were observed in the control and the fruits treated with 1.5% (w/v) CMC, respectively (Fig. 2A). In the present study, the coatings enriched with 0.2% (w/v) and 0.4% (w/v) *Zataria mulrifolra* essential oils were more effective in reducing metabolic processes in fresh pistachio compared with the other treatments.
The results indicated that there were significant differences in the carbohydrate content between uncoated and coated fruits during the storage period (Fig. 2B). It was found that treatment with 1.5% (w/v) CMC enriched with 0.2% (w/v) and 0.4% (w/v) *Zataria mulrifolra* essential oil showed the highest carbohydrate content in comparison with the other treatments in 32 days of storage.

3.3. Phenolic and Antioxidant activity

As shown in Fig. 3A, the lowest and the highest contents of phenolics were observed in the control (140.15 mg gallic acid per 100 g fresh weight) andthe fruits coated with 1.5% (w/v) CMC. Of the coatings containing 1.5% (w/v) CMC, the one enriched with 0.4% (w/v) *Zataria mulrifolra* essential oil showed the highest phenolics contents (221.59 mg gallic acid per 100 g fresh weight),which was found to be statistically significant. Antioxidant activity of the kernel was significantly affected by the treatment composition as well during storage (Fig.3B). During the storage period, the fruits treated with the coating containing 1.5% (w/v) CMC and 0.4% (w/v) essential oil showed the highest antioxidant activity in comparison to the other treatments. For example, on day 32, the fruits coated with 1.5% (w/v) CMC accompanied by 0.4% (w/v) essential oil showed 24.79 % antioxidant activity, while the control sample showed 14.66%.

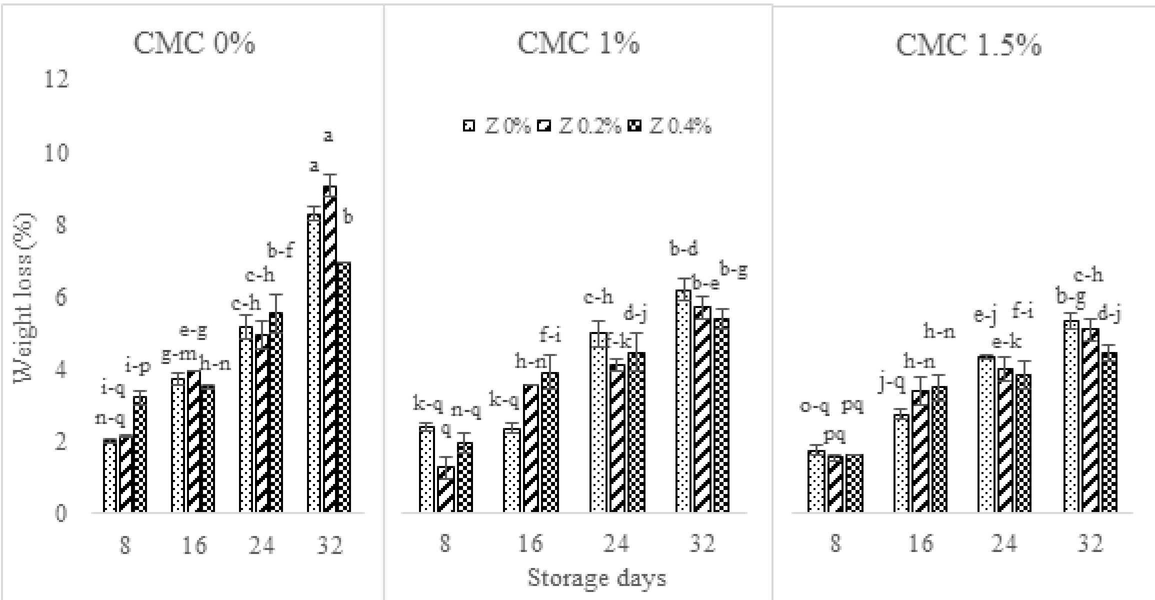

Fig. 1. Effects of different concentrations Carboxymethyl cellulose edible coating and *Zataria mulrifolra* essential oil on weight loss fresh pistachio during 32 days of storage at 3±1°C; 85±5% RH

