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ORIGINAL ARTICLE

Biocompatible Nanocomposite Film of Potato Starch/*Coleus scutellarioides* Anthocyanin Extract with Incorporated Nanorod-Zinc Oxide to Increase Sensory and Chemical Quality of Peanut

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KEYWORDS

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Biodegradable;
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Oxidation;
Quality;
Storage

ABSTRACT

In this work, the effect of potato starch/*Coleus scutellarioides* anthocyanin extract (CSAE) and potato starch/CSAE/1, 3, and 5% (w/w) ZnO nanorod (ZnO-N) packaging on chemical and microbiological and sensory properties of peanut kernels stored at 40 °C for 60 days were studied. Changes in chemical characteristics such as moisture content, peroxide value (PV), free fatty acidity (FFA), and sensory properties such as flavor and color indices and microbiological quality were tracked in the peanuts during storage in biodegradable packaging. Active packaging containing 5% ZnO-N inhibited primary products of lipid oxidation and it was showed by lower FFA and PV values with 0.33 g oleic acid/100 g oil and 0.66 meq O_2 kg^{-1} oil at the last preservation. On the 60 day, mold and yeast content was evaluated as 2.35 log CFU g^{-1} in peanuts packed with 5% ZnO-N whereas the mold and yeast content of kernels was measured as 3.47 log CFU g^{-1} packaged with potato starch/CSAE. The neat sample indicated the lowest moisture content of kernels (5.4%) than the 5% ZnO-N (6.9%). The flavor and color values of kernels packaged with potato starch/CSAE reached 2.7 and 2.5 respectively, and the highest values were observed in peanuts packaged containing %5 nanoadditives. Our findings indicate that bionanocomposite film could be utilized to extend shelf life, increase sensory and microbiological properties, and inhibit oxidation reactions of kernel peanuts during storage.

Introduction

Peanut kernel (*Arachis Hypogaea* L.) belongs to the legume family and has been cultivated world wide. Peanuts are grown in Georgia, India, China, the United States of America, Indonesia, Nigeria, Turkey, Argentina (Kassie *et al*., 2023; Thanh Tran *et al*., 2025). Raw peanuts are an excellent resource

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of polyunsaturated fatty acids, antioxidants proteins, vitamins, minerals, and other micronutrients, including Mg (Çiftçi and Suna, 2022; Thanh Tran *et al*., 2025).

Oxidation rancidity affects the quality of the product making it dissatisfaction or lees satisfaction for end user. Oxidation reactions form volatile compounds including hydrocarbons, furans, alcohols, aldehydes, and ketones (Vera *et al*., 2018). Also, high concentration of unsaturated fatty acids (UFA) make peanut kernels prone to autoxidation during storage, which can lead to quality loss, with toxic metabolite occurrence and undesirable effects (Yang *et al*., 2022). Oxidation reactions are influenced by unsaturated fatty acids, Fe, Mn and Cu, and antioxidants (Machado *et al*., 2023). Many factors can increase the oxidation lipid of peanuts. The bold extrinsic factors are including temperature, time, O_2 availability, relative humidity (RH), and exposure to light (Nader *et al*., 2021).

Hence, oxidative rancidity can decrease by the application of active film containing nanofillers and increase oxidation stability (Davoodi and Naji, 2018). Based on method mentioned, active packaging containing nanoparticles is a bold issue in food science. The aim of the application of active packaging containing nanofillers in different foods is prolonging shelf life by maintaining microbiological quality, reducing moisture and oxygen passage, and physichochemical reactions (Marvizadeh *et al*., 2021, Nobari *et al*., 2022). Nanoadditives have antifungal capacity and can also shift the edible packaging attributes to prevent O_2 passage (Javidi *et al*., 2022).

Among the natural molecules, that stand out as major materials to fabricate biocompatible films, there are lipids, proteins, and polysaccharides, proven replacement resources for the preservation of food, because of their fine barrier charactrictes to moisture content (Moosavian *et al.*, 2017; Chatrabnous *et al.*, 2018; Habibie *et al.*, 2019). Starch natural molecule is most commonly applied biopolymers in food packaging, due to its abundance in nature and low cost, as well as being simple and easy to fabricate biocompatible material (do Lago *et al*., 2020). low flexibility, resistance, and, hydrophilic behavior decrease the application of starch in food packaging (Majeed *et al*., 2023).

