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# Increasing the shelf life of fresh in-hull pistachio using nanocomposite packaging of zinc nanoparticles and pistachio green hull essential oil

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ABSTRACT

Fresh in-hull pistachio is one of the highly consumed but perishable forms of pistachio and can still be contaminated by aflatoxin-producing fungi. In this study, the packaging film prepared from an electrospun nanofibers was evaluated in order to increase the shelf life of raw pistachios. For this purpose, PVC and zinc nanoparticles combined with pistachio green hull essential oil (PGHEO) were used as the matrix and reinforcing phase of the composite, respectively. According to the results, monoterpenes such as α-pinene and limonene constitute more than 90% of PGHEO. The minimum inhibitory concentration (MIC) values of the essential oil ranged from 62.5 to 500 μg/mL which were more effective against *Aspergillus flavus*, a mycotoxigenic fungus affecting pistachio safety and quality, than other studied fungi. According to FE-SEM images, fibers were formed on film surfaces with diameters ranging from 89 nm to 295 nm. EDX spectra revealed some characteristic peaks for zinc indicating the existence of ZnO nanoparticles on the top surface of the composite. The prepared nanocomposite indicated the antifungal activity against *Candida albicans, Aspergillus flavus* and *Aspergillus parasiticus* with the growth inhibition percentage approximately 16.20, 9.60 and 2.88%, respectively. Finally, raw pistachios could be stored for 60 days in packaging made of nanocomposite, so that the amounts of aflatoxin $B_1$ and $B_2$ were lower than the allowed maximum level (< 12 ppb). It seems that the packaging used in this study can be a suitable solution to increase the shelf life of raw pistachios and reduce the waste caused by its spoilage.

## 1. Introduction

Pistachio is a valuable nut from both a nutritional and economic point of view. The growers and processors of the nut create a significant contribution in the economic output in the producing countries, generating jobs and spending across various sectors of their economies. Accordingly, pistachio nut world commercial production raised from 345,408 tonnes in 1994 to about 1.1 million tonnes in 2020 (i.e., more than 3-folds), with the United States, Turkey and Iran as leading producers, together accounting for 70% of the total (FAOSTAT, 2020). In comparison to other common nuts, pistachio has a higher ratio of essential amino acids (Hernández-Alonso et al., 2016). In addition, pistachios have the highest amount of monounsaturated fatty acids (MUFA) and the lowest ratio of polyunsaturated to saturated fatty acids, among nuts (Esteki et al., 2019) which suggests its cholesterol-reducing potential. It is also considered as a rich source of protein, dietary fibers, vitamins (B6, folate, A, E and K), minerals (phosphorous, magnesium, iron, zinc, calcium and selenium) and phytochemicals (carotenoids, such as lutein) and phenolic compounds) (Mandalari et al., 2021). As a result of these unique nutritional properties, it exhibits beneficial health

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effects including cholesterol-reducing potential, reduces the risk of diabetes and antioxidant activity (Hernández-Alonso et al., 2014; Sauder et al., 2015).

Raw pistachios are commercialized with or without hull, but the price of raw pistachios in hulled form is lower than that of the other form. The trade of raw in-hull pistachios in the harvest season is very prosperous due to the reasonable price of pistachio nuts in this period (Hokmabadi and Sedaghati, 2013). However, the major concern for this form of pistachio is also the contamination by toxin-producing molds, such as *Aspergillus flavus*, and their growth and subsequent formation of aflatoxins. These mycotoxins were confirmed by the International Agency for Research on Cancer (IARC) as a Group-1 carcinogen to humans (IARC, 2002), therefore imposing serious threat to human consumption. In particular, the moisture of the shell is relatively high so that it can be easily separated during consumption and the pistachio appearance is also preserved, so it is considered a perishable food product (Shayanfar et al., 2011). Furthermore, due to the poor hygiene of post-harvest pistachio treatment centers, there is a possibility of contamination with pathogens from various environmental sources (Al-Moghazy et al., 2014).

The use of fungicides such as sulfur and chemical agents such as nitric oxide and γ-aminobutyric acid may be used as one of the simplest strategies to increase the shelf life of in-hull pistachios (Gheysarbigi et al., 2020; Nazoori et al., 2022; Saeedi et al., 2022). Nevertheless, in order to overcome the health concerns of chemical tools, there is a growing interest in exploring alternative methods. For example, the potential of several polysaccharide coatings containing active agents including essential oils and salicylic acid to improve the postharvest quality of fresh in-hull pistachio was recently evaluated (Hashemi et al., 2021a, 2021b; Molamohammadi et al., 2020; Shakerardekani et al., 2021). In addition, in-hull pistachio storage in modified atmosphere packaging has been reported to extend the shelf life (Shayanfar et al., 2011; Sheikhi et al., 2019).

