



ORIGINAL ARTICLE

Effects of Active Packaging Based on Potato Starch/Nano Zinc Oxide/Fennel (*Foeniculum vulgare Miller*) Essential Oil on Fresh Pistachio during Cold Storage

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ABSTRACT

The purpose of this study was to evaluate the effects of a combination of zinc oxide nanoparticles (Nano-ZnO) and fennel essential oil (FEO) on the quality characteristics of fresh pistachio. The potato starch-based films containing different levels of Nano-ZnO (1, 3, and 5% w/w), and FEO (1, 2, and 3% w/w) were prepared by the casting method. The fresh Akbari pistachios were packed in these bio-nanocomposite films. The physicochemical and sensory properties and the growth of *Aspergillus flavus* and aflatoxin B1 production were investigated during 15 days of refrigerated storage (~ 4°C). Results showed that by packaging fresh pistachios in bio-nanocomposite films, the moisture, carbohydrate, and fat contents of pistachios and the sensory parameters (texture, flavor, appearance, and overall acceptability) of them were improved during refrigerated storage. The weight loss of control significantly was higher than packed samples in bio-nanocomposite films ($p < 0.05$). Usage of bio-nanocomposite films leads to a decrease in the growth of *Aspergillus flavus* and aflatoxin production in pistachios during storage periods. Finally, the results demonstrated that potato starch/5% Nano-ZnO/3% FEO bio-nanocomposite film improved the physicochemical and sensory properties and had the best effect on mold spoilage reduction of fresh pistachios; hence, it can be used as packaging for fresh nuts.

Introduction

Pistacia vera L. belongs to *Anacardiaceae* family and is one of the most important and widely used tree nuts in the world, which can be consumed both fresh and dried (Kola *et al.*, 2018; Molamohammadi *et al.*, 2020; Hosseini *et al.*, 2021). Fresh consumption of

pistachios accounts for about 10% of its total production (Hosseini *et al.*, 2019). Iran is known as the second-largest exporter of pistachios in the world, and the number of Iranian pistachio exports in 2020/2021 was reported to be about 185000 tons (Shahbandeh, 2021).

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Pistachio is an agricultural product rich in nutrients, including carbohydrates (16%), proteins containing essential amino acids (25%), oils containing unsaturated fatty acids (55%), dietary fiber (10%), and so on (Mokhtarian *et al.*, 2020). The growth of microorganisms, as well as biochemical and physiological changes in fresh pistachios, can reduce the shelf life of this product. During the storage period of fresh pistachios, microbial spoilage, texture softening, weight loss, changes in nutrient compositions, changes in surface color, and organoleptic characteristics can occur (Molamohammadi *et al.*, 2020). Pistachios become inedible due to the growth of molds, especially *Aspergillus flavus* and *Aspergillus parasiticus*, and the production of aflatoxins by them (Cheraghali *et al.*, 2007; Mahbobinejad *et al.*, 2019). Therefore, different strategies have been proposed to reduce the intensity of undesirable changes, microbial growth and increase the shelf life of fresh plant products including cold plasma technology (Abbaszadeh *et al.*, 2018), treatments (Fozi *et al.*, 2022; Sedaghat *et al.*, 2022), proper packaging (Moradinezhad and Jahani, 2016; Hadadinejad *et al.*, 2018; Talukder *et al.*, 2020), and use of edible films and coatings (Aziz *et al.*, 2021; Malekshahi and ValizadehKaji, 2021). These strategies are reconsidered solutions for improving the shelf life and the quality of pistachio and many other plant products (Esfahani *et al.*, 2020).

Biopolymers derived from natural sources, including carbohydrates, proteins, and lipids, can be used to manufacture edible films for food packaging applications (Mohammadi-Benaruiyeh and Sharifi-Sirch, 2021; Sharma *et al.*, 2018). Starch is one of the most important biopolymers for the production of edible coating and films. These biopolymers are non-toxic, safe, available, biodegradable and biocompatible (Hu *et al.*, 2019). Despite the optimal film-forming properties of starches, these films have poor mechanical and barrier properties (Yun *et al.*, 2019), and these

disadvantages can be overcome by using various additives such as natural essential oils and Nano-fillers (Riahi *et al.*, 2021). Biopolymer-based films are generally considered as suitable carriers for active agents such as antioxidants and antimicrobial compounds, and these active packaging films can be used to improve the shelf life of fresh food products (Basumatary *et al.*, 2020). In previous studies, different active edible films and coating have been used to improve the shelf life and quality of fresh pistachios (Kazemi *et al.*, 2020; Molamohammadi *et al.*, 2020; Nazoori *et al.*, 2018; Shakerardekani *et al.*, 2021).

Plant essential oils are a mixture of various active and volatile compounds such as polyphenols, terpenoids, short-chain fatty acids, etc. and often demonstrate notable and significant functional activities such as antioxidant and antimicrobial activities (Zahedi *et al.*, 2021). Fennel (*Foeniculum vulgare* Miller) belongs to the *Umbelliferae* (*Apiaceae*) family and is widely used in traditional medicine and pharmaceutical industry to treat different diseases such as abdominal pain, flatulence, stomach pain, gastritis, diarrhoea, ulcers, infantile colic, vomiting, cold, fever, insomnia, arthritis, liver disease, renal colic, and various types of cancer (Belabdelli *et al.*, 2020; Rather *et al.*, 2016). Two commercially important varieties of fennel are bitter fennel (*F. vulgare* var. *vulgare*) and sweet (*F. vulgare* var. *dulce*) (Ilić *et al.*, 2019). α -pinene, *o*-cymene, α -phellandrene and estragole have been identified as the major active constituents in fennel essential oil (Garzoli *et al.*, 2018). The antioxidant, antimicrobial and antifungal activities of fennel essential oil have been confirmed in different studies (Abdellaoui *et al.*, 2020; Khalid *et al.*, 2015; Shahat *et al.*, 2011; Zeng *et al.*, 2015). Antifungal activity and inhibition against grey mould of fennel essential oil to improve storage life of fruits and vegetables reported by other researchers (Jafarzadeh *et al.*, 2021; S. Mohammadi and Aminifard, 2013; Özcan *et al.*, 2006)