Nanoparticles are used to increase the functional behaviors of films based on starch biopolymers. Various fillers including nanoclay, cellulosic nanofiber, carbon nanotubes, Ag, Fe_3O_4, ZnO, SiO_2, MgO, and TiO_2 have been applied to improve the flexibility, resistance, and hydrophilic behavior of starch biofilms (Pires *et al*., 2021).

Due to their excellent attributes, colorants can be employed in various industries. Some of these characteristics include light barrier activity, antioxidant activity, antimicrobial and antifungal properties (NANSU *et al*., 2021).

Coleus scutellarioides is commonly farmed in gardens and also has application of medicinal in spacial nations like Indonesia, Mexico, and India, etc (Al-Mafarjy *et al*., 2024). Various active compounds, including flavonoids, alkaloids, volatile essential oils, saponinsand, and polyphenols, have been observed in the leaves of *Coleus scutellarioides* (Jakobina *et al*., 2024). The leaves of coleus contain high concentration of red anthocyanins. Also, this herb shows significant antioxidant and antimicrobial activity (Moektiwardoyo *et al*., 2019).

The aim of this investigation was to compare the chemical, sensory, and microbiological quality of raw peanut kernels packaged in potato starch/CSAE with respect to peanut kernels packaged in potato starch/CSAE containing 1, 3, and 5% ZnO-N during 60 days of storage at 40°C.

Materials and Method

Coleus scutellarioides anthocyanin extract

Based on Luchese *et al*. (2018) the anthocyanin pigment extract from *Coleus scutellarioides* was extracted. At 25°C, the plant leaves were submerged and dried. The *Coleus scutellarioides* leaves were then freeze-dried for 72 hours. The pigments were then extracted using 200-mesh *Coleus scutellarioides* powder. After obtaining 5g of powder, 100 ml of 50% ethanol was added, and the mixture was agitated at 600 rpm for 1 hour. Finally, the dispersion was separated using tissue paper (Whatman No. 4), and the *Coleus scutellarioides* anthocyanin extract that was produced was kept at 4°C in a dark cabinet.

Film preparation

To fabricate bionanocomposite film, ZnO-N at various categories (1, 3, and 5% w/w, based on starch) was added into 100 mL of deionized water and the nano-dispersion was stirred magnetically for 3 h, and the different nano-dispersions were exposed to ultrasound wavelength. Next, About 4g of starch (Marvizadeh *et al*., 2016), and mixture of plasticizer from sorbitol/glycerol (1.6 g) (Abdorreza *et al*., 2011) were combined with nano-dispersions. All the nano-dispersion were heated at 86°C for 45 min. During cooling period of the nao-dispersion, 7 mL of CSAE was added to the nano-dispersion. Finally, all nano-dispersions were decanted into plates (16 × 16 cm), and dried at room temperature to make active films.

Hazelnuts specimen packaging

Peanut kernels were weighed in 500 g portions in various films including potato starch/CSAE, potato starch/CSAE/1% ZnO-N, potato starch/CSAE/3% ZnO-N, and potato starch/CSAE/5% ZnO-N (thickness= 0.08 mm). Different packagings were stored for 60 days at 40 C and 60% RH in a dark room. The specimens were removed from conservation on days 0, 15, 30, and 60 for experiments.

Free fatty acidity (FFA)

Peanut kernel oil was extracted based on cold pressing of kernels using a 20-ton laboratory press (ZYP20. TMAX Co., Xiamen, China). Also, FFA value was estimated on peanut kernel oil and stated as g oleic acid/100 g oil (AOAC, 2010).

Moisture content

The moisture content of peanuts was estimated by laboratory oven model BO55 (Arta company, Iran), drying for 4 h at 105°C, to reach constant weight.

Peroxide value (PV)

Five grams of the specimen was mixed with 30 mL of acetic acid-chloroform and kept under magnetic stirring for 25 min. About, 0.5 mL of KI was added to the above dispersion at room temperature. Finally, 30 mL of distilled water was mixed and titration was carried out with sodium hydroxide (0.1 N).

