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**ORIGINAL ARTICLE**

# Novel Bio-nanopackaging Based on Polylactic Acid/Roselle Calyx Extract/ to Enhance Quality of Hazelnut

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K E Y W O R D S

Antifungal;
Bionanocomposite;
Chemical reaction;
Lipid oxidation;
Sensory properties

A B S T R A C T

Active biodegradable films prepared from polylactic acid (PLA)/roselle calyx extract (RCE)/Zinc Oxide Nanorod (ZnO-N) were fabricated as natural packaging materials for hazelnuts protection against microbial contamination and lipid oxidation. The PLA active films with ZnO-N (1, 3, and 5% w/w), and roselle calyx extract (0.5 g) were made by the casting solution technique. The fungal, microbial, chemical, and sensory properties of hazelnuts were measured during 9 months of conservation at 4°C. On the 6 and 9th months, moisture content was evaluated as %2.6 and 2.2% in the control sample whereas the moisture content of hazelnuts was measured as 3.6% and 2.9% packaged with PLA/RCE/%5 ZnO-N. At the last of experiments, the peroxide value (PV) was measured as 0.91 meq $O_2$ $kg^{-1}$ packaged with neat biocompatible films, whereas PV value was detected as 0.65 meq $O_2$ $kg^{-1}$ in packaged hazelnuts with %5 ZnO-N. Total microbial count ranged between 4.9 cfu $g^{-1}$ for hazelnuts packaged with neat PLA and 3.7 cfu $g^{-1}$ for hazelnuts specimens packaged with %5 ZnO-N after 9 months of storage. At 9 months, by increasing the level of ZnO-N from 1% to 5%, the mold count was reduced from 3.64 to 3.01 CFU $g^{-1}$. The fungal contamination in the neat specimens was 3.95 CFU $g^{-1}$. Sensory quality indicated that hazelnuts with ZnO-N had a significant impact on flavor and aroma, and the highest quality of sensory evaluation was related to hazelnuts packaged with PLA/5% ZnO-N/RCE. Flavor and aroma indices of hazelnuts packaged with %5 ZnO-N reached 3.5 and 4.5 respectively after 9 months of storage. The obtained findings indicate that biopackaging could be utilized to enhance microbial quality and inhibit oxidation reactions of hazelnuts during cold storage. Our results could be beneficial for introducing attractive characteristics to the biodegradable film packaging for example active film containing anthocyanin/nanofiller and can be applied when selecting a smart packaging for detection of food spoilage.

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**Introduction**

Hazelnut (*Corylus avellana*) is a well-known shrub (Rimamcwe and Chavan, 2017). Hazelnuts are cultivated in Asia Minor, European countries, and North America. Hazelnut is grown in different climates but prefers cold regions (Romero-Aroca *et al*., 2021). Hazelnuts such as other nuts are a rich resource in human nutrition including carbohydrates, vitamins, minerals, β-sitosterol, antioxidant phenols, oleic acid, dietary fiber, protein, and squalene (Roozban *et al.*, 2006; Sharifkhah *et al*., 2020; Sarikhani *et al*., 2021; Zhao *et al*., 2023, Hojjati *et al*., 2024). Oxidation rancidity is the most important reason for decreasing the shelf-life of the product. It reduces the nutrients of food, some vitamins and, some pigments decompose (Vera *et al*., 2022). The high amount of oil content in the hazelnut makes it susceptible to oxidation. Current works indicated that the oil content of hazelnut was 60% (based on dry weight) (Ghirardello *et al*., 2016). Mineral components such as Fe, Mn, Cu, α-tocopherol as antioxidants, and unsaturated fatty acids influence the rancidity of products (Machado *et al*., 2023). Humidity (Rh) oxygen gas availability and temperature, are the important external factors that affect hazelnut conservation (Adiletta *et al*., 2020, Hojjati *et al*., 2023).