Nano-fillers can improve the properties and performance of different biopolymer-based films (Xiao *et al.*, 2020). Metal and metal oxide nanoparticles with antimicrobial properties such as ZnO, CuO, TiO₂, Au, and Ag are widely used in active packaging (Tavakoli *et al.*, 2017). Nano-ZnO is one of the most well-known metal nanoparticles for food packaging applications that is safe and demonstrates good antimicrobial and improving properties (Kumar *et al.*, 2020). Kazemi *et al.* (2020) reported a shelflife improvement of fresh pistachio using nano-ZnO. Another research application of active films by using essential oils together with nanoparticles showed that it can improve the storage life of fresh pistachio (Esfahani *et al.*, 2020).

Due to the perishability of fresh pistachios and their economic importance, in this study, the synergistic effect of Nano-ZnO and fennel essential oil on the physicochemical properties and fungal spoilage of fresh pistachio was investigated.

Materials and Methods

Materials

Potato starch was purchased from SIM Company (Penang, Malaysia). Nano-ZnO and glycerol were purchased from Sigma Chemical Company (St. Louis, MO, USA). Fennel essential oil (FEO) was prepared from Barij-Essence Company (Iran). Akbari variety of pistachio was purchased from a local market in Damghan (Iran). Other chemicals were purchased from Merck Company (Germany).

Preparation of potato starch/Nano-ZnO/FEO bionanocomposite films

To prepare bio-nanocomposite films, at first Nano-ZnO were poured into distilled water (100 mL) at levels of 1%, 3% and 5% w/w (nanoparticle weight to dry weight of starch) and homogenized by an ultrasonic bath (BANDELIN SONOREX digitec, Germany) at 40°C for

20 min. To prepare the film solutions, 4 g of potato starch was added to Nano-ZnO solutions and mixed well. Glycerol at a level of 50% w/w was added to the solutions as a plasticizer (Babapour *et al.*, 2021). While stirring, the starch suspensions were heated to 90 °C, and complete gelatinization of potato starch was performed at this temperature for 30 min. During cooling of the film solutions at a temperature of 45°C, different concentrations of the FEO (1%, 2% and 3% w/w) were added to the film solutions and homogenized for 30 min. Finally, the film solutions (90g) were poured into Plexiglas plates and dried for 24 h in an oven (at 30°C and 50% RH) (Teymourpour *et al.*, 2015).

Preparation of pistachio samples

After preparing fresh pistachios, healthy pistachios were separated from defective and premature pistachios, and about 100 g of fresh pistachio fruit without spikes were used in each experimental unit. Fresh pistachio samples were packaged in the potato starch-based bionanocomposite films and stored in a refrigerator at 4 ± 1°C for 15 days under the same storage conditions, and the tests were performed on the pistachio samples every 5 days (Esfahani *et al.*, 2020).

Characterization of pistachio with active packaging

The moisture content of the pistachios was determined by the gravimetric method in an oven (Memmert, Germany) at 105 ± 2°C until reaching a constant mass (AOAC, 1994). The fat content of the samples was measured using the Soxhlet method (AOAC, 1994; Roozban *et al.*, 2005). The amount of soluble carbohydrate in the samples was determined by the sulfuric acid-phenol method and was reported as a percentage (g per 100g of dry matter) (Panahi and Khezri, 2011). To determine the weight loss percentage of the pistachios, their weight was measured before storage, and then re-weighed after certain intervals of storage and the weight loss was calculated by

$((\text{initial weight (g)} - \text{secondary weight (g)})/\text{initial weight (g)}) \times 100$ (Gao *et al.*, 2013).

To count the number of *Aspergillus flavus* colonies, the surface culture method, the potato dextrose agar medium (PDA), and the incubation temperature of 25°C for 4 days were used. To prepare the samples for microbial test, 25g of ground pistachio seeds on a magnetic stirrer was mixed with 225mL of peptone water (for 10 min). The results were reported in log CFU/g (Sayanjali *et al.*, 2011). The reverse-phase-high-efficiency liquid chromatography system (RP-HPLC) (Hewlett-Packard, England) was used to measure the aflatoxin B1 amounts in the samples (ISIRI, 2011).

Sensory analysis of pistachio during application of active packaging

The sensory evaluation of pistachio samples was performed using a 7-point hedonic scale test (1= very bad sample, and 7= excellent sample) and 10 trained panelists (5 male, 5 female) on the first and fifth day of storage. The studied sensory attributes include taste, physical appearance, firmness, and overall acceptance.

Statistical analysis

The mean of each parameter was analyzed by one-way analysis of variance (ANOVA) using the SPSS

22.0 software in a full factorial (n=3) design. Differences between treatments were expressed using Duncan's multiple range test at a 5% level ($p < 0.05$).

