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# Application of Bio-Nanocomposite Films Based on Nano-$TiO_2$ and Cinnamon Essential Oil to Improve the Physiochemical, Sensory, and Microbial Properties of Fresh Pistachio

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A R T I C L E I N F O

*Keywords:*

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A B S T R A C T

Bio-nanocomposite films based on sago starch containing 2% cinnamon essential oil and 3% titanium dioxide nanoparticles were used in the packaging of fresh pistachios and stored at different temperatures (4, 25, and 35 ˚C) and relative humidity (30, 50, and 70%) for 20 days, and pistachio properties were evaluated in five-day intervals. Using these films in packaging improved the physicochemical properties of pistachios, including moisture, fat, shrinkage, color characteristics, and sensory characteristics improved, and the growth of *Aspergillus flavus*, aflatoxin production, and the amount of hydro-peroxide increased much more slowly compared to the control samples. Temperature and relative humidity had significant effects on the physicochemical properties and deterioration of fresh pistachios ($p<0.05$) as, at higher levels of temperature and relative humidity, the count of *Aspergillus flavus*, peroxide index, and aflatoxin had the highest values. The most desirable conditions for fresh pistachio storage were the temperature of 4˚C and 30% relative humidity. Therefore, this active packaging can be utilized as a proper alternative to conventional packaging.

## Introduction

Pistachio is a very popular plant, native to Central Asia and East Asia. Arid and semiarid areas of Iran are currently one of the most significant pistachio producers in the world (Sharifkhah et al., 2020; Eslami et al., 2019). Under certain environmental conditions during pistachio harvesting, mold growth occurs with the production of secondary metabolites such as mycotoxins, the most important of which are aflatoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus* (Cheraghali *et al.*, 2007; Kershaw, 1985; Mahooney, 1996). Many chemical controllers have been deactivated due to the spread of new fungal species. Application these substances has also been limited because of their side effects such as carcinogenic properties, toxicity, and environmental pollution. Therefore, the use of an alternative method for controlling contaminations and microbial spoilage is recommended (Rasooli *et al*., 2006)

Active packaging technology, as an alternative method, includes interactions among food, packaging materials, or coatings and the inner gaseous atmosphere, which simultaneously preserves the quality, safety, and

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shelf life of the product (Labuza and Breen, 1988). The properties of active packaging are antimicrobial activity, oxygen scavenging, moisture or ethylene, and flavor or ethanol release (Ghanbarzadeh, 2006). The use of edible films and coatings with antimicrobial properties can be appropriate for improving the safety and quality of products because, despite the direct use of antimicrobials, they do not lose their effect on the product surface and are not immediately transferred to the product (Min, 2005). In general, coatings are directly formed on the surface of a product, while films are separately formed as a thin layer and then placed on the product (Aghaee, 2008). The purpose of producing these films and coatings is to prevent the migration of moisture, oxygen, carbon dioxide, or any other soluble material, and they are also utilized as carriers for food additives such as antioxidants or antimicrobials (Arvanitoyannis and Biliaderis, 1998). The promising alternatives include a wide range of biodegradable ones such as carbohydrates and proteins, which reduce the use of non-renewable resources and the amount of waste through biological recycling (Catherine *et al.,* 2002). Likewise, the use of biodegradable films and coatings improves some quality properties such as color and transparency (Mohammadi Nafchi *et al.,* 2012).

One of these biodegradable substances is starch, which is inexpensive and easily accessible. The popularity of starch in biopolymer science is due to its relatively low cost, abundance, and renewability. However, starch alone as an alternative in the polymer packaging material has some limitations such as brittleness and hydrophilic nature. Sago starch is a relatively unknown starch material. It is easily extracted from *Metroxylon sagu* palm tree at a very low cost compared to other starches. The functional properties of sago starch are between those of potato and cereal starches (Mohammadi Nafchi *et al.,* 2012). The use of nano-scale antimicrobials in packaging is superior efficiency against microorganisms due to their higher surface-to-volume ratio. Nano-particles also increase the mechanical, thermal, chemical, and microbial properties, permeability, and thermal resistance, and effectively prevent biochemical changes (Mohammadi Nafchi *et al.,* 2012). Some nanoparticles such as zinc oxide or titanium dioxide have antimicrobial properties and can also improve the packaging properties to prevent oxygen and moisture permeability; as a result, researchers have increasingly considered nano-based packaging and coatings, particularly in the food industry (Jafarzadeh and Jafari, 2020; Jafarzadeh *et al*., 2020; Hermawan *et al*., 2019; Jafarzadeh *et al*., 2019; Hadadinejad et al., 2018; Jafarzadeh *et al*., 2017) and also for improving postharvest longevity of horticultural crops (Kamiab et al., 2017).

Natural antimicrobials can control microbial contamination, increase shelf life, decrease the resestance of pathogenic microorganismsstrengthen immune cells in humans, and reduce antibiotic consumption (Tajkarimi *et al*., 2010). Among the natural antimicrobial compounds, essential oils as secondary aromatic volatile metabolites of some plants are naturally produced in specific cells or groups of cells (Oussalah *et al*., 2007). Natural essential oils from plants are recognized as a safe source of antimicrobial compounds. The antimicrobial and antioxidant activity of essential oils, especially cinnamon essential oil (CEO) applied in packaged bread or noodles has been reported in previous studies (Lopez *et al*., 2007; Quintavalla and Vicini, 2002; Esfahani *et al*., 2020). Cinnamon oil is an aromatic liquid obtained from the twigs, bark, and leaves of *Cinnamomum zeylanicum*. Extracts of cinnamon bark (CNB) and leaves (CNL) have been used extensively. The antimicrobial activity of bio-based films incorporated with different natural essential oils or extracts against bacteria and molds has been reported in several studies. Although the antimicrobial properties of natural extracts have been known for centuries, research has scarcely reported their applications in active packaging systems (Lopez *et al*., 2007; Quintavalla and Vicini, 2002; Esfahani *et al*., 2020).

Therefore, the main objective of this research was to investigate the effect of different levels of temperature and relative humidity on the quality, as well as the sensory and microbial properties of fresh pistachios covered by sago starch bio-composite films containing nano-particle titanium dioxide and CEO over 20 days of storage.

## Materials and Methods

The mechanical properties of the bio-nanocomposite films, including tensile strength (TS), elongation at break (EB), and Young's modulus (YM) were estimated based on the ASTM standard Method D882-18, and the results were reported in another published article (Esfahani *et al*., 2020).