Hence, oxidation reactions can decrease because of the application of bionanoconposite film and increased oxidation resistance (Davoodi and Naji, 2018). According to the solution mentioned, biopackaging containing nanofiller is an important issue in industrial packaging. The end of the usage of nano-packaging in perishable food is prolonging shelf life by preventing microbial and fungal contaminations, gas molecules traveling, and chemical reactions (Marvizadeh *et al*., 2021, Nobari *et al*., 2022). Nanoadditives such as metal and metal oxide have antifungal and antibacterial behavior and can also change the biocompatible film characteristics to prevent $O_2$ molecule penetration (Javidi *et al*., 2022).

Babapour *et al*. (2022) studied the antifungal effect of potato starch containing ZnO-N/fennel essential oil on pistachio nuts. It was found that film containing 5%nano-ZnO/3% fennel essential oil has higher antifungal properties than the pure sample. The impact of the film packaging with nano-$TiO_2$/fennel essential oil on the moisture content of fresh Abbas Ali pistachio was studied (Chavoshi *et al*., 2023). The findings represented that the use of nanofiller/essential oil causes conservation of the fresh pistachio up to 21 days. Moslehi *et al*. (2021) studied the use of various amounts of methylcellulose, on aflatoxin contamination of pistachio during four months of preservation. They stated that the 0.5% coated specimen has low levels of total aflatoxin compared to the control sample. Vera *et al*. (2022) demonstrated that packaging containing nano-$SiO_2$ decreases the oxidation chemical reactions of hazelnuts and improves the shelf life of the product.

Although synthesis polymers applied in the packaging industry protect food against physical and chemical damage, the major disadvantage are poor recyclability of plastic packaging (Velásquez *et al*., 2021).

Polylactic acid is considered as a biodegradable material for packaging due to its high transparency, fine mechanical characteristics, and low price (Vatansever *et al*., 2019). PLA is produced from lactic acid, which is fabricated using environmental resources for example sugar beets or corn microorganisms (Juturu and Wu, 2016). PLA is applied for food packaging and is generally distinguished as safe. Low thermal stability, gas barrier properties, and brittleness decrease the usage of PLA in food science (Roy and Rhim, 2020). The use of active compounds is used to increase the functional characteristics of PLA films (Moosavian *et al*., 2017). Various active compounds such as nanocellulose Ag, ZnO, $TiO_2$, nanoclay, essential oil, and herbal extract have been applied to enhance the physicochemical behavior of PLA films (Naskar *et *al*., 2023).

Biopigments can be applied in many industries due to their fine characteristics for example; antibacterial and antifungal behavior, antioxidant and light barrier activity (NANSU *et al*., 2021).

Flavonoid compounds are the predominant group of compounds in the RCE and sappan heartwood. The herb roselle is well-known worldwide and is commonly farmed in Thailand and other nations in Southeast Asia. The main ingredient in RCE is the anthocyanin pigment, which is also pH-sensitive. Hibiscus sabdariffa is red because the anthocyanin pigment undergoes a chemical change to become a flavylium cation (Etemadi Razlighi *et al*., 2023). According to studies, roselle monosaccharides can improve athletic performance over the long term. This is related to how polysaccharide molecules affect the metabolism of skeletal muscle, which can alter performance (Sadeghi *et al*., 2022).

In the current work, active bionanocomposite films based on polylactic acid/roselle calyx extract (RCE) containing 1%, 3% 5% ZnO-N were prepared. The purpose of the presentation work was to evaluate the changes in chemical, sensory, and microbial traits of packaged hazelnuts stored at 4°C.

## Materials and Methods

### *Materials*

Glycerol BHT and DG 18 agar were obtained from Sigma Chemical Co (USA). ZnO-N was supplied from US-NANO (USA). Polylactic acid (grade 3051D, d=1.25 g $cm^{-1}$) was obtained from Nature Works Ingeo (USA). Other chemical materials were purchased from Merck Co. (Germany). The *Corylus avellana* harvested in October 2022 in Iran was purchased from the producer from Gilan.