Results

The moisture content of pistachios

The moisture content of different Akbari pistachio samples during the 15-day storage period is compared with each other in Fig. 1 and shows that on the first day, the use of starch-based edible films containing different levels of nano-ZnO and FEO didn't have a significant effect on the moisture content of pistachio samples and the moisture content of these samples was 7.35%. Over time, the moisture content of samples gradually decreased ($p < 0.05$). The highest reduction of moisture was observed in control, and the sample pack in the film without nanoparticles and essential oil was after that. With increasing the concentrations of Nano-ZnO and FEO in the films, the moisture content of the pistachios was better preserved, and the effect of Nano-ZnO was more than the FEO. On the last day of refrigerated storage, the lowest moisture content was obtained in control (5.07%), followed by the sample packed in the film without nanoparticles and essential oil (5.41%), and the highest moisture was related to the pistachios packed in the film containing a combination of 5% Nano-ZnO and 3% FEO (6.55%).

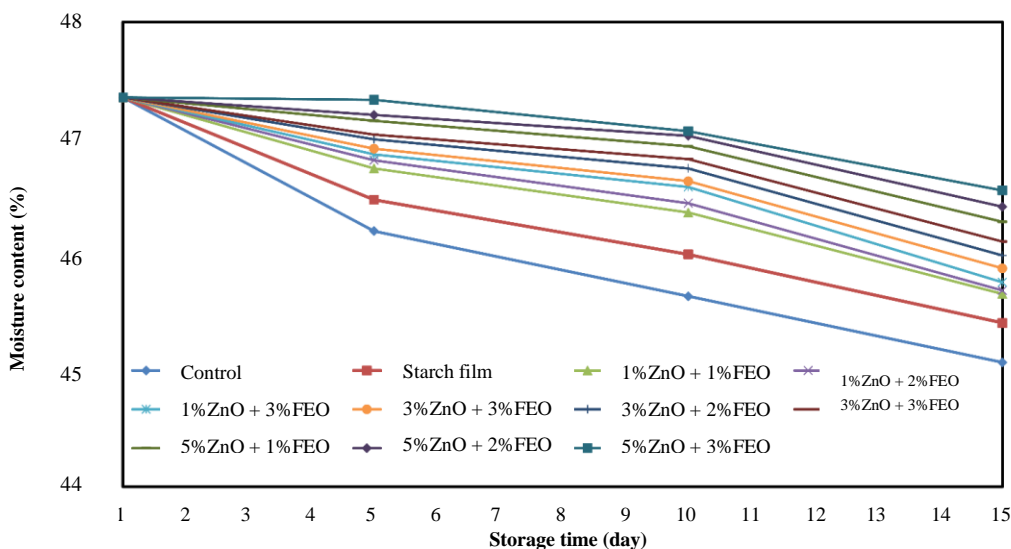


Fig. 1. Moisture content of different Akbari pistachio samples during the 15-day storage time.

The fat content of pistachios

Fig. 2 compares the average fat content of packaged pistachio samples in potato starch-based bio-nanocomposite films over a 15-day storage period. The results demonstrated that on the first day of storage, the use of bio-nanocomposite films had no effect on the fat content of pistachio samples, and pistachio samples on this day contained 59.64% fat. During the storage period, due to the respiration of pistachios and the decomposition of fats, and the production of free fatty acids, the fat content of different samples was reduced ($p < 0.05$). The highest reduction in fat content was observed in control, in which from the first day to the 15th day, the fat content decreased from 59.64% to 57.33%. By using potato starch-based films and adding different combinations of Nano-ZnO and FEO in the

films, by reducing the intensity of respiration and the rate of fat breakdown in pistachios, their fat content was maintained. On the last day of storage, the highest amount of fat was observed in pistachio samples packaged in the bionanocomposite films containing 5% Nano-ZnO in the combination of 1% and 2% FEO (59.19% and 59.08%, respectively). In general, the pistachio fat content increased by increasing the level of Nano-ZnO from 1% to 5% in the films by reducing the rate of respiration due to reduced oxygen permeability (Babapour, 2021) while increasing the concentration of FEO from 2% to 3% due to increasing the permeability of films to oxygen (Babapour, 2021) caused a significant reduction in the fat amount in the pistachios.

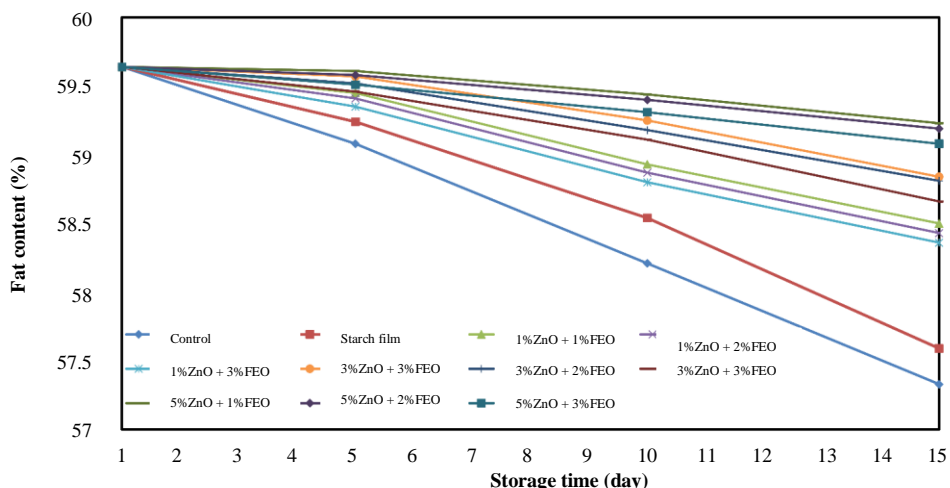


Fig. 2. The fat content of different Akbari pistachio samples during the 15-day storage time

Soluble carbohydrate content of pistachios

The soluble carbohydrate content of different Akbari pistachio samples during the 15-day storage period in a refrigerator is compared with each other in Fig. 3. On the first day, there was no difference between the carbohydrate content of pistachio samples, and over time, from the first to 15th day, due to the respiration of the pistachios and decomposition of sugars, the percentage of carbohydrates in pistachios significantly decreased ($p < 0.05$). The highest reduction in carbohydrates during the storage period was related to

the control sample, in which the number of carbohydrates was reduced from 9.25% to 5.80%. Bionanocomposite films based on potato starch containing different levels of Nano-ZnO and FEO by reducing the respiration rate of pistachios preserved the carbohydrate content in the samples. The highest amount of carbohydrates on the last day of storage was obtained in the starch films containing 5% Nano-ZnO in a combination of 1% and 2% FEO (8.35% and 8.26%, respectively).