### *Materials*

Sago starch (12% moisture and 25% amylose) was prepared from SIM Malaysia. Nano-scale titanium dioxide and glycerol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). CEO and Akbari pistachio were provided by Barij Essence Co, Kashan, Iran, and the local market of Damghan, Iran, respectively. Other chemicals (with the analytical grade or chromatography) and the culture medium were prepared from Merck, Germany.

### *Preparation of the bio-nanocomposite film of sago starch/cinnamon essential oil/nano-scale titanium dioxide*

First, a proper amount of titanium dioxide nanoparticle in 100 ml of distilled water was homogenized in an ultrasonic bath for 20 min to prepare a 3% nano-particle solution (nanoparticles/dry starch weights). Then, 4 g of sago starch and, subsequently, 2 g of glycerol (50% (w/w)) were added as a plasticizer; and the mixture was stirred until 90 ˚C to completely gelatinize for 30 min. As the mixture was cooled to 45˚C, 2% CEO was added to the film according to a previous research and homogenized for about 30 min. Then, the final mixture (92 g) was cast on Preplex plates and dried at 40 ˚C and 50% relative humidity for 24 h. Finally, the dried film was separated from the Preplex plate and stored for further use in certain desiccators with a relative humidity of 50-60% (provided with saturated magnesium nitrate at 25 ˚C) (Esfahani *et al*., 2020).

### *Preparation of fresh pistachio samples*

The fresh pistachios of well-known Akbari variety *(Pistacia vera L.)* with carton packaging were used. Two types of packaging treatment was used in this study including control samples as conventional packaging and treated samples consisting of conventional packaging plus bio-nanocomposite film sheets. In each packaging, 500-1500 g of pistachio was packed. In the treated sample, for every 500 g of pistachio, two sheets of starch bio-nanocomposite film (10×10 cm) were utilized. Three temperature levels (4, 25, and 35 ˚C) and three relative humidity levels of 30% (Damghan), 50% (Tehran), and 70% (northern regions of Iran with high humidity) were considered for storing the packs. Pistachio samples were stored at the intended temperature and relative humidity for 20 days, and the relevant tests were performed every five days.

### *Pistachio sample assays*

### *Measurement of moisture content*

Moisture assessment was performed by the weight measurement method (AOAC, 1994). Briefly, 3 g of each sample was weighed and dried in an oven (UFE 400-800 MEMMRET GmbH, a variable speed fan and a digital force gauge) at 105 ˚C for 3 h, and then cooled in a desiccator. The dried sample was weighed again and the moisture content (%) was calculated based on the following equation:

$$((W_1-W_2)/W_1)\times 100,$$

$W_1$ is the sample weight before drying.

$W_2$ is the sample weight (g) after drying.

***Measurement of fat content***

Soxella method was used to determine the fat content of the samples (AOAC, 1994).

***Measurement of shrinkage percentage***

First, the initial volume of pistachio (at the beginning of the storage period) was determined using the equation ($V_0 = 3.4\ \pi\ (D / 2)^3$), in which $V_0$ and D are the initial volume ($m^2$) and average geometric diameter of pistachio (m), respectively. Then, the percentage of pistachio shrinkage was calculated using the equation: $Sb = (1- (V / V_0)) \times 100$, in which Sb, $V_0$, and V were shrinkage percentage, initial volume, and the secondary volume of pistachios (after storage), respectively (Zarrin Nejad and Amiri Chaijan, 2013).

***Measurement of color***

The surface color of the pistachio fruits was measured by placing 5-7 fruits together, using Hanterlab (Colorflex, USA). The indicators of L* measuring light intensity, a* for red/green intensity, and b* for yellow/blue intensity of the pistachios were measured (Ozturk *et al*., 2016).

***Measurement of the peroxide index***

To determine the peroxide index of pistachio samples, first, the sample oil was extracted (National Organization for Standardization of Iran, 2009); according to the AOCS method, the peroxide index was measured with Cd8-53. Briefly, 5 g of the extracted oil was mixed with 30 ml of the acetic acid/chloroform solution (2:3), and then 0.5 ml of saturated potassium iodide was added. The mixture was frequently shaken in the dark for 1 min. Subsequently, 30 ml of distilled water along with a standardized amount of sodium thiosulfate solution (0.1 N) containing sodium carbonate (0.1 N) was added to the mixture, and titration was continued until the yellow color almost disappeared.

Next, 0.5 ml of the 1% (w/v) starch solution was used as an indicator of titration. Titration was continued by adding the sodium thiosulfate solution dropwise until the violet color disappeared, and the peroxide index was calculated by $((S-B) \times N \times 1000)/W$, where S is the sodium thiosulfate volume used to titrate the sample (ml), B is the sodium thiosulfate volume (ml) needed for the blank, N is the normality of the standardized sodium thiosulfate solution, and W is the sample weight (g). (AOCS, 1994).

***Counting A. flavus colony***

Initially, 25 g of fresh ground pistachio kernels was added to 225 ml of peptone water and homogenized on a magnetic mixer (Heidolph, Germany) for 10 min. The potato dextrose agar medium was used to grow the mold based on surface culture, after which it was incubated at 25 ˚C for four days. The results of mold counting were reported according to the log colony form unit per g (log CFU/g) (Sayanjali *et al*., 2011).

***Measurement of B group aflatoxin***

A high-efficiency liquid chromatography (HPLC) system was employed to evaluate the amount of aflatoxin produced in the pistachio samples during the storage period (National Standard Organization of Iran, 2011).

***Sensory evaluation***

The pistachio's sensory characteristics, including taste, textural firmness, physical appearance, and overall acceptance were compared by 10 trained panelists through a hedonic test on the second and eighth day of storage. The evaluation was carried out as a seven-point test (1 to 7) (*very bad* to *excellent*) for each qualitative property, and after scoring, the results were analyzed (Sedaghat *et al*., 2005).

### *Statistical analysis*

The mean of each parameter was analyzed by variance analysis in a completely randomized factorial design using SPSS 22.0. The differences between the treatments were evaluated via Duncan's test at a 95% probability level, and the relevant charts were plotted in Excel 2013.