The composite packaging film consists of a set containing the matrix phase and the reinforcing phase. Usually, the matrix component has a series of defects, such as low antimicrobial properties, high permeability to oxygen and/or moisture, which can be solved by adding a reinforcing component (Hsissou et al., 2021). Metal nanoparticles (Deng et al., 2020) and essential oils (Jiang et al., 2020) are among the most important active antimicrobial agents that are incorporated as reinforcing components in the structure of composite films. The solution casting, melt extrusion and electrospinning are three common approaches for entrapping the active agents in films (Lai and Wong, 2022). Although the potential of electrospinning in the film preparation for food packaging has already been demonstrated by several studies (Aydogdu et al., 2019; Li et al., 2021; Liu et al., 2021; Jiang et al., 2022), with the best of our knowledge, the use of electrospun films to extend the shelf life of fresh pistachios has not been reported. Therefore, the objective of this research was to prepare antimicrobial composite films for fresh in-hull pistachio packaging by electrospinning polyvinyl chloride (PVC), ZnO nanoparticles and pistachio green hull essential oil (PGHEO) on the surface of polyethylene film and to investigate its potential in reducing the fungal growth and aflatoxin production.

## 2. Material and methods

### 2.1. Materials and reagents

Pistachios cultivar 'Ahmadaghaei' were obtained from Pestaco Company located in Marvast, Yazd provinces of Iran. Commercial ZnO nanoparticles were purchased from Merck Chemical Co. (Darmstadt, Germany). Chemical reagents including polyvinyl chloride (PVC), dimethylformamide and Tween 80 were provided from Sigma-Aldrich chemical Company (St. Louis, MO). Microbial strains of *Staphylococcus aureus* (Collection No: ATCC 25,923), *Bacillus subtilis* (Collection No: ATCC 6051), *A. flavus* (Collection No: ATCC 24,109), *Candida albicans* (Collection No: ATCC 10,231), *Aspergillus parasiticus* (Collection No: ATCC 28,285) were purchased from the Iranian Research Organization for Science and Technology (IROST) microbial collection.

### 2.2. Extraction of the essential oil

Extraction of the essential oil was carried out by Clevenger apparatus through hydrodistillation method. In summary, pistachio green hull powder (500 g) was immersed in water and then the volatile oil was collected after 4 h.

#### 2.2.1. Chemical composition of the essential oil

The essential oil was analyzed using Agilent 6890 series gas chromatography device connected to Agilent 5973 Network Mass Selective Detector. PGHEO was detected in a capillary column with a length of 30 m and an inner diameter of 250 μm and a thickness of the inner layer of 0.25 μm with a temperature program of 60 °C for 2 min and then with a gradual increase of 5 °C to 280 °C. The carrier gas was helium, at a rate of 1 mL/min. The detector had ionization energy of 70 eV. The identification was carried out by the library method through the retention time calculation of the Kovats index and Wiley 229 and 1998 NIST library information.

### 2.3. Antimicrobial effectiveness of PGHEO

The broth microdilution method was applied to investigate the antimicrobial activity of PGHEO against two foodborne pathogen strains (*S. aureus* and *B. subtilis*) and pistachio contaminating fungi. Two-fold dilutions of the essential oil (1000, 500, 250, 125, 62.5 and 31.25 μg/mL) in Mueller-Hilton Broth (MHB, for the bacteria) medium or Sabouraud Dextrose Broth (SDB, for the fungi) medium were added to microtitration plates in a volume of 100μl. Then, each well was inoculated by $5 \times 10^5$ colony-forming units (CFU) of the microbial inoculum (bacteria or fungi) prepared in the same mediums. After 24 h (for the bacteria) or 48 h (for the fungi) incubation at 37 °C, the minimal inhibitory concentration (MIC) was determined in terms of μg of PGHEO per mL for each microorganism. Finally, 100 μL of the dilution representing the MIC and two concentrations higher than it, were plated on Mueller-Hilton Agar (MHA, for the bacteria) or Sabouraud Dextrose Agar (SDA, for the fungi) and incubated at 37 °C overnight to determine the viable CFU/mL. The minimal bactericidal concentration (MBC) or minimal fungicidal concentration (MFC) was the lowest concentration with no visible growth.

Positive control without sample and negative control with media alone were considered for each set of experiments.