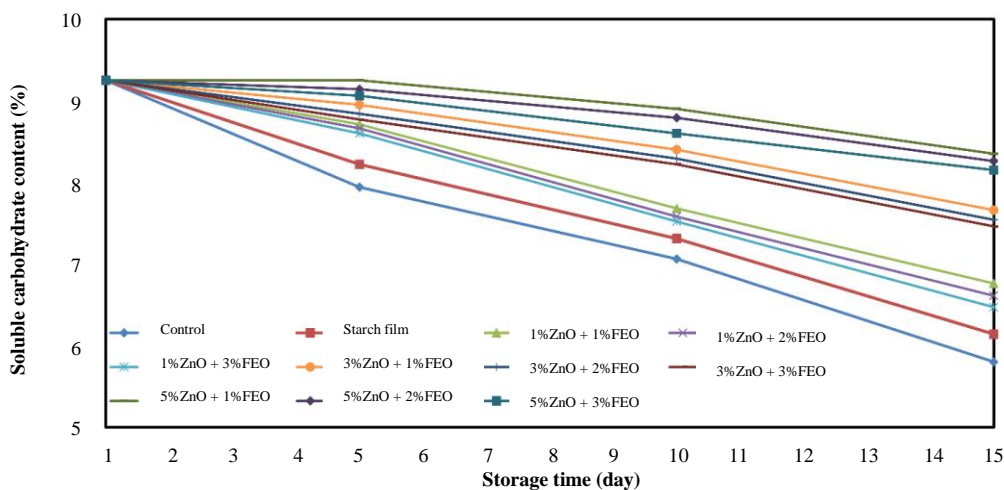


Fig. 3. Soluble carbohydrate content of different Akbari pistachio samples during the 15-day storage time.

Weight loss of pistachios

The weight loss percentages of different pistachio samples during the 15-day storage in the refrigerator are shown in Fig. 4. The use of bionanocomposite films as well as storage period demonstrated a statistically significant effect on the weight loss of fresh Akbari pistachios ($p < 0.05$). As expected, on the fifth day of storage, the highest amount of weight loss was observed in control (1.96%), and packaging of pistachios with starch-based bio-nanocomposite films significantly reduced the weight loss of pistachios ($p < 0.05$).

Increasing the levels of Nano-ZnO and FEO in the films reduced the weight loss percentage of pistachios by preserving the moisture content. During the refrigerated storage, the weight loss percentage of different samples increased significantly ($p < 0.05$). On the last day, the highest weight loss was related to the control (4.40%), and the pistachios packed in the starch film containing 5% Nano-ZnO in combination with 3% FEO showed the lowest amount (2.54%).

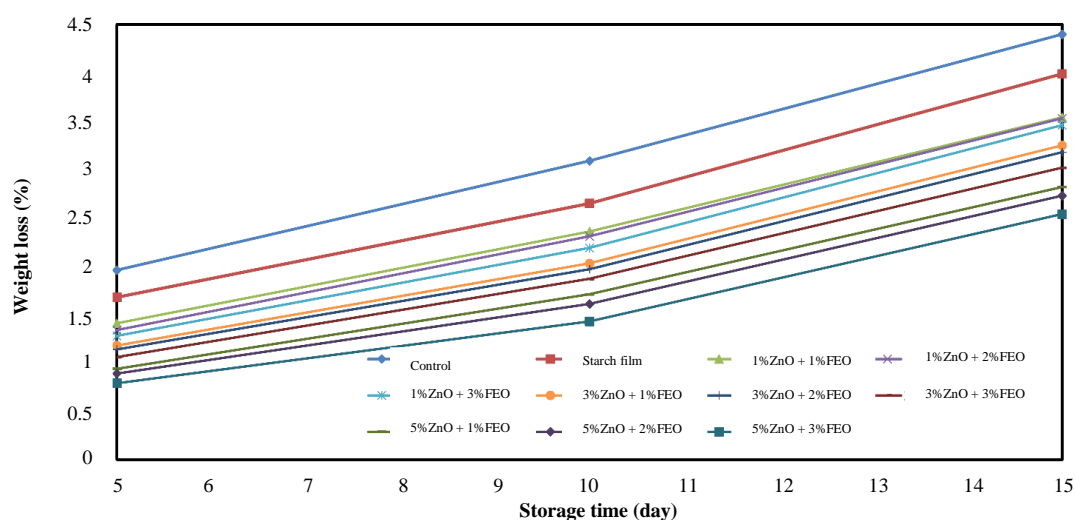


Fig. 4. Weight loss of different Akbari pistachio samples during the 15-day storage time.