## Results

### *Moisture content*

Moisture has a positive effect on the degree of kernel crispiness, particularly after roasting; besides, it affects the growth of aflatoxin-producing fungi (Mortazavi *et al*., 2014). According to Fig. 1, there was no significant difference between the moisture content of various pistachio samples on the first day, but the moisture content of all the samples gradually decreased during the storage period. The highest moisture loss was 42.06% compared to the initial level of the control kept at 35 ºC and 30% relative humidity, in which the moisture content decreased from 6.68% to 3.87% during the 20-day storage period. However, the lowest moisture loss content, 8.38%, compared to the initial content belonged to the bio-nanocomposite samples from 6.68% to 6.12%, kept at 4ºC and relative humidity of 70%. In general, the use of bio-nanocomposites preserved the moisture content of fresh pistachio and provided a higher moisture content. Due to the addition of CEO and especially titanium nanoparticles into the starch bio-nanocomposite film, the film's permeability to water vapor and gases was reduced, as a result of which less moisture was removed from the product surface (Esfahani *et al*., 2020). The highest moisture content was related to the pistachios stored at 4 ºC, and as the storage temperature increased, the moisture content of the pistachio samples significantly decreased ($p<0.05$). In a study with similar results, the amount of moisture and fresh pistachio nutrient reduced, and weight loss increased during the storage period; however, the use of aloe vera coating reduced the weight loss of pistachio samples (Ahmadi *et al*., 2013). Moreover, similar result obtained in another study on strawberry, in which sodium alginate and ascorbic acid coating was shown to extend the storage life of strawberry through reducing water loss (Nazoori et al., 2020).

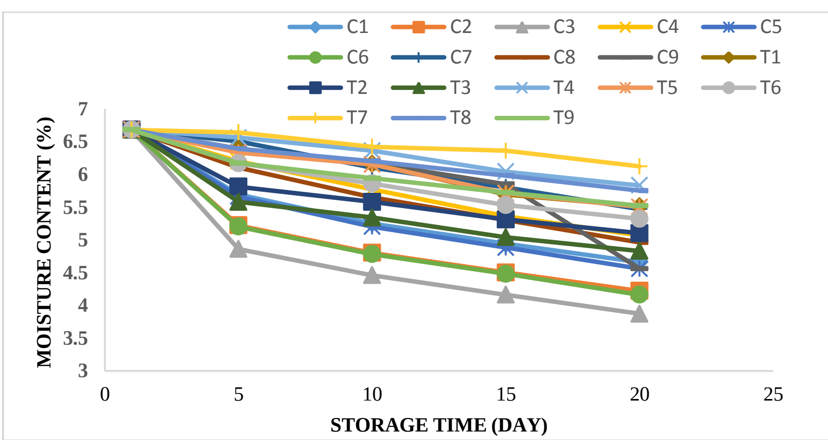


**Fig. 1.** Changes in moisture content (%) of different pistachio samples during the 20-day storage period (C1: control + 4˚C and RH 30%; C2: control + 25˚C and RH 30%; C3: control + 35˚C and RH 30%; C4: control +4˚ C and RH 50%; C5: control + 25˚C and RH 50%; C6: control + 35˚C and RH 50%; C7 : 4˚C and RH 70%; C8: control + 25˚C and RH 70%; C9: control + 35˚C and RH 70%; T1: Bio-nanocomposite + 4˚C and RH 30%; T2: Bio-nanocomposite + 25˚C and RH 30%; T3: Bio-nanocomposite + 35˚C and RH 30%; T4: Bio-nanocomposite +4˚C and RH 50%; T5: Bio-nanocomposite + 25˚C and RH 50%; T6: Bio-nanocomposite +35˚C and RH 50%; T7: Bio-nanocomposite +4˚C and RH 70%; T8: Bio-nanocomposite +25˚C and RH 70%; T9: Bio-nanocomposite +35˚C and RH 70%)

***Fat content of the pistachio samples***

The results showed the significant effect of packaging type, relative humidity, temperature, and storage time on the fat content of fresh pistachio samples ($p<0.05$). According to Fig. 2, on the first day, the amount of fat in all the treatments was similar. Over time, the fat content of all the samples showed a significant decrease ($p<0.05$). The highest rate of fat reduction compared to the initial rate, 6.81%, was associated with the control sample kept at 35 ºC and the relative humidity of 70%, in which the amount of fat varied from 55.81 to 52.1% during the 20-day storage period. However, the lowest reduction in fat percentage, 2.78%, belonged to the sample packed with bio-nanocomposite films and stored at 4 ºC and 30% relative humidity, decreasing from 55.81 to 54.26%. The pistachio samples packed with the bio-nanocomposite films showed a higher fat content than those in the control packages. A decrease in some nutrients, such as fats and carbohydrates, can result from the effect of the respiration process into the stored fruits' texture in terms of providing the energy needs (Nazeri *et al*., 2014). The fat content of the pistachio samples significantly decreased as relative humidity and storage temperature increased ($p<0.05$) because lipase activity and fat lipolysis in the pistachios increased and, subsequently, the fat content decreased. In addition, the fat content significantly decreased as the storage time of the pistachios increased ($p<0.05$). This decrease is not only related to the fat degradation during the storage period and an increase in the free fatty acid value, but is the consequence of the respiration process and consumption of fats (Nazeri *et al*., 2014). The results of this study are similar to the study on freshly stored pistachios in 45 days, in which the fat content decreased due to the samples respiration (Nazeri *et al*., 2014). Other studies showed similar results, indicating that proper packaging protects fresh pistachios from fat oxidation (Ozturk *et al*., 2016).