Microbial and mold assay

Counts of mold and yeast were evaluated by culturing specimens in SDA (Sabouraud Dextrose Agar, BritaniaLab). Mold and yeast count of kernels was done using of pour-plated method. About 10 g of each milled kernel were mixed and homogenized in 90 mL of peptone water in sterile bags. All samples were incubated for 5 days at 25°C.

Sensory properties

Kernels were roasted at 150 C for 30 min in an oven (BO55, Arta company, Iran) and blanched before sensory assay. The sensory attributes of peanuts were estimated by 9 panelists (7 men and 2 women) on the 1st and 60th day of the storage. Sensorial properties including flavor and color were evaluated as the acceptability of the final product by the panelist, using 5-point scale, 5 being the very good and 1 the very bad. All specimens were estimated in partitioned small room under sunlight at 25°C.

Statistical analysis

ANOVA test was carried out to compare data of mold and yeast count, oxidation indices, and sensory properties of peanuts at $p<0.05$. Different data of the examination were analyzed by Minitab version 21.4.2.

Results

Free fatty acidity (FFA)

Changes in FFA are illustrated in Fig. 1. Significant differences ($p < 0.05$) were indicated in the FFA of peanuts with bionanocomposite film compared with the control packaging during the preservation time. After 60 days of preservation FFA reached 0.79 g oleic acid/100 g for peanuts packed with potato starch/CSAE films. Kernels wrapped with 5% nanoadditive had very low FFA, 0.33 g oleic acid/100 g in peanuts. Moreover, the FFA of peanuts wrapped with 5% ZnO-N was lower than that packaged with neat film.

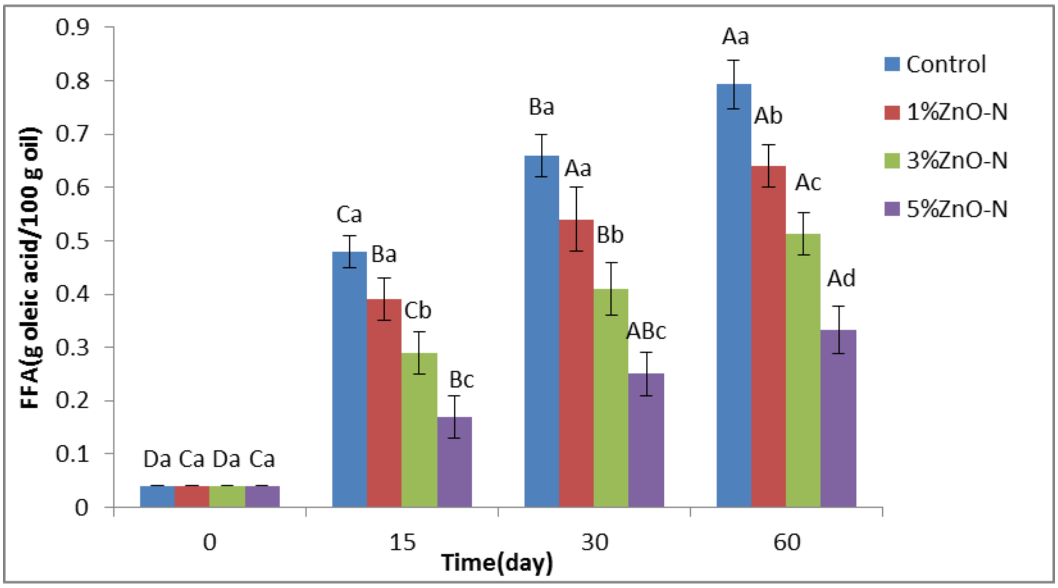


Fig.1. FFA of peanuts. Control: peanuts packed with potato starch/CSAE. Different small and capital letters illustrate significant difference among means ± SD (n=3) of various concentrations of ZnO-N and one specimen during preservation respectively

Moisture content

Fig. 2. exhibits the effect of ZnO-N during 60 days of preservation on the moisture content of peanuts. The use of ZnO-N had a significant impact ($p< 0.05$) on the moisture content of peanuts. The findings illustrated that the moisture content of peanuts had the reducing trend for 60 days; there was significant ($p< 0.05$) between all treatments during storage time. The moisture content of peanuts packed with potato starch/CSAE decreased from 8.8% to 5.4% after 60 days of conservation.