Aspergillus flavus count in pistachios

The mean logarithmic changes of the number of *A. flavus* fungi in pistachio samples during refrigerated storage are compared in Fig. 5 and demonstrate that over time from the first to the 15th day of storage, the number of molds in the fresh pistachios increased significantly ($p < 0.05$), and the use of starch-based bio-nanocomposite films containing a different combination of nano-ZnO and FEO significantly reduce the growth rate of molds in the samples ($p < 0.05$). Increasing the

concentration of nanoparticles and essential oil had a positive effect on reducing the growth rate of fungi in the fresh pistachios. Nano-ZnO showed higher antifungal activity than FEO. On the last day of refrigerated storage, the highest and lowest molds numbers were observed in the control sample (3.31 log CFU/g) and packaged sample in the film containing 5% Nano-ZnO in a combination of 3% FEO (1.67 log CFU/g), respectively.

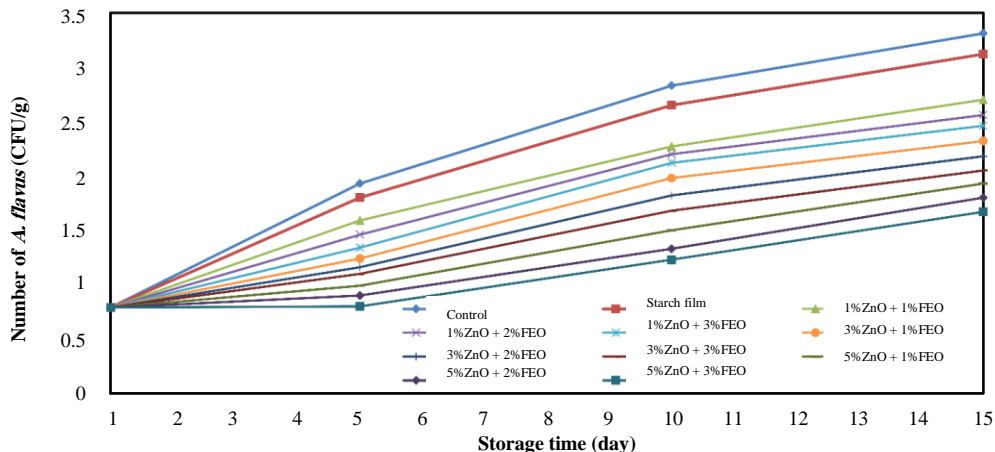


Fig. 5. The mean logarithmic changes of the number of *A. flavus* fungi in pistachio samples during the 15-day storage time.

Aflatoxin content of pistachios

The aflatoxin content of different pistachio samples during the 15-day storage are presented in Fig. 6. The use of bio-nanocomposite films as well as storage period demonstrated a statistically significant effect on the aflatoxin amounts of fresh Akbari pistachios ($p < 0.05$). On the first day of storage, the use of bio-nanocomposite films did not affect the molds numbers in pistachio samples and therefore didn't show any effect on aflatoxin levels. During the storage period, with increasing the number of molds in pistachios, the

amount of aflatoxin production in the samples also increased significantly ($p < 0.05$). With increasing the concentration of nano-ZnO and FEO, the antifungal activity of the starch films increased, and the production of aflatoxin in the pistachios decreased ($p < 0.05$). On the last day, the control had the highest amount of aflatoxin (5.10 ppb), and the lowest amount was for pistachios packed in the films containing 5% nano-ZnO in a combination of 3% FEO (2.80 ppb).

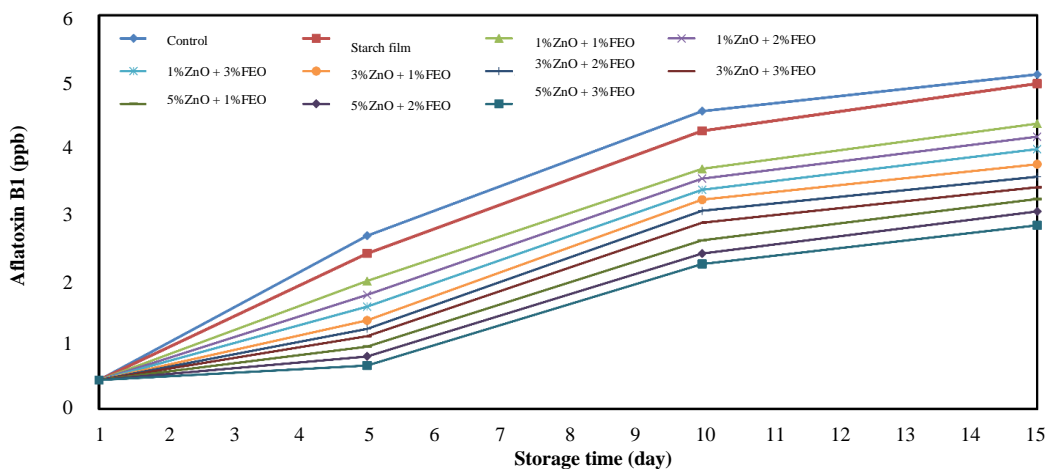


Fig. 6. The aflatoxin content in pistachio samples during the 15-day storage time.

Sensory evaluation of pistachios

The average scores of texture, taste, physical appearance and overall acceptability of different samples of Akbari pistachio are compared in Fig. 7. On the first day, all samples obtained complete sensory scores, and there was no difference between the pistachio samples. Over time, the sensory properties of all pistachio samples gradually decreased, and the rate of sensory scores changes in the control and subsequently in the sample packed in the potato starch-

based film without nano-ZnO and FEO was significantly higher than other samples ($p < 0.05$). Increasing the concentration of nano-ZnO in the films resulted in better maintenance of sensory characteristics of pistachios during storage, so that on the last day of storage, samples packed in the films containing 5% nano-ZnO in combination with all three levels of FEO obtained the highest sensory scores and the lowest score was for the control.