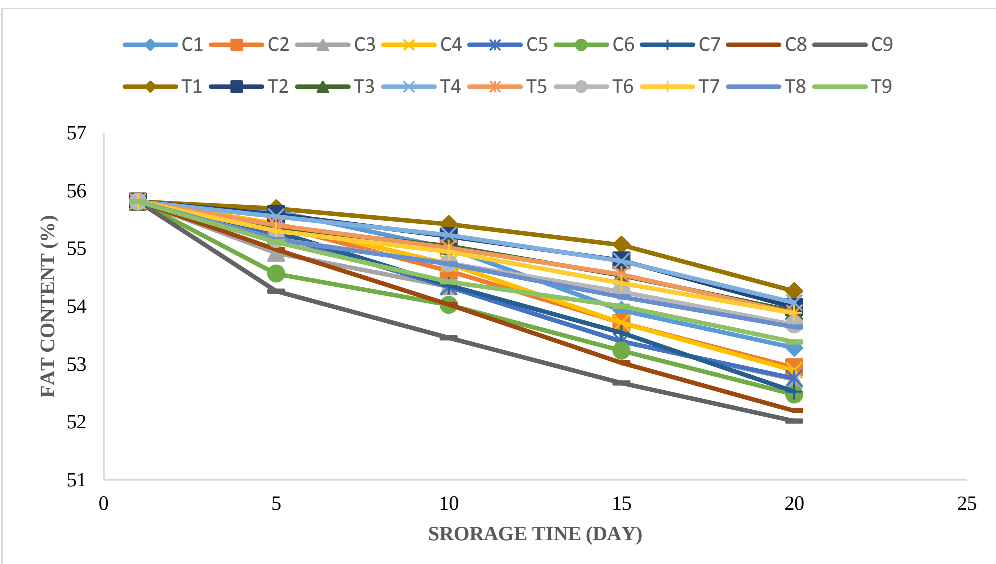


**Fig. 2.** Average changes in the fat values (%) of different pistachio samples during the 20-day storage period

***The shrinkage percentage of the pistachio samples***

The results showed the significant effect of packaging type, relative humidity, temperature, and storage time on the shrinkage rate of fresh pistachio samples ($p<0.05$). Fig. 3 displays the changes in the mean values of different pistachio samples during the 20-day storage period in different storage conditions. On the first day, the rate of shrinkage in all treatments was zero due to the lack of volume difference. During the storage period, the amount of shrinkage significantly increased due to a decrease in the moisture content of all the samples (p<0.05). Over time, the highest increase in the shrinkage percentage was observed in the control samples kept at 35 ºC and relative humidity levels of 30% and 50%, in which the shrinkage rate increased from zero to 2.00 and 1.90%, respectively. There was no significant difference between the two samples. The lowest increase in the shrinkage percentage was related to the bio-nanocomposite sample kept at 4 ºC and 70% relative humidity, which increased from 0 to 0.78%. The moisture content significantly increased and, subsequently, the shrinkage rate was reduced with an increase in the relative humidity and moisture absorption and the reduction in the rate of moisture evaporation from the samples (p<0.05). The moisture content of the samples significantly decreased and the shrinkage percentage significantly increased due to increased temperature and storage time (p<0.05). It has been reported that the shrinkage level depends on the relative humidity of the surrounding medium; as temperature increases, the moisture content of samples is evaporated more quickly and, consequently, shrinkage occurs more significantly (Zarrinnejad and Amiri Chayjan, 2016).

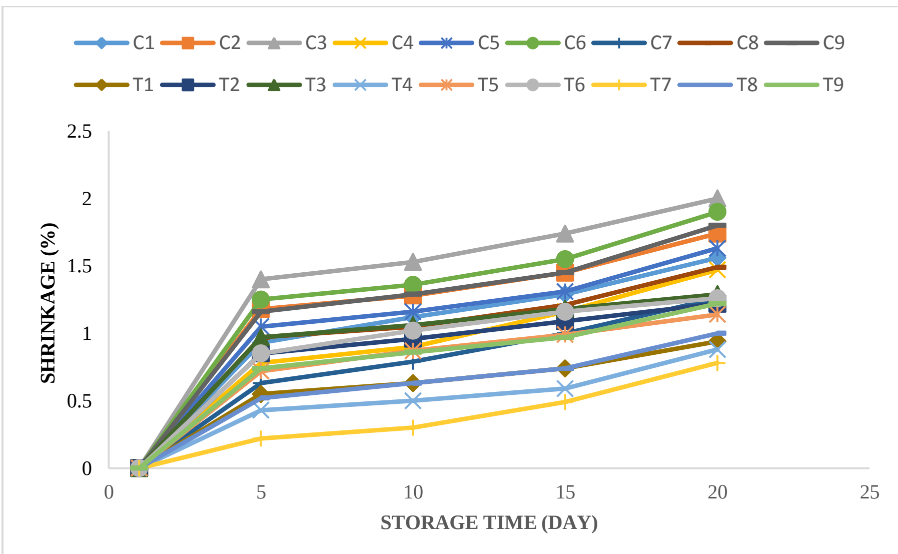


**Fig. 3.** Average changes in the amount of shrinkage (%) of different pistachio samples during the 20-day storage period

### *Color indicators of the pistachio samples*

Color is one of the most important factors affecting consumer acceptance. The results showed the significant effect of packaging type, relative humidity, temperature, and storage time on the values of L*, a*, and b* index of fresh pistachio samples (p<0.05). On the first day, the L*, a*, and b* indicators were similar in all the treatments. Over time, the light intensity of the pistachio color significantly decreased due to the increased brown enzymatic index in the texture (p<0.05) (Table 1). The highest decrease in the L* index belonged to the control sample kept at 35 ºC and relative humidity of 30%, in which this index varied from 53.74 to 44.69 during the storage period, and decreased by 16.84% compared to the initial value. However, the lowest decrease in this index belonged to the sample packed with bio-nanocomposite films and stored at 4 ºC and relative humidity of 70%, which decreased by 5.75%. The higher L* index of the pistachio samples packed with bio-nanocomposite films compared to the control samples is due to a reduction in the browning reaction in the sample texture (Ozturk *et al*., 2016). The L* index value of the pistachio samples significantly decreased with a decrease in relative humidity and an increase in temperature and storage time due to an increase in browning reaction intensity in the texture ($p<0.05$).