According to the results, it can be proven that in all peanuts, the moisture content change of peanuts packaged with potato starch/CSAE/5% nanofiller is lower than other treatments.

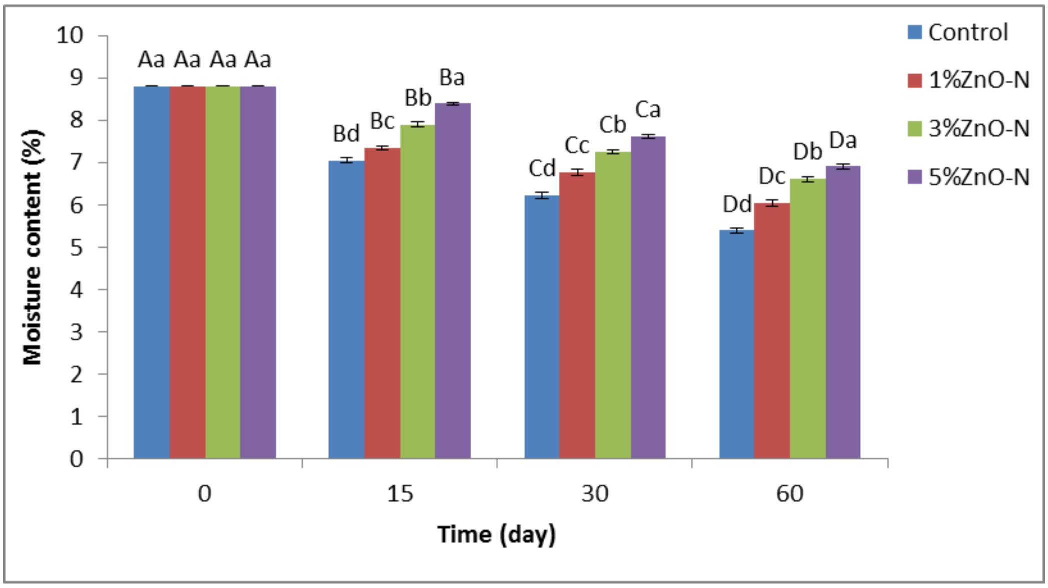


Fig.2. Moisture content of peanuts. Control: peanuts packed with potato starch/CSAE. Different small and capital letters illustrate significant difference among means±SD (n=3) of various concentrations of ZnO-N and one specimen during preservation respectively.

Peroxide value (PV)

PV changes in peanuts were studied for 60 days, and the averages are exhibited in Fig 3. The PV of all samples increased during preservation, and the increase in kernels packaged with potato starch/CSAE was the highest. After 60 days of preservation, PV was found as 0.99 meqo$_2$ kg^{-1} oil in the peanuts packaged with potato starch/CSAE, whereas the PV of the kernels packaged with 5% nanoaditive was evaluated as 0.66 meqo$_2$ kg^{-1} oil. The minimum PV was related to 5% ZnO-N, and the mentioned sample were significantly different from the PV of the control specimens. The lowest PV was related to 5% and 3% nanofiller.