### 2.4. Preparation of nanocomposites

Firstly, 10 g PVC and 100 mL dimethylformamide (DMF) were stirred for about 1 h at 28 °C until the PVC was completely dissolved. PGHEO, ZnO nanoparticles and Tween 80 (200, 40 and 20 mg/g PVC, respectively) were added to the PVC solution, and then the solution was vigorously mixed. Then, the upper surface of a neat polyethylene film was covered by the polymeric solution using a laboratory immersion electrospinning unit made by Industrialization Center for Applied Nanotechnology (ICAN, Tehran, Iran). In this device, by applying a high voltage (26 kV), an electromagnetic field was created, which led to the stretching of the polymer solution along the length of the device through the wires embedded on the cylindrical drum and the collection of nanoscale fibers on the collector (Fig. 1). The drum speed was set to 300 rpm and its distance from collector was 10 cm. This device produced nanofibers without using a syringe nozzle but with the help of a solution bath (150–200 ml), so it had the ability to produce faster and at a higher scale in a shorter period of time.

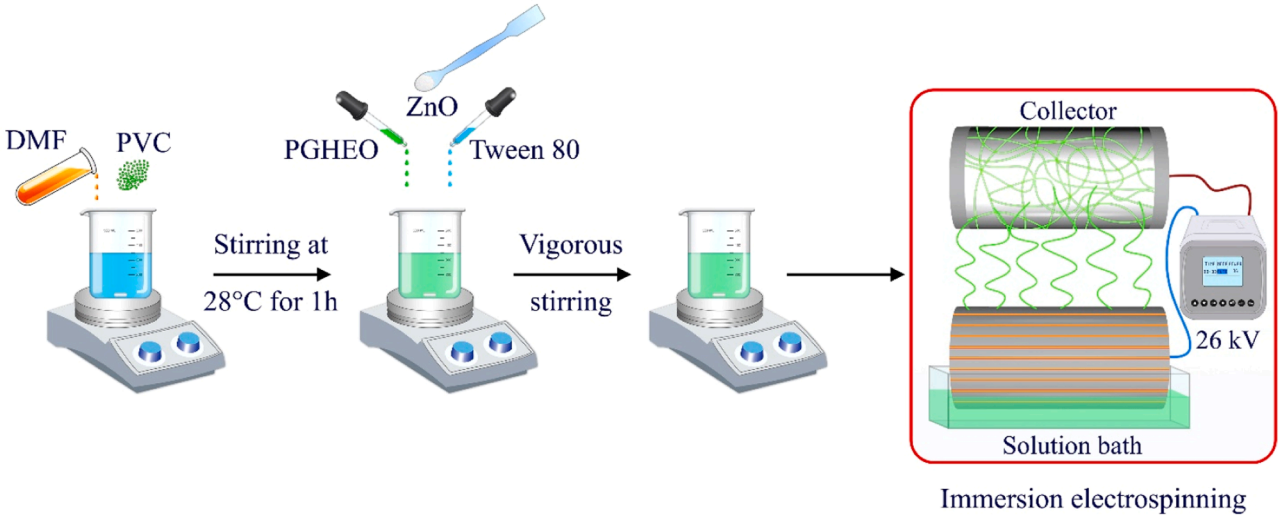


**Fig. 1.** Schematic of the preparation process of ZnO and PGHEO nanocomposite.

### 2.5. Characterization of nanocomposites

#### 2.5.1. Surface composition and morphological analysis

The morphological characterization of the prepared composite was carried out using Field Emission Scanning Electron Microscopy (FE-SEM) (Sigma VP, ZEISS Germany). The samples were attached on metal stubs and consequently coated with an ultrathin gold palladium layer. Moreover, energy-dispersive X-ray spectroscopy (EDX) (FEI NOVA NANO SEM 450, Germany) was used to study the chemical composition of the nanofiber formed on the film.

#### 2.5.2. Evaluation of antifungal properties

The antifungal activity of the nanocomposites was tested by a method based on the indirect measurement of cell numbers with some modifications (Ebadzadsahrai et al., 2020; Kurapati et al., 2016). For this purpose, the prepared nanocomposite (1 × 1 cm) was first sterilized in 70% ethanol aqueous solution and dried up using a sterilized incubator at 37 ºC. Next, the sample was introduced into three sterilized tubes containing selected fungi at a concentration of $10^6$ CFU/mL ($10^4$ Spore/mL) in SDB culture medium. 3 tubes containing the naked film piece (film without nanocomposite coating; 1 × 1 cm) and the same fungi at a concentration of $10^6$ CFU/mL in the same culture medium without the scaffold were used as negative control. In addition, SDB culture media without the fungi were used as blank. The tubes were incubated in a shaker-incubator at 150 rpm speed at 37 °C for 24 h. To compare the turbidity of each tube, the absorbance of each of them was read at 600–660 nm. The growth inhibition percentage (GIP) was obtained by the following formula:

$$\text{GIP}\ (\%) = \frac{\text{OD}_{\text{negative control}} - \text{OD}_{\text{treatment}}}{\text{OD}_{\text{negative control}} - \text{OD}_{\text{blank}}} \times 100$$