### *Roselle calyx extract*

The roselle extract was applied based on the changed technique illustrated by Giusti, *et al*.'s [13]. The pH of the mixture was then brought down to pH 2 using HCl after being mixed with roughly 15 mL of alcohol (80% ethanol) and roselle calyx powder (1 g). The specimens were centrifuged for 5 min at 3000 rpm after being heated for 50 min at 50°C to produce the final extract. Using a Rotary Evaporator (Heidolph, GER), the solvent was separated at 50°C.

### *Film preparation*

PLA/ZnO-N/RCE biocompatible films were fabricated using casting technique with slight modifications to the technique illustrated by Rhim *et al*. (2006). Solution based on PLA was fabricated by dissolving 5 g of PLA resins in chloroform (100 ml) and kept under stirring for 4 h. About 0.5g of RCE was added into chloroform (5 mL) and dispersed in an ultrasonic homogenizer for 50 min. Different mixtures of PLA/ZnO-N biocompatible films were achieved by adding 1, 3, and %5 (w/w) of ZnO-N to PLA suspension. PLA/ZnO-N solutions were stirred for 16 h at 25°C. The bionanocomposite solution was put in an ultrasonic bath for 50 min. Bionanocomposite solution was added to the RCE solution. The solutions containing ZnO-N were cast on glass casting plates (16 ×16 cm) and then dried for 30 min at 75°C.

### *Hazelnuts specimen packaging*

Packaged hazelnut samples were weighed in 30 g portions. Prepared specimens were stored at 4°C for 9 months. Specimens were evaluated on the day of biopackaging and every 3 months during conservation; four replicates were done per test.

### *Moisture content*

Moisture content was measured by laboratory mill model RRH350 (Azin lab company, Iran), drying for 3 h at 105°C, and then weighing.

### *Peroxide value (PV)*

About 5g of the sample was added to 30 $cm^3$ acetic acid-chloroform and kept under stirring for 15 min. Also, 0.5$cm^3$ of KI saturated was dispersed in the mentioned mixture at 25°C. At last, distilled water (30 $cm^3$) was added and titration was performed with NaOH (0.1 N) and using of starch as an indicator.

### *Microbial and mold assay*

About 10 g of hazelnut was weighed from each biopackaged and was homogenized in a sterilized bag with 90 mL of peptone water (0.1%) in the Bag mixer for 3 min. Then, serial dilutions were fabricated in the diluent solution (9 ml). International standards were used as the foundation for plate counting (for total microbial count: 30-300 colonies and mold assay between 25-250 colonies) (Tavakoli *et al*., 2017). A sample of 0.1 mL was taken from each dilution, placed on BHI agar, and then incubated at 35°C for 24–48 h.

To mold assay, 0.1 mL of dilution was placed on DG18 agar and incubated at 25°C, for 3-5 days.

### *Sensory properties*

The sensory quality of hazelnuts was evaluated by 11 panelists on the first and 9th month of the conservation period. They scored two parameters from 1 (very bad) to 5 (very good). To experiment, six hazelnut specimens were put on a glass plate and proposed to panelists to assay sensory quality.

### *Statistical analysis*

ANOVA test and independent-sample t were performed to compare data of microbial, chemical, and sensory properties of hazelnuts at $p<0.05$. Data from experiments were analyzed by Graph Pad Prism 9.5.1.733.

## *Results*

### *Moisture content*

Changes in moisture content are indicated in Fig. 1. The conservation period and biodegradable film containing ZnO-N, significantly ($p < 0.05$) affected moisture content. Significant differences ($p < 0.05$) were found in the moisture content of hazelnuts with active biocompatible films containing ZnO-N compared with the neat sample during the storage time. After 9 months of conservation, moisture content reached 2.2% for hazelnuts packed with neat films. Hazelnuts wrapped with 5% ZnO-N had very high moisture content, 2.9% in hazelnuts. Moreover, the moisture content of hazelnuts wrapped with the neat film was lower than that packaged with 5% nanofiller.