(A)

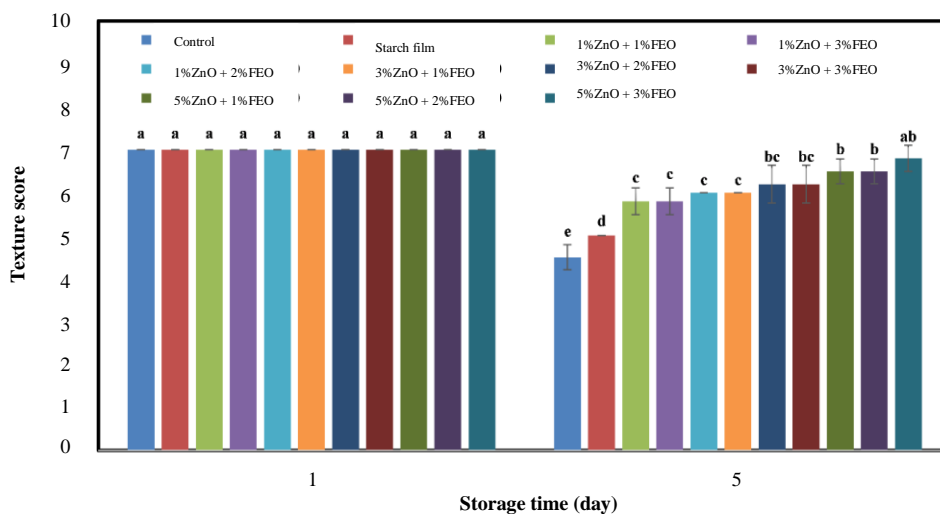
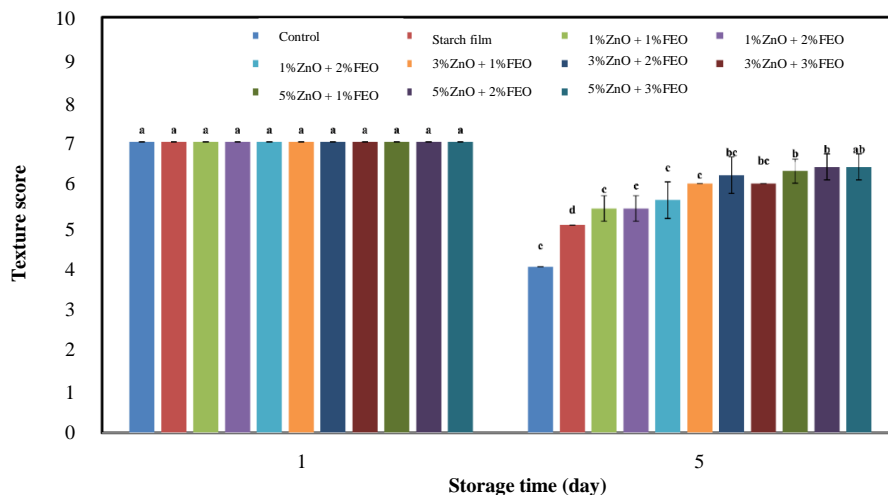
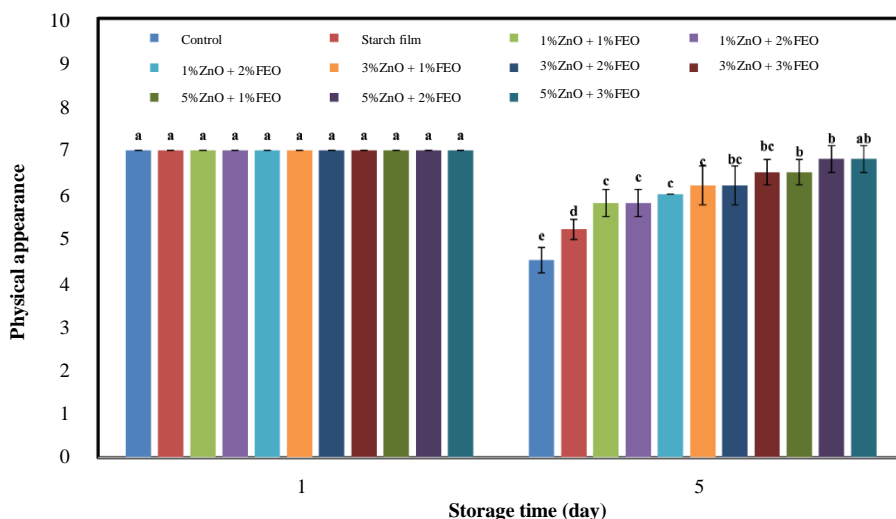


Fig. 7. Sensory attributes (A: Texture, B: Taste, C: Physical appearance, and D: Overall acceptability) of fresh pistachio packed in active packaging after 5 days.

(B)



(C)



(D)

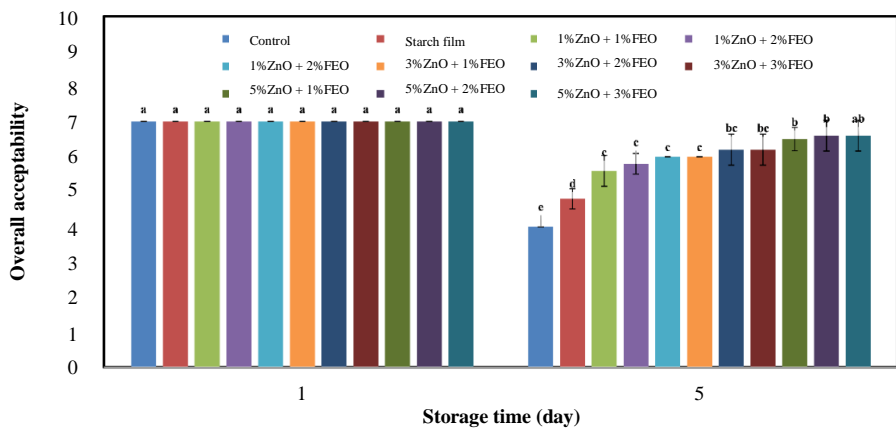


Fig. 7. Continued.