### 2.6. The use of nanocomposite film in the packaging of pistachios

ZnO/ PGHEO nanocomposites coated films was used as the top layer of a cube-shaped polyethylene package. For this purpose, first, 250 g of pistachio was poured into the bag and then it was sealed with a layer of nanocomposite film under modified atmosphere (MAP) conditions. The gaseous composition of the packaging was 2% oxygen, 70% carbon dioxide and 30 nitrogen. The control sample consisted of pistachios packed in a completely polyethylene MAP package. All samples were stored at refrigerator temperature.

### 2.7. Determination of aflatoxin levels in pistachio

#### 2.7.1. Extraction and isolation of aflatoxins

The packed samples were allowed to reach 25 ± 2 °C at room temperature. The extraction method of aflatoxins was based on the previously reported procedure with some modification (Trucksess et al., 2008). First, a sufficient amount of the pistachio nuts was finely ground and 25 g of the sample were then mixed with 5 g NaCl and 100 mL methanol/water solution (80:20) for 2 min in a high speed blender. The crude sample extract was filtered through a paper filter Fisher QL 125 (Scientific Ltd. Loughborough, Uk). Filtered extract (10 mL) was mixed with 70 mL phosphate buffer solution containing 10% Tween 20. For adjusting the solution pH to 7.4, 2 M NaOH was used and the diluted extract was again passed through the filter paper to remove interfering substances. The filtrate (70 mL) was finally passed through the immune-affinity column (AflaOchraTest column; G1017; Vicam, Watertown, MA) with a flow rate of 2 mL/min followed by washing using 20 ml phosphate buffer. The mycotoxins were eluted from the column using 1 ml of methanol, transferred into a vial for HPLC-FID analysis.

#### 2.7.2. HPLC-FLD condition

The analysis of aflatoxin concentration in the pistachio samples was carried out using a HPLC system (Waters Alliance 2690, Milford, MA, USA), coupled to a fluorescence detector. This device was attached to a Kobra cell R chamber (Rhône diagnostics, Glasgow, UK) for post-column derivatisation reaction. The chromatographic separation was performed at 30 °C by an Inertsil ODS-2 Analytical column (C18 column; 5 µm, 150×4.6 mm) protected by a Hichrome column (Hichrom Ltd., Reading, UK). The detector was set at an excitation wavelength (λex) of 365 nm and an emission wavelength (λem) of 435 nm. The isocratic mobile phase was methanol and water blend with a composition of 40/60 (ν/ν) stabilized by 350 µl nitric acid (4 mol/l) and 120 mg potassium bromide at a flow rate of 1 ml/min.

### 2.8. Statistical analysis

Each experiment was carried out in triplicate. Statistical analysis was performed using SPSS (SPSS Inc., Chicago, IL, version 15) and the LSD test was used to compare the mean values at a confidence level of $\alpha<0.05$.

## 3. Results and discussion

### 3.1. Chemical composition of PGHEO

18 components of the extracted essential oil were identified by GC–MS device which included 100% of the total components of PGHEO (Table 1). Among these, hydrocarbon monoterpenes (93.87%) and oxygenated monoterpenes (6.13%) were the major groups of the essential oil extracted from the pistachio green hull without any hydrocarbon sesquiterpenes and oxygenated sesquiterpenes. The dominant component of PGHEO was α-pinene (39.59%); significantly higher compared to other major components including limonene, terpinolene, camphene, L-bornyl acetate and β-Pinene. Chahed et al. (2007) identified the main components of pistachio hull essential oils from three tunisian localities as α-pinene (30.44–43.87%), α-terpinolene (32.2–35.77%), δ−3-carene (3.99–4.52%), bornyl acetate (1.32–4.68%) and limonene (2.84–3.09%) (Chahed et al., 2007). Moreover, the major constituents identified in the pistachio green hull essential oil harvested from trees in Turkey were α-pinene (54.40%), terpinolene (18.91%), limonene (6.62%) δ−3-carene (3.97%), and camphene (3.20%) (Küsmenoglu et al., 1995). By comparing these data with the results of the present study, it is clear that the higher amount of limonene is the distinguishing feature of Iranian PGHEO.