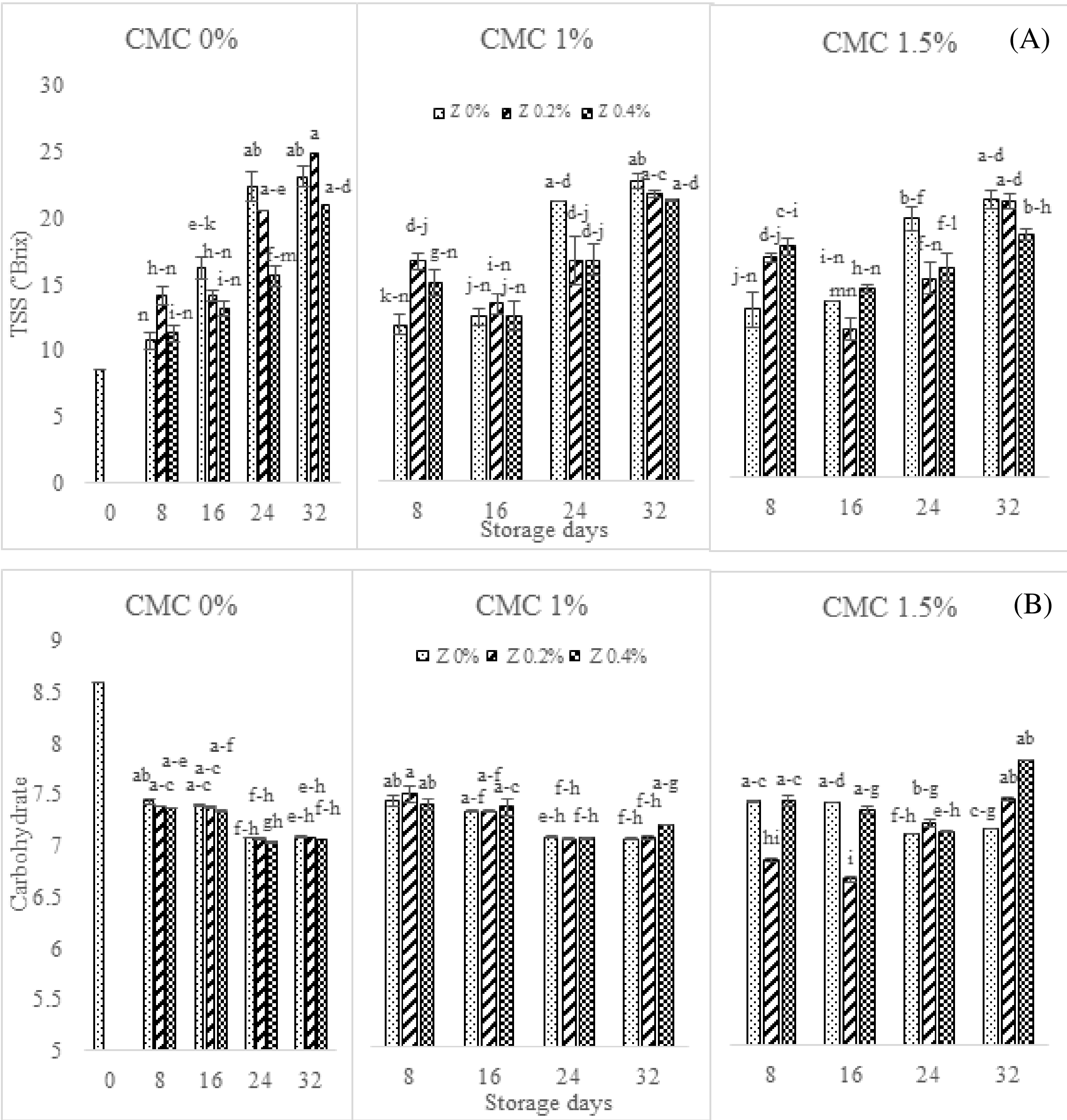

Fig. 2. Effects of different concentrations Carboxymethyl cellulos edible coating and *Zataria mulrifolra* essential oil on (A) total soluble solids (TSS) and (B) carbohydrate of fresh pistachios during 32 days of storage at 3±1°C and 85±5% RH. (CMC: Carboxymethyl cellulos edible coating; Z: *Zataria mulrifolra* essential oil)