Discussion

Moisture is one of the most important and effective factors in increasing the shelf life and quality of nuts such as pistachios. By increasing the moisture content, the spoilage reactions in the nuts are accelerated, and its shelf life is reduced (Cha and Chinnan, 2004). Chemical and microbial spoilage, as well as the production of the mycotoxin, occur at the high moisture content (Boghorri *et al.*, 2020). Edible films and coatings act as a barrier to the transfer of water to the nut tissues and prevent the acceleration of the lipid hydrolysis reaction by preventing moisture from entering the foods. Packaging the product in active coating and films reduces the passage of water molecules through this dense network and improves the quality of the nuts, and increases their

shelf life by better preserving the moisture of the product and preventing the absorption of moisture from the environment (Pokorny and Dieffenbacher, 1989; Chatrabnous *et al.*, 2018). Hydrophobic compounds of FEO and Nano-ZnO acted as a barrier against moisture transfer by improving the barrier properties of the potato starch-based films against water vapor (Babapour *et al.*, 2021). Jafari and Javadi (2020), in the study of the effect of coating fresh pistachios with chitosan containing walnut leaf extract, observed that by increasing the concentration of the extract in the coating, the moisture content of the pistachio samples was better preserved during the storage period. Colzato *et al.* (2011) also noticed that the presence of hydrophobic compounds in

the solution of coating and films causes an inhabitation property in them. Nur Hanani *et al.* (2012) found that the films and coating based on 4% gelatin were good protective coating against moisture.

Pistachio kernels are a rich source of oil, and the content of pistachio oil varies between 40-63% (Arena *et al.*, 2007). Triacylglycerols are the most important acylglycerols in pistachio oil and make up more than 90% of the total fat in pistachio oil (Chahed *et al.*, 2008). These triacylglycerols are rich in polyunsaturated fatty acids (PUFA) such as linoleic acid and linolenic acid, which are essential for the human diet (Ballistreri *et al.*, 2010). The fat and carbohydrate retention of pistachios due to the use of bio-nanocomposite films can be attributed to the reduction of oxygen permeability of the films due to the incorporation of nano-ZnO, because by reducing the penetration of oxygen into pistachios, the respiration rate of the product decreases and the decomposition of storage materials such as carbohydrates and fats also decreases. In general, it can be stated that the incorporation of nanoparticles into edible films and coatings can reduce the possibility of exchange and penetration of water vapor, oxygen and carbon dioxide between two inside and outside packing spaces (Moraru *et al.*, 2003). On the other hand, bio-nanocomposite films containing nano-ZnO and FEO reduced the speed of UV light transmission to the packaged product (Babapour *et al.*, 2021), thereby reducing the rate of destructive oxidation of fats in pistachios. Ahmadi *et al.* (2014) similarly showed that coating fresh pistachio with aloe Vera gel-based edible coating prevented fat loss of samples during storage and also reduced the rate of carbohydrate reduction. Shakerardekani, *et al.* (2021), in the study of the effect of sodium alginate coating containing thyme essential oil on the amount of soluble sugars in fresh pistachios, reported that no significant change in soluble sugars was observed during storage periods. Hashemi *et al.* (2018) also observed that the use of CMC-based films

containing 0.2% and 0.4% w/v *Zataria multiflora* essential oil reduced the intensity of carbohydrate degradation in the fresh pistachios.

The results of weight loss of fresh Akbari pistachios samples showed that the use of starch-based bio-nanocomposite films containing a combination of Nano-ZnO and FEO significantly reduced the weight loss of pistachio samples ($p < 0.05$). In general, moisture loss is the main reason for weight loss of products, which occurs as a result of post-harvest respiratory and transpiration processes in the product, and depends on the force caused by the difference in water vapor pressure between the kernel tissue and the surrounding air, as well as the resistance of the tissue to this force (Joo *et al.*, 2011). The weight loss of the products is also related to the water vapor permeability of the packaging films (Conte *et al.*, 2009). Active films act as a barrier against the passage of gasses and water vapor and reduce weight loss by reducing moisture loss of pistachios and reducing the breakdown of carbohydrates and fats in the kernels. According to Shakerardekani, *et al.* (2021), the application of sodium alginate coating containing thyme essential oil was effective in decreasing the weight loss of fresh pistachio samples during storage. Molamohammadi *et al.* (2020) and Mirdehghan *et al.* (2021) also reported a decrease in the weight loss of pistachio samples due to the use of chitosan coating incorporated with salicylic acid and carboxymethyl cellulose coating containing clove essential oil during the storage period, respectively. Reducing the weight loss of peanuts due to the use of whey protein concentrate coatings containing thyme essential oil was also observed by Boghori *et al.* (2020). In the research conducted by Nasef (2020), the weight loss in bell pepper samples decreased due to the addition of fennel essential oil.