**Table 1. L* indicator values of different pistachio samples during the storage time**

| Packaging | RH (%) | Temperature (˚C) | Days | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1st Day | 5th Day | 10th day | 15th day | 20th day |
| Control | 30 | 4 | 53.74± 0.16$^{a}$ | 51.17± 0.18$^{de}$ | 49.95±0.19$^{efg}$ | 48.20±0.18$^{f}$ | 46.30±0.14$^{gh}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 50.15± 0.13$^{g}$ | 48.53±0.15$^{i}$ | 47.29±0.21$^{h}$ | 44.38±0.15$^{jk}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 49.54± 0.15$^{h}$ | 47.54±0.14$^{i}$ | 46.54±0.15$^{i}$ | 44.69±0.69$^{k}$ |
| | 50 | 4 | 53.74± 0.16$^{a}$ | 51.82± 0.16$^{c}$ | 50.41±0.18$^{e}$ | 48.61±0.14$^{e}$ | 47.30±0.20$^{d}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 50.51± 0.14$^{f}$ | 48.90±0.13$^{h}$ | 47.79±0.18$^{g}$ | 45.49±0.17$^{ij}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 50.08± 0.19$^{g}$ | 48.19±0.22$^{i}$ | 47.07±0.18$^{h}$ | 45.15±0.21$^{j}$ |
| | 70 | 4 | 53.74± 0.16$^{a}$ | 52.22± 0.15$^{b}$ | 50.95±0.19$^{d}$ | 49.09±0.19$^{cd}$ | 47.80±0.14$^{c}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 51.15± 0.22$^{de}$ | 49.72±0.20$^{fg}$ | 48.21±0.17$^{f}$ | 46.05±0.19$^{h}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 49.44± 0.23$^{h}$ | 48.82±0.13$^{h}$ | 47.74±0.16$^{g}$ | 45.57±0.15$^{i}$ |
| Bio-nanocomposite | 30 | 4 | 53.74± 0.16$^{a}$ | 52.91±0.21$^{a}$ | 51.54±0.22$^{c}$ | 50.32±0.20$^{b}$ | 49.19±0.20$^{c}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 51.53± 0.18$^{cd}$ | 50.31±0.21$^{e}$ | 48.71±0.22$^{de}$ | 46.83±0.25$^{ef}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 50.82± 0.16$^{e}$ | 49.63±0.23$^{g}$ | 48.06±0.32$^{fg}$ | 45.56±0.23$^{ij}$ |
| | 50 | 4 | 53.74± 0.16$^{a}$ | 52.97±0.25$^{a}$ | 52.00±0.22$^{b}$ | 50.81±0.23$^{a}$ | 49.95±0.30$^{b}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 51.84± 0.19$^{c}$ | 50.98±0.26$^{d}$ | 49.13±0.21$^{c}$ | 47.11±0.25$^{de}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 51.09± 0.15$^{e}$ | 50.14±0.22$^{ef}$ | 48.47±0.22$^{ef}$ | 46.52±0.24$^{fg}$ |
| | 70 | 4 | 53.74± 0.16$^{a}$ | 53.27± 0.15$^{a}$ | 52.65±0.18$^{a}$ | 51.24±0.20$^{a}$ | 50.65±0.26$^{a}$ |
| | | 25 | 53.74± 0.16$^{a}$ | 52.30± 0.20$^{b}$ | 51.74±0.19$^{bc}$ | 49.34±0.24$^{c}$ | 47.50±0.23$^{cd}$ |
| | | 35 | 53.74± 0.16$^{a}$ | 51.50± 0.21$^{cd}$ | 50.78±0.17$^{d}$ | 48.80±0.23$^{de}$ | 46.86±0.30$^{def}$ |

Each value is the mean ± standard deviation of three replicates. In each column for the samples, different superscript letters mean significant differences ($p<0.05$).

Moreover, over time, the a* index significantly decreased ($p<0.05$) (Table 2). The highest decrease in the a* index belonged to the control sample kept at 35 ºC and relative humidity of 70%, in which the a* index decreased by 15.23% during the storage time. Nevertheless, the lowest decrease in this index was associated with the sample packed with bio-nanocomposite films and stored at 4 ºC and 30% relative humidity, which decreased from 19.31 to 18.36 by 4.92% in comparison with the initial rate. In general, the pistachio samples packed with the bio-nanocomposite films showed a higher a* index value than the control one did. As the relative humidity, temperature, and storage time increased, the a* value of the pistachio samples significantly decreased ($p<0.05$).

**Table 2. The a* indicator values of different pistachio samples during the storage time**

| Packaging | RH (%) | Temperature (˚C) | Days | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1st Day | 5th Day | 10th day | 15th day | 20th day |
| Control | 30 | 4 | 19.31±0.11 a | 18.98±0.10 abc | 18.29±0.08 e | 17.62±0.11 e | 17.10±0.11 e |
| | | 25 | 19.31±0.11 a | 18.82±0.09 cd | 18.23±0.07 ef | 17.52±0.10 efg | 16.86±0.15 ef |
| | | 35 | 19.31±0.11 a | 18.71±0.12 def | 18.08±0.10 fg | 17.43±0.09 efg | 16.71±0.11 fg |
| | 50 | 4 | 19.31±0.11 a | 18.88±0.14 bcd | 18.32±0.12 de | 17.62±0.17 ef | 16.97±0.20 ef |
| | | 25 | 19.31±0.11 a | 18.73±0.10 def | 18.13±0.12 ef | 17.34±0.13 fgh | 16.76±0.16 fg |
| | | 35 | 19.31±0.11 a | 18.56±0.12 fg | 18.08±0.13 efg | 17.32±0.10 gh | 16.55±0.13 gh |
| | 70 | 4 | 19.31±0.11 a | 18.64±0.13 efg | 18.25±0.11 ef | 17.47±0.17 fgh | 16.55±0.09 gh |
| | | 25 | 19.31±0.11 a | 18.76±0.07 de | 18.06±0.10 fg | 17.36±0.08 g | 16.80±0.17 f |
| | | 35 | 19.31±0.11 a | 18.43±0.09 g | 17.86±0.12 g | 17.15±0.07 h | 16.37±0.11 h |
| Bio-nanocomposite | 30 | 4 | 19.31±0.11 a | 19.13±0.06 a | 18.92±0.09 a | 18.61±0.09 a | 18.36±0.07 a |
| | | 25 | 19.31±0.11 a | 19.06±0.05 ab | 18.69±0.11 bc | 18.53±0.10 bc | 18.16±0.12 bc |
| | | 35 | 19.31±0.11 a | 19.00±0.06 b | 18.51±0.10 cd | 18.24±0.11 bc | 17.97±0.10 cd |
| | 50 | 4 | 19.31±0.11 a | 19.02±0.08 ab | 18.82±0.09 ab | 18.55±0.08 a | 18.19±0.10 ab |
| | | 25 | 19.31±0.11 a | 18.97±0.10 abc | 18.69±0.07 b | 18.36±0.09 b | 18.15±0.08 bc |
| | | 35 | 19.31±0.11 a | 18.92±0.09 bcd | 18.40±0.06 d | 18.13±0.12 cd | 17.86±0.07 d |
| | 70 | 4 | 19.31±0.11 a | 18.92±0.11 bcd | 18.70±0.08 b | 18.40±0.05 b | 18.07±0.09 bc |
| | | 25 | 19.31±0.11 a | 18.80±0.10 cd | 18.51±0.09 cd | 18.28±0.07 bc | 18.01±0.09bcd |
| | | 35 | 19.31±0.11 a | 18.66±0.13 ef | 18.29±0.08 e | 17.94±0.09 d | 17.84±0.10 d |

Each value is the mean ± standard deviation of three replicates. In each column for the samples, different superscript letters mean significant differences ($p<0.05$).

In addition, over time, the b* index of the samples significantly decreased ($p<0.05$) (data not shown). The highest decrease in the b* index was related to the control sample stored at 35 ºC and relative humidity of 50 and 70%; this index decreased from 11.42 to 9.01 during the storage period and was reduced by 21.10%. However, the lowest decrease in this color index was associated with the sample packed with bio-nanocomposite films and stored at 4 ºC and 30% relative humidity, which varied from 11.42 to 9.86 and showed a decrease of 13.66%. In general, the pistachio samples packed with the bio-nanocomposite film showed a higher b* index than the control ones. As the relative humidity, temperature, and storage time increased, the b* index of the pistachio samples significantly decreased ($p<0.05$).