### 3.2. Investigating the antimicrobial properties of PGHEO

The antimicrobial activity of the essential oil against some common microorganisms using the broth microdilution method are shown in Table 2. PGHEO was able to have inhibitory and lethal effects on all 4 investigated microorganisms, with its antifungal activity was far more than its antibacterial effects. In this regard, the most sensitive strains were *A. flavus*, a mycotoxigenic fungus which can affect pistachio quality via producing aflatoxin B or as a result of fungal colonization. It may be mentioned here that the incubation temperature used in the current study was slightly higher than the optimum growth range (25–35 °C) of filamentous fungi (Císarová et al., 2016). This can somewhat affect the growth pattern and mycotoxin production due to the temperature stress. Despite the existence of a report on the effectiveness of hull essential oil of *Pistacia vera* L. variety Bronte on inhibiting the growth of *S. aureus* and *E. coli* (MIC = 7110 μg/mL) (Smeriglio et al., 2017), with the best of our knowledge, there have been no other reports on the antifungal potential of PGHEO. As mentioned above, monoterpenes (such as α-pinene and limonene) constitute more than 90% of PGHEO and therefore play a major role in the antifungal activity of the essential oil. Due to their hydrophobic nature and low molecular weight, they can easily penetrate the cell membrane and disrupt its function (Nazzaro et al., 2017). Terpenoids have also been reported to disrupt mitochondrial function and cause subsequent deleterious consequences (such as elevated levels of reactive oxygen species and altered ATP levels) (Haque et al., 2016).

**Table 1**
Chemical components of PGHEO along with their amount (%), retention time (min) and Kovats index.

| No. | Compounds | Retention time (min) | Kovats index | Percent |
|---|---|---|---|---|
| 1 | Tricyclene | 10.91 | 926 | 1.49 |
| 2 | α-Thujene | 11.07 | 930 | 2.81 |
| 3 | α-Pinene | 11.49 | 939 | 39.59 |
| 4 | Camphene | 12.34 | 954 | 5.52 |
| 5 | Sabinene | 13.52 | 975 | 0.35 |
| 6 | β-Pinene | 13.79 | 979 | 4.52 |
| 7 | Myrcene | 14.36 | 990 | 1.20 |
| 8 | δ−3-Carene | 15.35 | 1011 | 2.75 |
| 9 | α-terpinene | 15.84 | 1017 | 0.71 |
| 10 | ρ-Cymene | 16.35 | 1024 | 0.36 |
| 11 | Limonene | 16.52 | 1029 | 19.41 |
| 12 | β- Phellandrene | 16.63 | 1030 | 1.11 |
| 13 | Z-β-Ocimene | 17.38 | 1037 | 0.29 |
| 14 | γ-Terpinene | 18.03 | 1059 | 0.57 |
| 15 | Terpinolene | 19.43 | 1088 | 13.19 |
| 16 | Borneol | 24.10 | 1169 | 0.30 |
| 17 | Terpinen-4-ol | 24.43 | 1177 | 0.42 |
| 18 | L-bornyl acetate | 29.23 | 1289 | 5.41 |
| Total identified | | | | 100 |

**Table 2**
Determined minimum inhibitory (MIC) and lethal concentrations (MBC or MFC) of PGHEO for different microbial strains.

| Microorganism | Strain | MIC (μg/mL) | MBC (μg/mL) | MFC (μg/mL) |
|---|---|---|---|---|
| ***Staphylococcus aureus*** | ATCC 25,923 | 500 | 500 | – |
| ***Bacillus subtilis*** | ATCC 6051 | 250 | 500 | – |
| ***Aspergillus flavus*** | ATCC 24,109 | 62.5 | – | 125 |
| ***Aspergillus parasiticus*** | ATCC 28,285 | 125 | – | 250 |

### 3.3. Surface and morphological analysis of the nanocomposite

Elemental composition of the prepared nanocomposite obtained from EDX analysis is illustrated in Fig. 2. Peaks due to chloride, carbon, oxygen and zinc in EDX spectra are representing the starting materials i.e. PVC, PGHEO and ZnO nanoparticles. Peaks due to zinc element confirm the existence of ZnO nanoparticles on the top surface of the composite. Furthermore, no nitrogen peak was observed in the spectrum, indicating complete removal of DMF, the solvent used in the electrospinning, from the nanocomposite during the process. DMF with a boiling point of 153 °C is a suitable substitute for acetic acid in the electrospinning process, because the vapors of this acid in the workshop cause sensitivity to the workers. The presence of residual solvent in the composite structure is important in terms of safety when it comes in contact with food in the form of packaging.