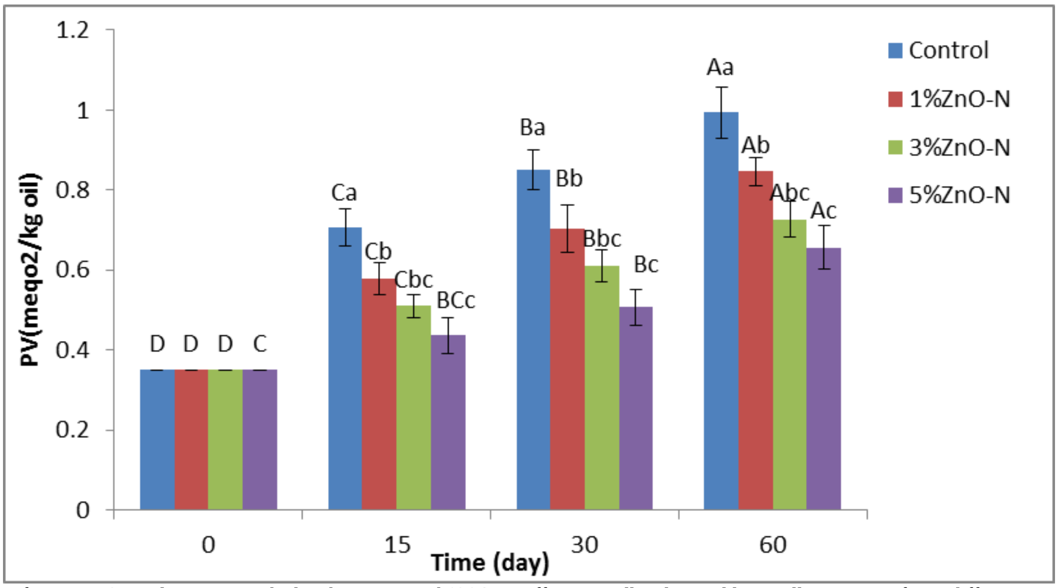


Fig.3. PV of peanuts. Control: peanuts packed with potato starch/CSAE. Different small and capital letters illustrate significant difference among means ± SD (n=3) of various concentrations of ZnO-N and one specimen during preservation respectively.

Mold and yeast count

Changes in mold and yeast content of kernels packaged with potato starch/CSAE and potato starch/CSAE/ZnO-N are illustrated in Fig.4. Mold and yeast content increased significantly ($p<0.05$) during preservation time for all samples but this increase was greater in control sample than treatments. The initial mold and yeast content of kernels were very low. The mentioned index increased with conservation time increased, and significant differences ($p<0.05$) were observed between different packagings. The mold and yeast of kernels only indicated significant differences ($p<0.05$) for 30 days for all samples, but no significant differences ($p> 0.05$) between 3 and 5% ZnO-N at 60 days.

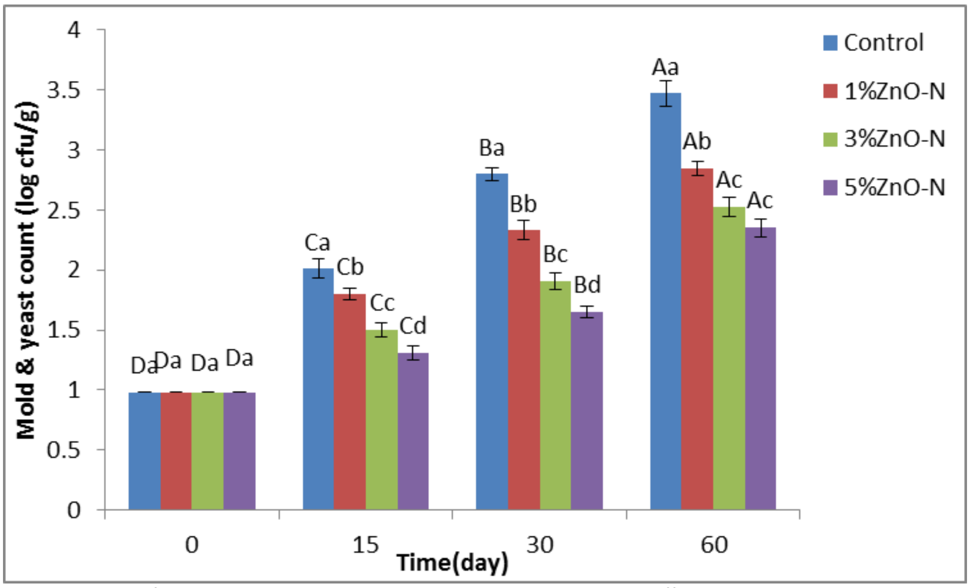
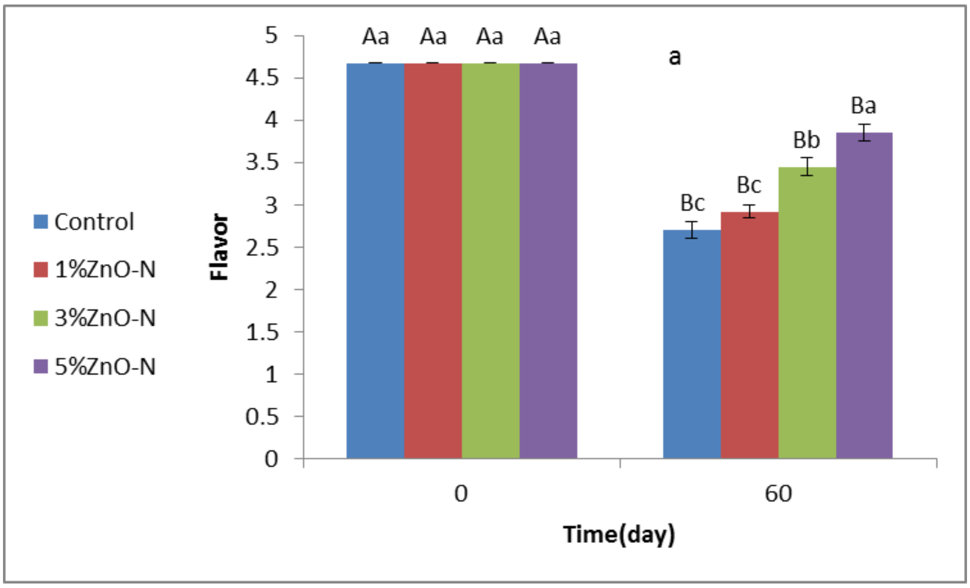