3.4. Sensory analysis

Sensory evaluation was conducted based on hull color, taste, odor, strange flavor and odor, juiciness texture, and overall visual and flavor acceptance for both coated and uncoated samples during the storage period of 32 days at 3±1°C (Fig. 4). The incorporation of 0.2% (w/v) and 0.4% (w/v) *zataria mulrifolra* essential oil into the CMC-based coating formulation had desirable effects on the sensory attributes of the coated fruits. Of all the coatings, the one enriched with 0.4% (w/v) *zataria mulrifolra* showed more positive effects on the overall acceptability of samples (11.41).

4. Discussion

In 2012, the Wittaya Group reported that edible coatings containing plasticizers are good inhibitors for moisture loss, and they can specifically reduce the rate of moisture loss from seeds [16]. The weight loss of fresh pistachios coated by the CMC in our study was consistent with the results reported by the Albanese Group in 2007 [17], who demonstrated that the immersion of citrus fruits in CMC coating effectively reduced weight loss. It was suggested that the positive effect of this coating was capable of reducing respiration rate due to the development of an amorphous glass on the citrus surface which held back water evaporation. In 2007, the Lins Group stated that edible coatings provided effective barriers against oxygen, carbon dioxide, and water vapor transmissions and, as a result, they could reduce moisture loss [18].