***Peroxide index of the pistachio samples***

The most important destructive reactions of pistachio leading to quality loss during storage are oxidation and hydro-peroxide formation (Taub and Singh, 1997). The presence of unsaturated fatty acids in pistachios increases oxidation. In addition, lipase and lipoxygenase enzymes are often found in pistachios, which also cause destructive oxidation reactions (Tavakolipour *et al*., 2010). Fig. 4 shows the mean changes in the peroxide index values in different pistachio samples during the storage time. On the first day, there was no difference between the peroxide index of different pistachio samples. The peroxide index of all samples increased during storage ($p<0.05$). The highest increase in peroxide index was related to the control sample kept at 35 ºC and 30% relative humidity, which varied from 0.73 to 5.51 meq/kg and increased by 86.75%, while the lowest increase of this oxidative index belonged to the bio-nanocomposite sample kept at 4 ºC and 70% relative humidity, which varied from 0.73 to 2.71 meq/kg and increased by 73.6%.

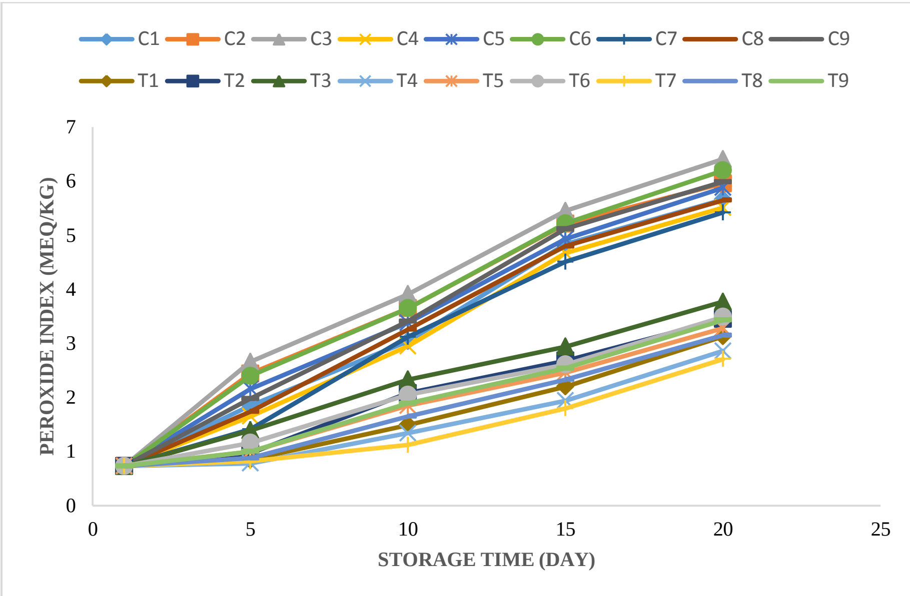


**Fig. 4.** Average changes in the peroxide index (meq/kg) values of different pistachio samples during the 20-day storage period

### *The A. flavus colony count in the pistachio samples*

Packaging type, relative humidity, temperature, and storage time significantly affected the count of *A. flavus* colonies in fresh pistachio samples ($p<0.05$). Fig. 5 depicts the mean changes in the logarithm of the number of molds in different pistachio samples during the storage period. On the first day, there was no significant difference between the number of molds in different pistachio samples. The number of mold colonies in all the samples significantly increased during the storage period ($p<0.05$). The highest increase in the mold colony number was related to the control sample kept at 35 ºC and 70% relative humidity, varying from 0.72 to 5.11 log CFU/g and increasing by 85.91%, whereas the lowest number of mold colonies was related to the sample packed with bio-nanocomposite films stored at 4 ºC and 30% relative humidity, which varied from 0.72 to 1.94 log CFU/g and showed an increase of 62.88%.

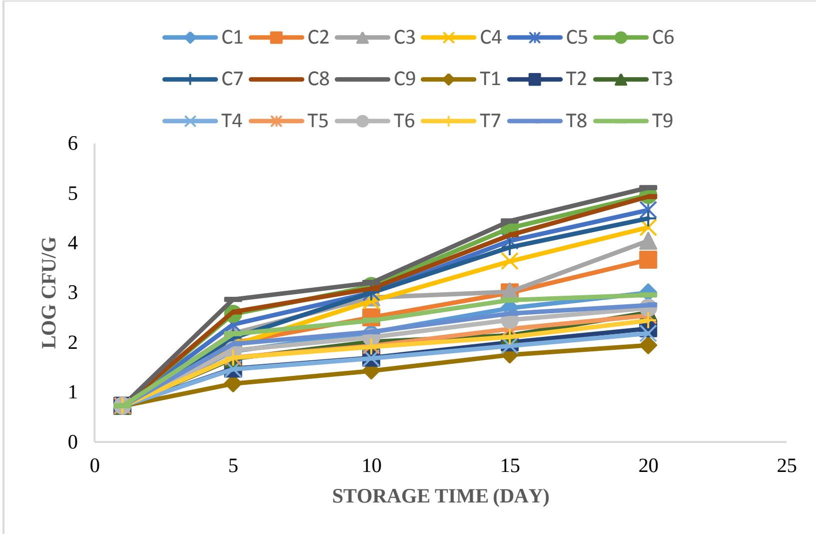


**Fig. 5.** Average changes in the count of *A. flavus* (log CFU/g) in pistachio samples during the 20-day storage period

As the relative humidity and temperature increased, the number of molds in the samples increased significantly ($p<0.05$), which could be due to the increased respiration rate and, subsequently, the production of higher moisture and removal of more moisture from the product, which increased the relative humidity around the samples and thus increased the mold growth. In one study, the use of carboxyl methyl cellulose-based edible films reduced the growth of *A. flavus* in fresh pistachios due to the reduction of respiratory gas transfer (Sayanjali *et al*., 2011). Also, the use of edible coating containing sage extract at a concentration of >4500 ppm and coatings containing cumin extract at a concentration of >6000 ppm prevented the production of aflatoxin in pistachio (Tavakolipoor, 2019)

***Aflatoxin content of the pistachio samples***

A significant effect of packaging type, relative humidity, temperature, and storage time was found on the aflatoxin content of fresh pistachio samples ($p<0.05$). The mean changes in aflatoxin values of different pistachio samples during storage in different conditions are illustrated in Fig. 6. On the first day, there was no difference between the aflatoxin levels of different pistachio samples. During storage, the aflatoxin content of all the samples significantly increased ($p<0.05$). The highest increase in aflatoxin was related to the control sample stored at 35 ºC and 70% relative humidity, which varied from 0.36 to 6.48 ppb during the storage period and showed an increase of 94.44%. However, the lowest aflatoxin level belonged to the sample packed with bio-nanocomposite films kept at 4º C and 30% relative humidity, which varied from 0.36 to 2.38 ppb and showed an increase of 84.87%.