As is evident in Fig. 3(a–e), fibers were formed on film surface with diameter ranging from 89 nm to 295 nm. ZnO nanoparticles with sizes about 40–60 nm (Fig. 3e) were agglomerated on the fiber surfaces so that distributed well throughout the fibrous network.

### 3.4. Investigating the antifungal properties of the nanocomposite

The results of the antifungal nanocomposite test are shown in Table 3. As it is apparent, the nanocomposite indicated antifungal activity so that the sensitivity of the three investigated fungi decreased in the following order: *C. albicans* > *A. Flavus* > *A. parasiticus*. It seems that PGHEO and ZnO nanoparticles incorporated into nanocomposite

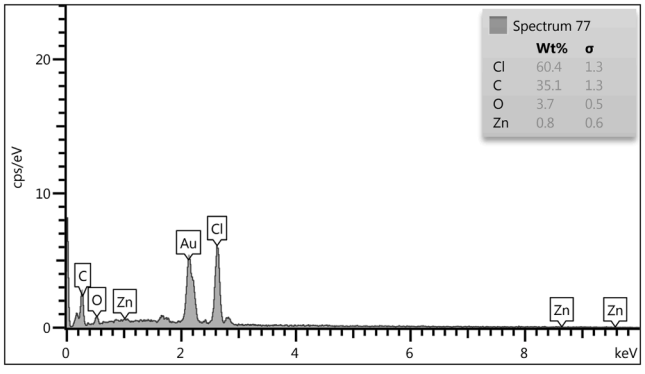


**Fig. 2.** EDX elemental analysis of the prepared nanocomposite; the nanocomposite showed 60.4%wt chlorine, 35.1%wt carbon, 3.7%wt oxygen and 0.8%wt zinc.