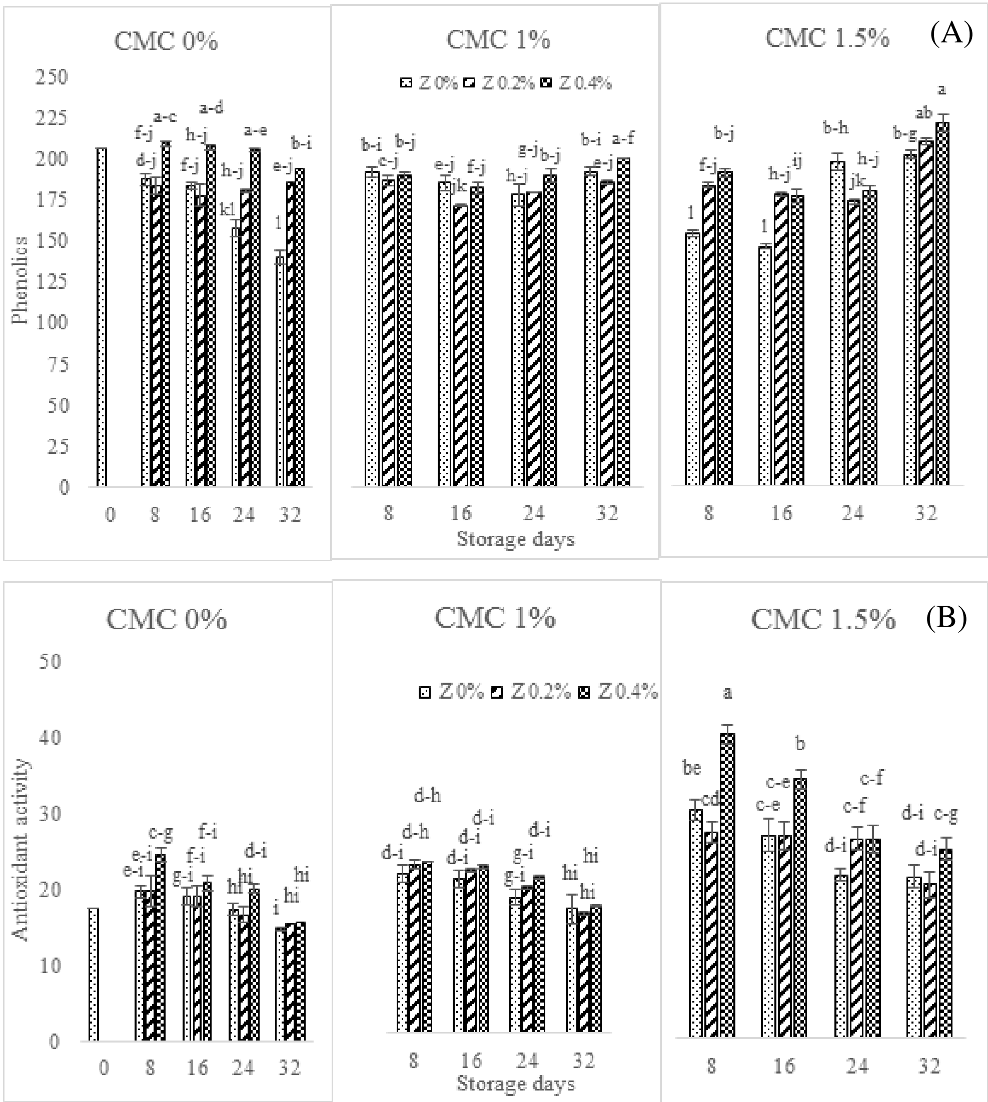

Fig. 3. Effects of different concentrations of carboxymethyl cellulos edible coating and *Zataria mulrifolra* essential oil on (A) phenolics and (B) antioxidant activity of fresh pistachios during 32 days of storage at 3±1°C and 85±5%RH.CMC: Carboxymethyl cellulos edible coating; Z: *Zatariamulrifolra* essential oil)

Also, in 2013, the Athmaselvi Group reported that tomatoes coated with *Aloe vera* showed a gradual decrease in the TSS during storage [19]), a result in line with the findings of our study. This may be due to the break-up of pectin and carbohydrates, partial hydrolysis of protein, and decomposition of glycosides into subunits during respiration, which causes a decrease in the total soluble solids [19]. Furthermore, the Athmaselvi Group reported that sugar content in coated tomatoes was higher compared to the corresponding control, due to the controlled atmosphere around the fruit created by the edible coating that in turn was obtained by reducing respiration and transpiration loss [19].

Phenolic compounds are natural antioxidants that can be found in different vegetable sources [20]. The relationship between the amount of the total phenolics and the antioxidant properties has been studied in many fruits and vegetables [20]. It is believed that phenolic components are considerably involved in the antioxidant capacity [20]. The Guerreiro Group previously reported that the antioxidant capacity of *Arbutus unedo* fresh fruits increased when the fruits were treated with alginate edible coatings enriched with eugenol and citral essential oils [4], which is in line with our results. The decrease in antioxidant activity during storage may be attributed to the destruction of cell structures as the fruit undergoes senescence [21]. Edible coatings may provide a barrier to reduce oxygen consumption and therefore reduce oxidative processes [22]. The antioxidant effects of some essential oil compounds may also contribute to the maintenance of antioxidant activity in fruits [23]. Furthermore, in 2008, the Oms-Oliu Group found that using edible coatings was effective in reducing total phenolic compounds and antioxidant capacity in fresh-cut melon during storage [24].In 2015, the Dashipour Group reported that the highest total phenolic and antioxidant activity was observed in CMC films incorporated with *Zataria multiflora* essential oil [5].