The amount of aflatoxin produced significantly increased with increasing the relative humidity, temperature, and storage time due to the increased growth of *A. flavus* ($p<0.05$), which was in agreement with the results of Shahrbabaki's research (Shahrbabaki, 2001). Moreover, by using edible coating based on whey protein concentrate containing Shirazi thyme extract at concentrations of >400 ppm, the growth of *Aspergillus* fungus was prevented and the production of aflatoxin toxin was reduced in pistachio (Tavakolipoor *et al*., 2012). Another study confirmed the anti-fungal activity of biopolymers containing essence, that prevented aflatoxin production in samples (Tavakolipoor *et al*., 2019).

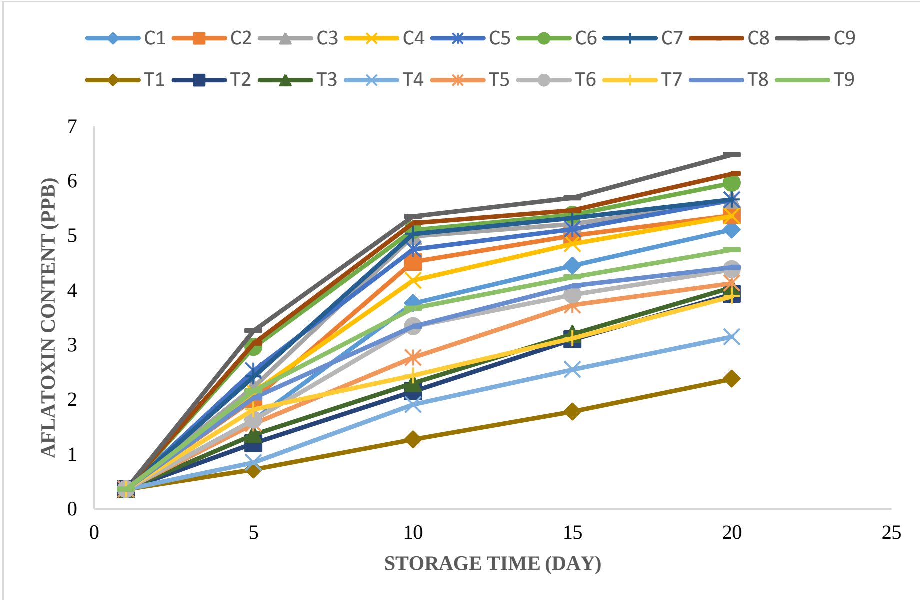


**Fig. 6.** Average changes in aflatoxin (ppb) values of different pistachio samples during the 20-day storage period.

### *Sensory evaluation in the pistachio samples*

Fig. 7 shows a comparison of the average taste scores of different samples. The use of bio-nanocomposite films improved the taste of fresh pistachio, such that on the eighth day of storage, these samples had a significantly more desirable taste than the control ones. In both types of packaging, the taste score decreased with increasing the storage temperature. Overall, on the eighth day, the highest taste score belonged to the samples packed with bio-nanocomposite films and stored at 4 ºC and 30, 50, and 70% relative humidity, and no significant differences were observed between the three samples. The control samples kept at 35 ºC and the relative humidity of 50% and 70% had the lowest taste score.

The mean tissue stiffness scores of different samples are compared in Fig. 8. The use of bio-nanocomposite films improved the stiffness of fresh pistachio texture during the storage time. In both types of the studied packaging, the firmness of the pistachio tissue decreased with increasing the storage temperature and relative humidity. Overall, on the eighth day, the highest tissue stiffness score was related to the sample packed with bio-nanocomposite films and stored at 4 ºC and 30% relative humidity. The control sample stored at 35 ºC and relative humidity of 70% gained the lowest tissue stiffness score.

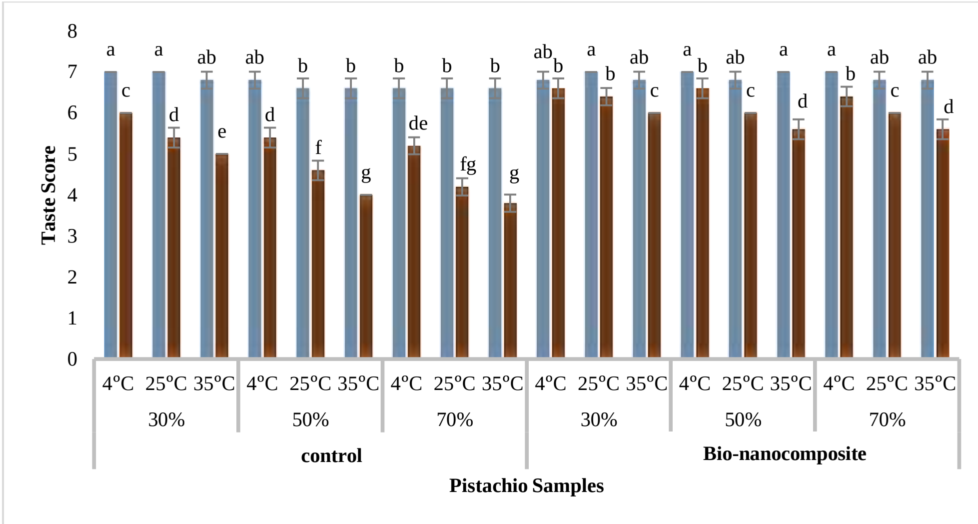


**Fig. 7.** Comparison of the taste scores of different samples on the second and eighth day of storage (blue: 2nd day, red: 8th day) (Columns indicate the mean ± standard deviation. The difference in the letters on the columns indicates a significant difference at the probability level of 5% ($p<0.05$).)