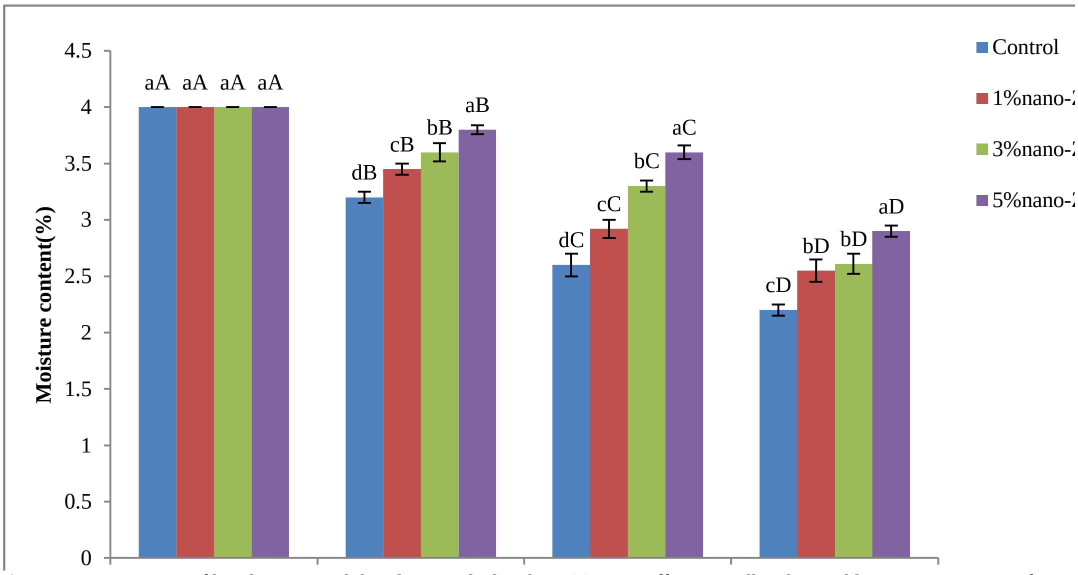


**Fig.1.** Moisture content of hazelnuts control: hazelnuts packed with PLA/RCE. Different small and capital letters represent significant differences among means±SD of various levels and one specimen during conservation respectively

***Peroxide value***

Fig. 2. indicates the effect of nanofiller during 9 months of conservation on the PV of hazelnuts. The use of nanoadditive had a significant impact on the PV of hazelnuts ($p<0.05$). The findings represent that the PV of hazelnuts had an enhancing trend for 9 months; there was no significant between 1% nanofiller and 3% nanoadditive on the last month of storage. The PV of hazelnut packed with PLA/RCE was decreased from 0.31 to 0.91 meq$O_2$ kg$^{-1}$ after 9 months of conservation.

Regarding Fig. 2. it can be observed that in all hazelnut samples, the PV change of hazelnut packaged with PLA/RCE/5% ZnO-N is lower than other hazelnut specimens.