Fig.4. Mold and yeast content of peanuts. Control: peanuts packed with potato starch/CSAE. Different small and capital letters illustrate significant difference among means±SD (n=3) of various concentrations of ZnO-N and one specimen during preservation respectively.

Sensory properties

Changes in the sensory quality are presented in Fig 5 (a and b). The initial flavor and color of peanuts were 4.67 and 5 at first storage, respectively. It reached 2.7 and 2.5 for peanuts packaged with potato starch/CSAE after 60 days of storage. The sensory attributes of all samples decreased over time, and the decrease in control categories was highest. The flavor and color scores of kernels exhibited significant differences ($p < 0.05$) among the treatment and control categories during preservation. On 60 days, the highest flavor and color scores of peanuts were observed in kernels wrapped with the highest nanoadditives. Flavor and color averages reached maximum scores of 3.85 and 4.51 respectively in peanuts packed with 5% additives. Kernels packed with 5% ZnO-N exhibited the highest sensory quality.



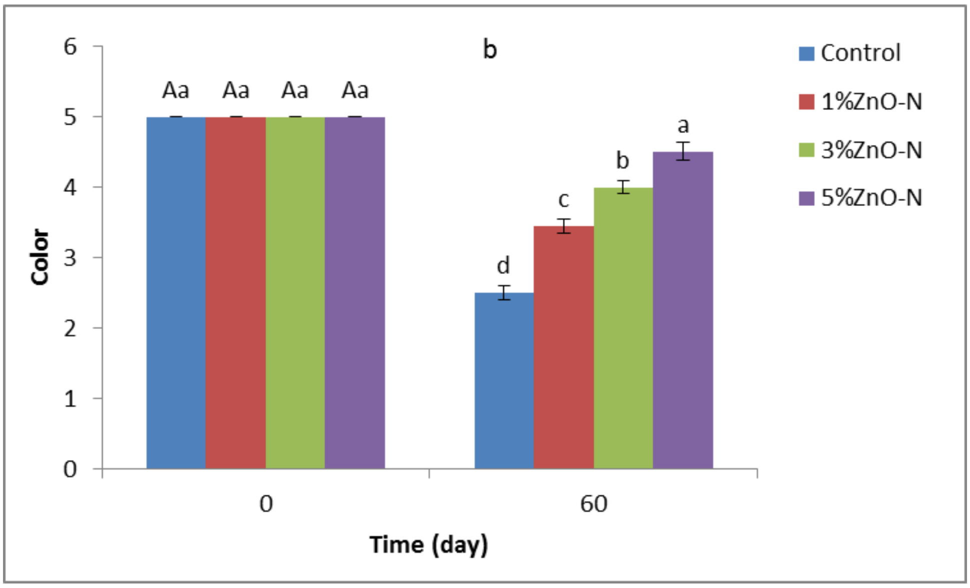


Fig.5. Flavor (a) color (b) of peanuts control: peanuts packed with potato starch/CSAE. Different small and capital letters illustrate significant difference among means ± SD (n=3) of various concentrations of ZnO-N and one specimen during preservation respectively.