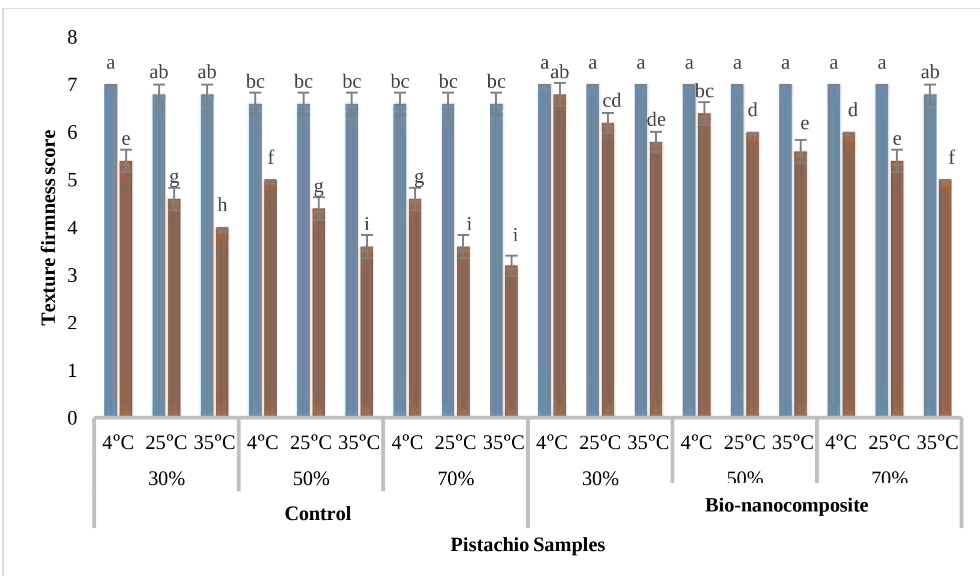


**Fig. 8.** Comparison of the texture stiffness scores of different samples on the second and eighth day of storage, (blue: 2nd day, red: 8th day) (Columns indicate the mean ± standard deviation. The difference in the letters on the columns indicates a significant difference at the probability level of 5% ($p<0.05$).)

In both types of the studied packaging, the physical appearance and overall acceptance decreased as temperature and storage time increased, which can be attributed to the discoloration and reduced strength and texture firmness, but the reduction rate in samples packed with bio-nanocomposite films was significantly less than that in the control ones. Overall, on the eighth day, the highest score of physical appearance and overall acceptance was related to samples packed with bio-nanocomposite films stored at 4 ºC and relative humidity of 30, 50, and 70%. The control sample kept at 35 ºC and the relative humidity of 70% showed the lowest score of physical appearance. This sample also had the lowest overall acceptance score at the same temperature and the relative humidity of 50 and 70%. Other researchers reported similar results by pointing out that packaging had a positive effect on the sensory quality of fresh pistachio samples (Ozturk *et al*., 2016).

**Discussion**

The results showed the significant effect of packaging type, relative humidity, temperature, and storage time on the moisture content of fresh pistachio samples ($p<0.05$). Bio-nanocomposite films significantly prevented weight change in fresh pistachio (Maghsoudloo *et al.*, 2012). They also have a significant inhibitory effect on the moisture and weight loss of jujube fruit (Lei and Ton That., 2006). Nanoparticles present in the bio-nanocomposite films reduced the respiration rate of pistachios and preserved the pistachio fat content by decreasing the permeability and transmission of carbon dioxide, oxygen, and water vapor (Esfahani *et al*., 2020). Furthermore, these films were able to reduce the rate of light transmission which, in turn, decreased the lipid oxidation in pistachio (Esfahani *et al.,* 2020). In fact, the shrinkage rate was reduced by preserving the pistachio moisture content using bio-nanocomposite packaging (Esfahani *et al.,* 2020).

As the relative humidity, temperature, and storage time increased, the a*, b*, and L* values of pistachio samples significantly decreased ($p<0.05$). In general, the pistachio samples packed with the bio-nanocomposite films showed higher a*, b*, and L* index values than the control one. These results were similar to a report in which bio-nanocomposite coating positively influenced color indices (Ozturk *et al.,* 2016). Also, the results of another research indicated that the coatings provided a glossy appearance that made the color of nuts very distinctive from the uncoated product (Javanmard, 2008). Due to the barrier properties of this packaging against gases and light, no significant change was observed in the peroxide value of the coated samples (Ozturk *et al.,* 2016)

The use of bio-nanocomposite films decreased the growth rate of aerobic *A. flavus* in pistachio samples due to the appropriate inhibitory properties against gases such as oxygen. On the other hand, nano-titanium dioxide and cinnamon essential oil present in bio-nanocomposite films have significant antimicrobial activity and prevent the growth of molds and other microorganisms, as titanium dioxide binds to microorganism membrane and prolongs the delay phase (Atmaca *et al.,* 1998). Likewise, trans-cinnamaldehyde, the most important compound in cinnamon essential oil, effectively prevents fungal growth. Due to the presence of other active ingredients in this essential oil (cinnamaldehyde, benzene propanol, propane acid, 2-methyl-3-phenylpropyl ester, etc.), the synergistic activity of these compounds has been observed, as the inhibitory activity of cinnamaldehyde and trans-cinnamaldehyde has been against the synthesis of enzymes from fungal walls (Xing *et al.,* 2011; Wang *et al*., 2005). The same antimicrobial effect of essential oil and nanoparticles was also reported in tissue culture for disinfection of Bermudagrass explants (Taghizadeh et al., 2014). Overall, the samples packed with the bio-nanocomposite films had significantly lower aflatoxins than the control samples, which is due to a decrease in the number of molds in the samples.

The beneficial effect of bio-nanocomposite films and the low storage temperatures on the taste of fresh pistachio is probably due to reduced respiration rate, consumption of carbohydrates and fats, and moisture retention; these factors create an optimal balance among chemical compounds in fresh pistachio as the permeability to water vapor and gases and fat oxidation are decreased, particularly at low temperatures. In all the pistachio samples, the taste score and tissue stiffness decreased over time, but the rate of decrease in the samples packed with bio-nanocomposite films was significantly lower than that of control. Likewise, the use of bio-nanocomposite films preserved the physical appearance and overall acceptance of fresh pistachio in different storage conditions, such that in comparison with the control sample, both features were significantly improved.

## Conclusions

The effect of bio-nanocomposite films and storage conditions, including temperature and relative humidity, on the physicochemical, antimicrobial, and sensory properties of fresh pistachios during the 20-day storage period were investigated. By using sago starch bio-nanocomposite films containing cinnamon essential oil and nano-scale titanium dioxide, the moisture, fat, color characteristics, and sensory properties of fresh pistachios were preserved in a better way and the shrinkage of samples decreased during the storage time. The use of bio-nanocomposite films reduced the formation of hydro-peroxide and aflatoxins in pistachios and delayed the growth of *A. flavus* due to its antioxidant and antifungal properties. The results of this research demonstrated that the shelf life of fresh pistachio can be increased and its physicochemical characteristics can be enhanced by reducing the temperature and relative humidity levels of storage and applying these bio-nanocomposite films.

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