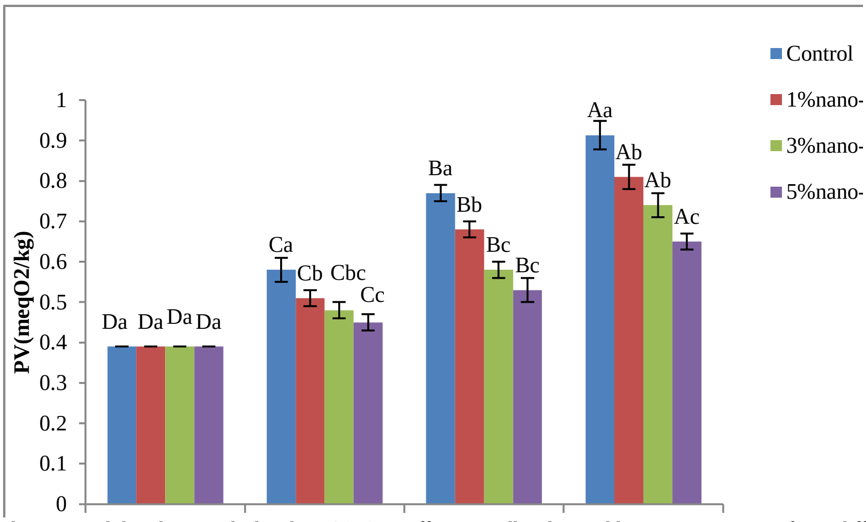


**Fig.2.** PV of hazelnuts control: hazelnuts packed with PLA/RCE. Different small and capital letters represent significant differences among means±SD of various levels and one specimen during conservation respectively

***Total microbial count and mold assay***

Changes in the total microbial count and total mold are presented in fig 3 and 4. The initial total microbial count and total mold of hazelnuts packaged with neat film were 2.6 and 2.4 cfu g$^{-1}$ at the beginning of the test, respectively. It reached 4.9 and 3.95 cfu g$^{-1}$ for hazelnuts packaged with PLA/RCE after 9 months of storage. The total microbial count and total mold of all samples increased during conservation, and the increase in neat specimens was the highest. The microbial and mold assay results represented significant difference ($p < 0.05$) between the control and treatment samples during storage. At the last of the conservation, the lowest total microbial count and mold count of hazelnuts were observed in specimens wrapped with 5% ZnO-N. Total microbial count and total mold reached maximum levels of 3.7 and 3.01 respectively in hazelnut packaged with 5% nanofiller. PLA biocompatible film containing 5% ZnO-N showed the highest antimicrobial activity.

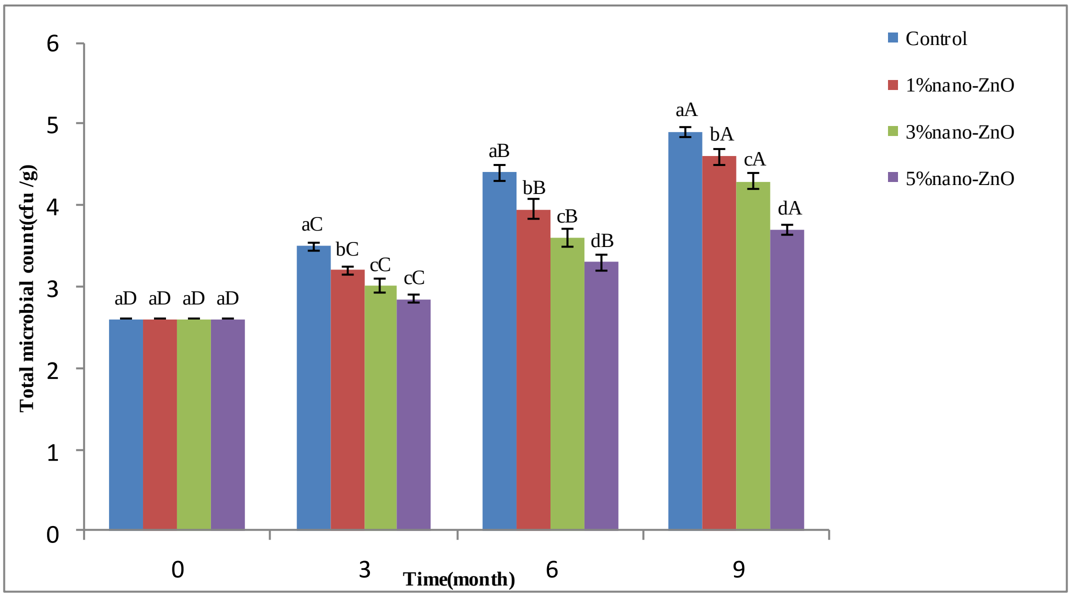


**Fig.3.** Total microbial count of hazelnuts Control: hazelnuts packed with PLA/RCE. Different small and capital letters represent significant differences among means±SD of various levels and one specimen during conservation respectively