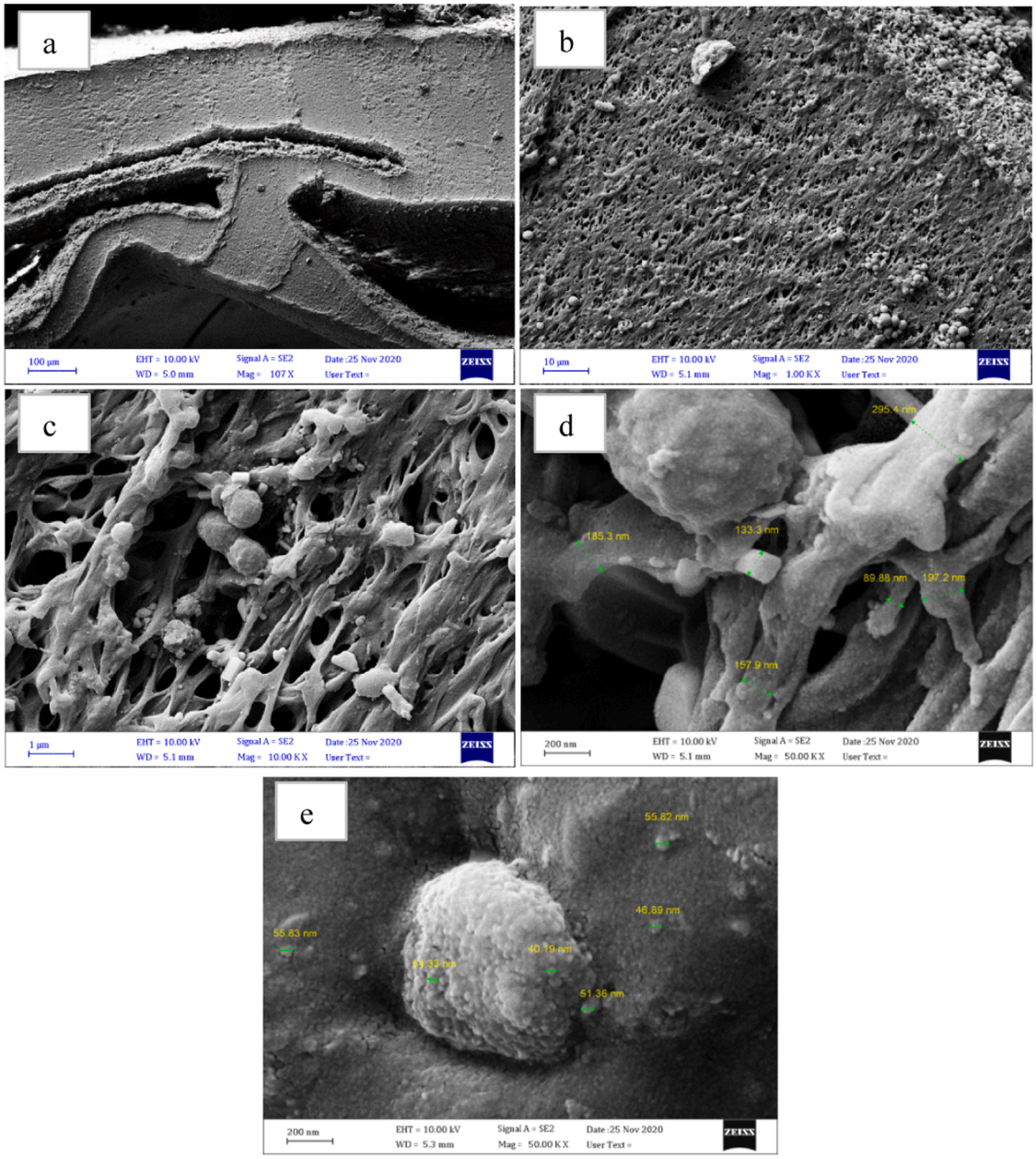


**Fig. 3.** SEM images of the prepared nanocomposite based on different magnifications (Mag in a–e is 107 X, 1 KX, 10 KX, 50 KX (cross-section) and 50 KX (surface-section) respectively.

**Table 3**
Indirect measurement of cell numbers (OD) and the growth inhibition percentage (GIP) resulted from the prepared nanocomposite.

| Fungi | $OD_{treatment}$ | $OD_{Negative\ control}$ | $OD_{blank}$ | GIP (%) |
|---|---|---|---|---|
| ***Aspergillus parasiticus*** | 0.950 ± 0.020 | 0.977 ± 0.030 | 0.052 ± 0.004 | 2.88 ± 4.84[a] |
| ***Aspergillus flavus*** | 0.963 ± 0.035 | 1.060 ± 0.065 | 0.053 ± 0.005 | 9.60 ± 4.82[b] |
| ***Candida albicans*** | 0.850 ± 0.053 | 1.003 ± 0.015 | 0.058 ± 0.003 | 16.20 ± 5.79[c] |

Various small letters at each column represent significant difference ($p<0.05$).

network by electrospinning process have been able to show their antimicrobial properties well. These results are in agreement with those observed for the antimicrobial properties of PGHEO. As mentioned in Section 3.2, PGHEO indicated a significant antifungal activity, so that its effect on *A. flavus* was more evident than on *A. parasiticus*. In addition, several studies previously reported the antifungal effect of ZnO nanoparticles (Kumari et al., 2019; Raj et al., 2021). The origin of this effect of nanoparticles can be attributed to the induction of oxidative stress on fungal strains, which in turn leads to the activation of metalloproteases and ultimately proteolysis and uncontrolled cell destruction (Raj et al., 2021).

### 3.5. Investigating the effectiveness of nanocomposite packaging in the shelf life and amount of aflatoxin of pistachios

Fig. 4 demonstrates the visual appearance of the pistachio nuts packed in three food packages during storage. As it is shown in Fig. 4(a, b), pistachios packed in conventional polyethylene film gradually lost their bright color appearance so that they became dark with increasing storage time and finally turned into completely black as a result of both