Discussion

FFA vlaue shows the level of glycerid decomposition. These compounds create by lipase enzymes of fungi in the seeds at high temperatures (Cakmak-Arslan, 2022).

Figs. 1 and 2 Indicate FFA and PV values of peanuts, during 60 days of storage. The FFA and PV values of kernels was enhanced significantly ($p<0.05$) during preservation time maximum FFA and PV value of peanuts (0.79 g oleic acid/100 oil and 0.99 meq O_2 kg^{-1} oil) was attributed to the kernels packaged with tapioca starch/CSAE during conservation. These trends accord with the investigation from Martín *et al*. (2016), in which the initial FFA and peroxide values were 0.08 g oleic acid/100 oil 0.38 meq O_2 kg^{-1} oil respectively for samples.

With respect to FFA, the maximum value in raw kernels is lower than 1.00%. All samples were below this value at 60 days. However, tapioca starch/CSAE specimens developed higher FFA than tapioca starch/CSAE/ZnO-N specimens throughout the storage. Also, lowest PV value reached for peanut kernels packaged in tapioca starch/CSAE/5% ZnO-N at last conservation was within allowable limits (2 meq O_2 kg^{-1} oil). The active film containing nanoparticles has shown resistance against oxygen transfering in walnuts packaged with tapioca starch/bovine gelatin/roselle calyx extract/nano-ZnO (Aghayan *et al*., 2024). Pistachio packaged with aluminum containing ZnO-N reduced the PV of kernels compared to untreatment samples (Kazemi *et al*., 2020). The chitosan biofilms with nano-ZnO have strong barrier properties against oxidation reactions and improve the PV and FFA values of fresh pistachio (Taghipour *et al*., 2024). The trend of PV value was in accord with another article of Ebrahimian *et al*. (2024) that stated the PV value of pistachio was reduced upon the incorporation of nanoencapsulated pistachio green peel extract.

Edible films are good barriers against the transfer of O_2 and reduce the concentration of O_2 in packaging, and the presence of O_2 is demanded in oxidation reactions, the reduction of O_2 causes an increase in oxidative stability. Phenolic compounds also prevent autoxidation because of their antioxidant activity (Huntrakul *et al*., 2020). Also, it was illustrated that CSAE improves the gas barrier characteristic of edible films (Hematian *et al*., 2022).

The moisture content of product is a bold qualitative factor in dried foods (Marvizadeh *et al*., 2014, Marvizadeh *et al*., 2017).

The impact of ZnO-N was investigated on the peanut's moisture content for 60 days (Fig. 2). Moisture content decreased during storage for all samples but this trend was greater in potato starch/CSAE than treatments (Fig. 2). The moisture content decreased is related to temperature of storage (40 °C). The presentation study show that the initial moisture content was 8.8% while Martín *et al*. (2016) demonstrated 7.8% moisture content for peanuts. Also, Martín *et al*. (2018) represented lower moisture content for raw peanuts, namely 6.9% on the first day. According to Fig 2. biofilm based on potato starch/CSAE/5%ZnO-N had higher moisture content (6.9%) than treatments. The kernels packaged with potato starch/CSAE displayed the lower moisture content (5.4%), which is compatible with the trends of pistachio packaged with cassava starch/bovine gelatin/Nano-TiO_2/fennel essential oil (Chavoshi *et al*., 2023). They represented that cassava starch/bovine gelatin containing nano-TiO_2/fennel essential oil maintains the moisture content of pistachios compared with untreatments.

These trends exhibit the fine barrier of nanoparticles against moisture passage between peanuts and the environment.

The hydrophobic additive of ZnO-N acts as an excellent barrier against moisture passage when used in rye starch biofilms (Fallah *et al*., 2022). After adding nanofiller, the barrier behavior of the biofilm improved because nano-ZnO was incorporated into the biopolymer structure's tortuous pathway, which allowed molecules of gas to pass through (Marvizadeh *et al*., 2018). Also, The barrier behavior improved in film based on fish gelatin containing CSAE might be attributed to form hydrogen bonds between CSAE and biopolymer (Hematian *et al*., 2022).