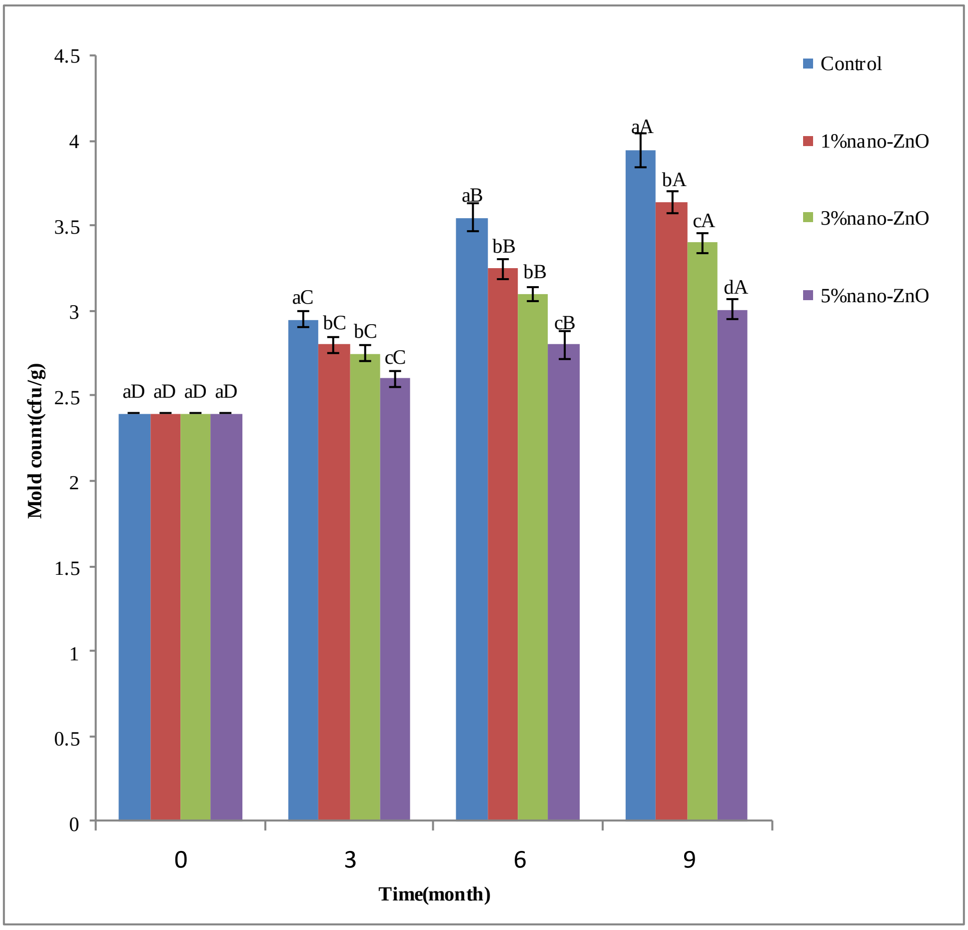


**Fig.4.** Mold count of hazelnuts Control: hazelnuts packed with PLA/RCE. Different small and capital letters represent significant differences among means±SD of various levels and one specimen during conservation respectively

### *Sensory properties*

Sensory quality changes in hazelnuts were studied for 9 months, and the findings are represented in Fig 5. The sensory quality of all samples decreased during conservation, and the decrease in neat specimens was highest. After 9 months of the conservation, flavor, and aroma score were found as 2.5 and 3 respectively in the control sample, whereas the flavor and aroma of the hazelnuts packaged with 5% nano-ZnO were measured as 3.5 and 4.5 respectively. The minimum flavor and aroma score was related to the neat specimens, and they were significantly ($P < 0.05$) different from the sensory quality of treatment specimens.