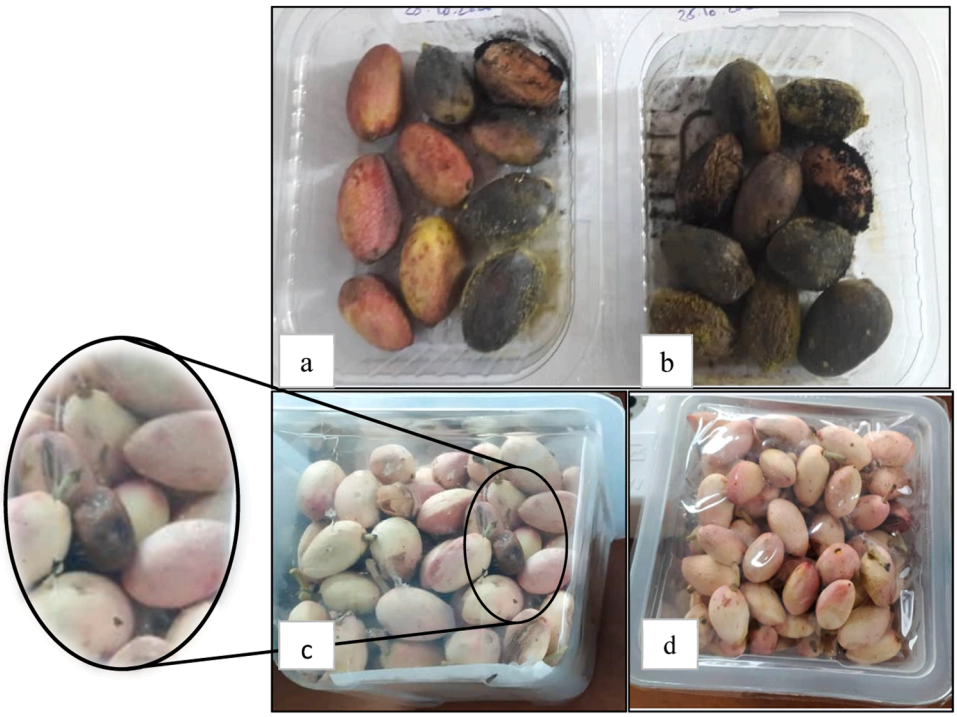


**Fig. 4.** Comparison of the visual appearance of the pistachios packed in polyethylene package (a and b: on 10 and 15 days of storage, respectively), polyethylene MAP package (c: on 60 days of storage) and nanocomposite package (d: on 60 of storage).

oxidation and the fungi growth on the 15th day of storage. Also, the traces of mold are clearly visible on the pistachio surfaces. Both samples packed in polyethylene MAP package and nanocomposite film indicated higher shelf life compared to polyethylene film. However, nanocomposite film preserved the pistachios better. Evidence for this finding is the darker color of the pistachios, as well as the presence of rotten pistachios (as shown enlarged in Fig. 4c) in the MAP package. In a recent study, on passive- and active-modified atmosphere packaging fresh in-hull pistachios, shelf life of approximately 45 days was achieved under active MAP condition of 5% $O_2$ + 45% $CO_2$ (Sheikhi et al., 2019). In addition, Shayanfar et al. (2011) reported maximum shelf stability of about 42 days for fresh pistachio samples preserved in various MAP conditions (Shayanfar et al., 2011). Therefore, it can be concluded that the results of this study are unique and more prominent than the previous reports as the shelf life of up to 60 days was achieved.

The HPLC analysis of aflatoxins in pistachios preserved in two types of packaging (MAP and nanocomposite) is presented in Table 4. The concentration of aflatoxin $B_1$ in pistachios packed in MAP film was about 128 fold its value in samples preserved in nanocomposite packages. Furthermore, the value of about 9 ppb for samples packed in MAP film was slightly higher than the maximum levels set by the Iranian National Standardization Organization (INSO, 2020) and EU regulatory standard (EC, 2006) for Aflatoxins in pistachio nuts (8 ppb for aflatoxin $B_1$ and 10 ppb for sum of $B_1$, $B_2$, $G_1$ and $G_2$). While aflatoxin $B_2$ could not be measured in the samples packed in nanocomposite, its value was around 0.5 ppb in the pistachios preserved in MAP film. Moreover, aflatoxin $G_1$ and $G_2$ were not detectable in any of the samples (Table 4). It seems that the fungus *A. flavus* is only active in the production of aflatoxin derivatives type $B_1$ and $B_2$.

**Table 4**
The concentration of 4 types of aflatoxins measured in pistachios packed in nanocomposite and control packages (MAP without nanocomposite).

| Packaging | Aflatoxin concentration (ng/g) | | | |
|---|---|---|---|---|
| | $B_1$ | $B_2$ | $G_1$ | $G_2$ |
| Nanocomposite | $0.073 \pm 0.014^a$ | UN | UN | UN |
| Control | $9.353 \pm 0.001^b$ | $0.567 \pm 0.001$ | UN | UN |

UN: Unmeasurable.
Various small letters at each column represent significant difference ($p<0.05$).

## 4. Conclusion

This study aimed to provide a packaging material capable of long-term storage of raw pistachios. In this regard, first by adding zinc nanoparticles and pistachio green hull essential oil to the PVC matrix phase, a nanocomposite was produced by electrospinning method. Then the prepared nanocomposite was used as the top layer of cubic polyethylene pistachio packaging. The results showed that the essential oil with the main component of α-pinene exerted the greatest effect on inhibiting the growth of *A. flavus*. Electron microscopy studies confirmed the formation of fibers with a diameter of up to 295 nm. Finally, the packaging prepared with nanocomposite was able to create a higher shelf life of pistachios (60 days) and also a much lower amount of aflatoxin $B_1$ and $B_2$ compared to the MAP packaging prepared with polyethylene film.

## CRediT authorship contribution statement

**Mohammad Nejatian:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization. **Amir Pouya Ghandehari Yazdi:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization. **Sepideh Khorasani:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization, Supervision. **Jesus Simal-Gandara:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – review & editing, Visualization, Supervision.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

## Data availability

Data will be made available on request.

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