Aspergillus flavus is one of the fungi that produce aflatoxin in human and animal food. According to the previous research, different species of *Aspergillus* fungi

have been isolated from soft and hard shells and kernels of Iranian pistachios (Rahimi *et al.*, 2008). The most important aflatoxin-producing fungi isolated from the pistachios are *A. flavus* and *A. parasiticus*, which are capable of producing different aflatoxins. Infection of pistachios with *Aspergillus* species occurs during the ripening process in the garden. The potato starch-based bio-nanocomposite films containing Nano-ZnO and FEO have good barrier properties against gases (Babapour *et al.*, 2021) and reduce the rate of growth and multiplication of fungi by reducing oxygen permeability because molds are completely aerobic microorganisms and need oxygen in sufficient concentration to grow and multiply. On the other hand, both Nano-ZnO and FEO have significant and remarkable antimicrobial activity and prevent the growth of molds and other microorganisms (Babapour *et al.*, 2021). According to the findings of Todd *et al.* (2013), the chemical compounds in essential oils can accumulate in the fat-rich atmosphere of the cell membrane structure and causes its function to deteriorate. Methylchavicol and E-anethole are the major components of FEO, which belongs to phenylpropanoids and act as strong fungal agents in the fermentative stages of fungi and have similar effects (Fujita and Kubo, 2004). Proposed mechanisms for antimicrobial function of nanoparticles include effect on the enzyme active site, DNA and the ribosomes activity, interferences with metabolic activity (Bruna *et al.*, 2012), induction of reactive oxygen species induction (such as hydroxyl radicals, hydrogen peroxide, and superoxide) (Emamifar *et al.*, 2010), as well as the destruction of the cell wall (Li *et al.*, 2011). Shakerardekani, *et al.* (2021) showed a significant decrease in fungal growth in fresh pistachios due to the use of sodium alginate coating containing thyme essential oil. Other researchers similarly have suggested that the use of edible coatings and films reduced the mold growth in nuts by reducing moisture and oxygen

permeability (Boghorri *et al.*, 2020; Hashemi *et al.*, 2021; Javanmard and Ramezan, 2009; Razavi *et al.*, 2021; Ziani *et al.*, 2010). Tavakoli *et al.* (2017) found that Nano-silver films could reduce the mold numbers compared to control, and by increasing the Nano-silver levels, their inhibitory activity was increased. Shafiee Nasab *et al.* (2019) reported antifungal activities of polylactic acid films containing ZnO nanoparticles against *A. flavus*. Increasing the levels of Nano-ZnO from 1% to 5% led to a decrease in the mold numbers significantly. In the previous studies, FEO has shown significant antifungal activity (Barkat and Bouguerra, 2012; Khalid *et al.*, 2015; Sharifi *et al.*, 2008; Thakur *et al.*, 2013).

One of the main problems in the field of pistachio exports is the contamination with *A. flavus* and the production of aflatoxins, which can threaten this product and prevent the country from competing in the global market. Aflatoxins are a large group of mycotoxins that are secondary metabolites of molds. Cereals and nuts are the most susceptible agricultural products and nuts for mold contamination and aflatoxin production due to their high fat and carbohydrate content. As expected, in the present study, the use of starch-based bio-nanocomposite films containing a combination of Nano-ZnO and FEO significantly reduced the growth of molds and aflatoxin amounts in pistachio samples ($p < 0.05$). Moslehi *et al.*, (2021) similarly showed a significant decrease in the aflatoxin production in pistachio samples coated with methylcellulose compared to the uncoated sample (control). Mohammadi *et al.* (2020) also observed that the pistachios coated with a gelatin-CMC solution containing *Dianthus barbatus* essential oil had no aflatoxin growth in different concentrations of essential oil (300, 450, and 600 ppm), while the control sample (uncoated pistachio) demonstrated a mild growth. Tavakolipour *et al.* (2020) observed the significant effect of whey protein isolate coating containing Shirazi thyme, cumin, and sage essential oils

on reducing aflatoxin production in the pistachios. Esfahani *et al.* (2020) also reported the reduction in aflatoxin content of fresh pistachios due to the use of bio-nanocomposite films containing a combination of Nano-TiO₂ and cinnamon essential oil.

The results of the sensory evaluation showed that, in general, the use of potato starch-based active films containing a combination of Nano-ZnO and FEO by blocking the penetration of oxygen into the pistachio kernels tissue prevented unwanted oxidation reactions and discoloration of color and flavor of the product. Pistachio texture is also affected by moisture content so that in samples that had less moisture loss during storage, the texture was better preserved. Maghsoudlou *et al.* (2012) stated that the use of chitosan-based edible coatings had no significant effect on the organoleptic properties of pistachio including color, texture, and overall acceptance. Hashemi *et al.* (2018) found that incorporating 0.2 and 0.4% w/v Shirazi thyme to CMC-based coating had favorable effects on the sensory characteristics of fresh pistachios. Esfahani *et al.* (2020) also reported the lack of significant effect of using bio-nanocomposite films containing a combination of Nano-TiO₂ and cinnamon essential oil on the texture and flavor of fresh pistachios.

Conclusions

In this study, the effect of bionanocomposite films based on potato starch/Nano-ZnO/FEO on the physicochemical and fungal spoilage of fresh Akbari pistachios during refrigerated storage was investigated, and the obtained results demonstrated that packaging of pistachios with bionanocomposite films increased the storage life of pistachios by reducing the growth and proliferation rate of *A. flavus* fungi and as a result produced lower amounts of aflatoxin. Packing pistachios in active bio-nanocomposite films also preserves moisture, carbohydrate, and fat of pistachios during storage and reduces the weight loss percentages,

and in addition, maintains the sensory characteristics of the pistachios. Since the lowest fungal contamination and aflatoxin production, as well as the least changes in chemical composition and sensory properties during storage, were observed in the packaged pistachios in the film containing 5% Nano-ZnO in combination with 3% FEO, therefore, this sample was introduced as the best treatment.

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Conflict of interests

There is no conflict of interest.

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