There is a total decrease in the sensory factor of all specimens during conservation. The lowest flavor score was related to 1% ZnO-N and control samples. The mentioned samples were significantly different from the flavor score of other treatments.

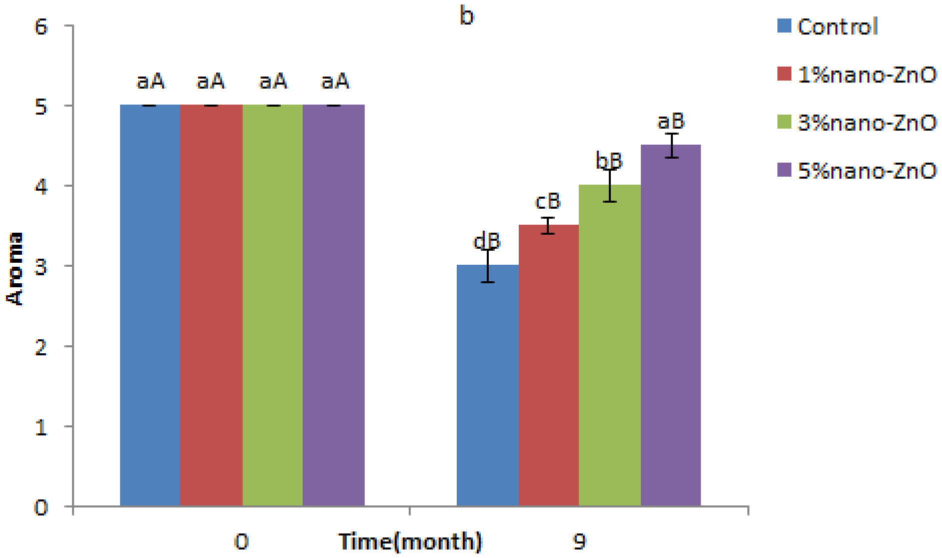


**Fig.5.** Flavor(a) and Aroma (b)of hazelnuts Control: hazelnuts packed with PLA/RCE. Different small and capital letters represent significant differences among means±SD of various levels and one specimen during conservation respectively

## Discussion

An important qualitative index in dried food is moisture content (Marvizadeh *et al*., 2014, Marvizadeh *et al*., 2017).

The impact of nano-ZnO was studied on the moisture content of hazelnuts for 270 days (Fig. 1). Obtained results indicate that the initial moisture content was 4% while Schlörmann *et al*. (2015) stated 4.70% moisture content for samples. Whereas, Guiné *et al* Almeida and Correia (2014) indicated lower moisture content for samples, namely 4.04–4.1% before conservation. Based on Fig 1. film based on PLA/RCE containing 5%ZnO-N had higher moisture content (2.9%) than other specimens. The sample packaged with PLA/RCE represented the lower moisture content (2.2%), which is consistent with the obtained results of Kirse-Ozolina *et al.* (2019). They exhibited that active PLA enhances the moisture content of hazelnuts compared to neat specimens.

This finding displays the fine-preventing impact of nanofiller against moisture traveling between hazelnuts and the surrounding atmosphere.

Hydrophobic filler of ZnO-N and RCE act as a fine barrier against moisture migration by enhancing the barrier behavior of the PLA biocompatible films against water vapor (Marvizadeh *et al*., 2016, Sadeghi *et al*., 2023). The barrier behavior enhanced after the addition of nanofiller related to the incorporation of nano-ZnO in the tortuous pathway of biopolymer structure for gas molecules to pass through (Fallah *et al*., 2022, Marvizadeh *et al*., 2018). Also, The barrier properties improvement in film containing RCE might be attributed to the enhancement in the interactions between extract and PLA (Sadeghi *et al*., 2023).

Free fatty acids content, high oil, and damages resulting from the process are the phenomenons for hazelnut susceptibility to reactions oxidation (Cakmak-Arslan, 2022).