The initial mold and yeast count of peanuts were 0.98 log CFU g^{-1} (Fig. 4), which was enhanced in different samples during preservation. The lowest increases were observed in peanuts wrapped with potato starch/CSAE/5% ZnO-N while the greatest changes were found in peanuts packaged with potato starch/CSAE.

The emission of Zn cation in the matrix of the biofilm may be the cause of the nanofiller's antibacterial capacity (Sun *et al*., 2020). Moreover, the Zn cation in ZnO might damage the bacterial cell wall. Additionally, it was discovered that nano-ZnO produced hydrogen peroxide on the surface of the cell wall, causing oxidative stress in the presence of moisture(K *et al*., 2019). Several variables, such as temperature, the presence of oxygen gas in the packaging, the moisture content of the product, and relative humidity, might influence the growth of fungi. The active film inhibits mold growth by reducing oxygen passage (Fallah *et al*., 2023). Tapioca starch/bovine gelatin with RCE/ZnO-N has strong barrier capacity against O_2 gas and reduces mold growth by reducing in oxygen passage (Aghayan *et al*., 2024).

Moreover, coleus grass leaves anthocyanins improve the antibacterial capacity of chitosan/fucoidan against E.coli and *Staphylococcus aureus*. Antibacterial characteristics of chitosan/fucoidan with coleus grass leaves anthocyanins have been related to anthocyanin pigment and protocatechuic acid (Wang *et al*., 2023).

The total viable count of hazelnut kernels was measured by Tavakoli *et al*. (2017). The authors showed that the total viable count of kernels ranged from 3.7 and 5.6 log CFU g^{-1} in polyethylene with 3% nano and without nanofiller, respectively. In another paper on pistachio packaged with carboxymethyl cellulose/soy protein isolate, it was stated that the total fungi count was highest in comparison with samples packed with nanoencapsulated pistachio green peel extract during conservation (Ebrahimian *et al*., 2024). Ras cheese packaged with active packaging containing roselle extract and roselle extract/nanoaditive-ZnO increased the antibacterial of packaging with 3% extract/nanofiller compared with untreatments (El-Sayed *et al*., 2020).

It is global interest to lower aflatoxin in peanuts because of the association between the toxin and carcinogenicity in animals and humans. It is proven that moisture content is one of the factors in the contamination of peanuts. Aflatoxin is commonly correlated with peanut moisture contents of 10% or higher (Martín *et al*., 2016). The moisture content of peanuts observed in the current investigation (day 0= 8.8%, day 60= 6.9%) is considered as allowable limit for peanuts.

Sensory attributes were carried out by 9 members of panelists. They measured flavor and color values from 1 to 5 after the storage period. The sensory attributes of peanuts are the bold index in kernels and are influenced by the physicochemical quality and constituent compounds. The final results displayed that, the use of nano-packaging containing ZnO-N by barrier characteristics against O_2 passage into the peanuts increases oxidative stability and improves sensory attributes. The flavor score of peanuts packaged with tapioca starch/CSAE reached 2.5 on 60 days. However, the color score of bionanocomposite film with 5% ZnO-N was 4.51 at the last storage (Fig. 5b).

According to Vera *et al*. (2018) flavor index of hazelnut kernels decreased with enhanced preservation period. They demostrated that the aroma index (after 42 days) of hazelnuts are 3 and 4 in the pure specimen and laminate with nano-selenium, respectively, and the laminate/nanoadditive shows higher sensory attributes compared with the hazelnuts packaged with pure laminate.

Kazemi *et al*. (2020) represented higher sensory attributes for pistachios packed with cinnamon essential oil/nano ZnO compared with kernels packed with neat film during storage.

Conclusions

Biodegradable film based on tapioca starch/CSAE, along with ZnO-N, effectively contribute to improve the chemical and sensory quality of peanut kernels in comparison to neat samples. The results reported in this investigation also indicate that peanut kernels packaged in tapioca starch/CSAE/5% ZnO-N in a good way microbial quality during preservation. The tapioca starch/CSAE containing 5% ZnO-N prolonged the shelf-life of peanuts in better conditions sensory, microbiological, and chemical characteristics of these products.

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Conflict of interests

The authors declare that there is no conflict of interest.

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