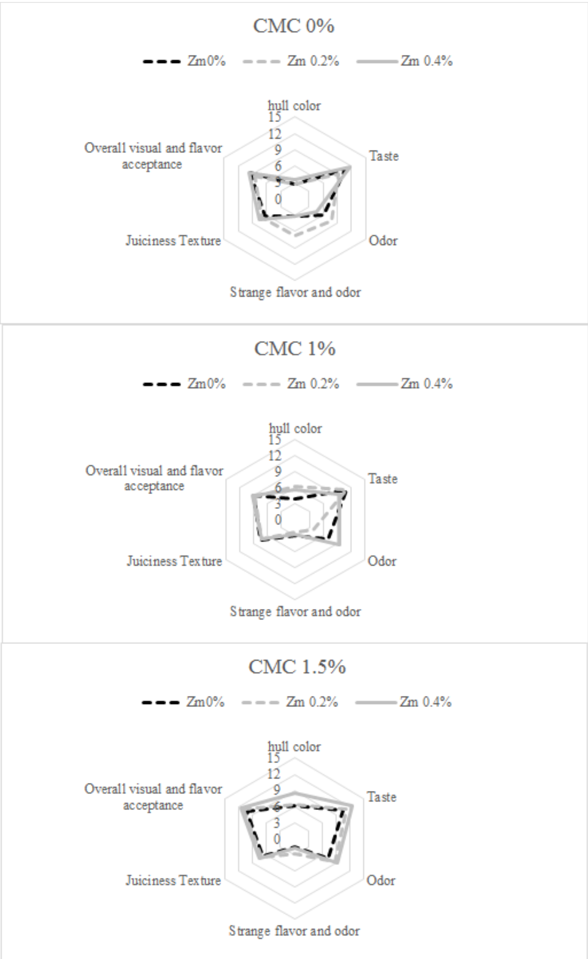

Fig. 4. Effects of different concentrations of carboxymethyl cellulos edible coating and *Zataria mulrifolra* essential oil on the sensory properties of fresh pistachios after 32 days of storage at 3±1°C and 85±5%RH. (CMC: Carboxymethyl cellulos edible coating; Z: *Zataria mulrifolra* essential oil)

In our study, the increase in antioxidant activity was related to the enhancement of phenolics in the pistachio kernel. Also, some researchers have stated that the accumulation of phenolic compounds can be a result of an increase in antioxidant activity [24-25].

The addition of essential oils to edible coatings instead of the direct application of the essential oils can cause the oils to be released gradually. This improves their performance and efficiency. Our results are in line with those previously reported by the Khoshnodinia Groupin 2013 who showed that the application of antioxidant-gelatin combined with an edible coating on pistachio had greater effects on the sensory scores in comparison with the absence of the antioxidant [26]. Since edible coatings are usually eaten along with the fruits, investigation of their sensory properties is of great importance [27]. The sensory results obtained in the present study are also in harmony with those reported by the Arnonand the Tzoumaki groups [12, 28]. In 2006, the Baldwin Group indicated that CMC can influence the sensory parameters related to the pecan kernel [10]. CMC-containing coatings also improve the appearance and gloss of the coated fruit, maintain the fruit quality and flavors, and fully cover the fruit surface [28]. Moreover, this coating is deemed economically affordable and is considered a homogeneous and transparent material. Also, CMC-based coatings possess good stability and can be easily prepared [28].

Thymol and carvacrol are the most important and abundant components in thyme essential oil. These components belong to the group of phenols and have antifungal properties [29]. It has been also reported that the phenolic compounds available in thyme have inhibitory effects on the growth of *Aspergillus flavus* [30]. In a study by the Baraiya Group in 2012, the application of CMC in combination with clove essential oil effectively increased the shelf life of tomato by delaying the ripening of the fruit and postponing their senescence [31].

5. Conclusion

This study showed that the CMC edible coating enriched with *Zataria multiflora* essential oil can maintain the fruit quality of fresh pistachios during storage. Furthermore, this treatment prevents excessive weight loss and improves the fruit quality in terms of phenolics, antioxidant activity, carbohydrates, and TSS. We concluded that using an edible coating containing 1.5% (w/v) CMC enriched with 0.4% (w/v) *Zataria multiflora* essential oil can extend the duration of storage and maintain the quality of fresh pistachios for 32 days.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

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