Fig 2. indicates hazelnuts'PV, during conservation. The mentioned factor was enhanced significantly during conservation period maximum PV of hazelnuts (0.91 meq $O_2$ $Kg^{-1}$) was attributed to the neat film during conservation. These findings accord with findings from (Kang *et al*., 2013), in which the initial peroxide value was 0.38 meq $O_2$ $Kg^{-1}$ for samples.

Chlebowska-Śmigiel *et al*. (2008) stated an enhancement from 0.11 to 0.0.5 meq $O_2$ $Kg^{-1}$after 90 days of conservation. Also, Ghirardello *et al*. (2014) indicated PV of 0.17 meq $O_2$ $Kg^{-1}$ after 240 of days conservation and 0.62 meq $O_2$ $Kg^{-1}$ after 1 year of conservation of hazelnuts. The lowest PV was attributed to the 5% PLA/RCE with 5% ZnO-N. Nano additives have shown behavior as a fine barrier to oxygen transfering in hazelnuts packaged with TPA (Fallah *et al*., 2023). Hazelnuts packaged with polyethylene containing nano-silver reduced the PV of hazelnuts packed with 3% nanofiller compared to neat specimens (Tavakoli *et al*., 2017). These obtained findings of PV were in accord with another research by Kazemi *et al*. (2020) that demonstrated the PV of fresh pistachio was reduced significantly upon the addition of nanofiller.

The initial total microbial count and mold count of hazelnuts were 2.6 and 2.4 cfu $g^{-1}$ respectively (Figs. 3 and 4), which enhanced in all biopackaging during conservation. The greatest increases were found in pure samples while the least changes were hazelnuts packaged with 5% ZnO-N.

The antimicrobial behavior of ZnO-N could be attributed to the release of Zn cation in the biocompatible film matrix (Sun *et al*., 2020). Also, ZnO can disrupt bacterial cell wall by Zn cation. Furthermore, it is found that, in the presence of moisture, ZnO nanoparticles created oxidative stress in cell wall through the generation of hydrogen peroxide on its surface (K *et al*., 2019). There are different factors, that can affect fungi growth, including, RH, moisture content of production, and temperature and presence of $O_2$ in the packaging, The biocompatible film inhibits fungi growth by reducing oxygen traveling (Chavoshi *et al*., 2023). The biocompatible film containing ZnO-N/RCE has strong barrier behavior against $O_2$ and reduces the population of mold by reducing in oxygen transmission (Sadeghi *et al*., 2023).

On the other hand, the antibacterial properties of compounds in the RCE constitute a potential change for controlling bacteria contamination in food production. Antibacterial characteristics have been related to compounds including anthocyanin and protocatechuic acid (Rangel-Vargas *et al*., 2017).

The total microbial count of hazelnuts was evaluated by Tavakoli *et al*. (2017). They demonstrated that the total count (24 months) of specimens ranged from 3.7 and 5.6 log CFU $g^{-1}$ in polyethylene containing 3% nano and neat group, respectively. In another study on saffron packaged with 5% nano-silver, it was demonstrated that microbial count was lowest in comparison with neat specimens during conservation (Ahari *et al*., 2013).

The impact of biocompatible film with RE and RE/nano-ZnO on the microbial count of Ras cheese has been investigated in another study by El-Sayed *et al*. (2020). They stated that the increasing of roselle extract levels led to enhanced antibacterial properties, while the highest antimicrobial activity was detected with the active biocompatible film with 3% RE-ZnO.

Sensory evaluation was performed by panelists composed of 11 members. They scored two factors from 1 to 5: aroma, and flavor after 9 months of storage. The sensory quality of hazelnuts is the key factor in hazelnuts packaged and is influenced by the chemical reaction and constituent compounds. The obtained results represented that, the use of active packaging with nano-ZnO by barrier behavior against $O_2$ transfer into the hazelnuts decreases oxidation reactions and improves quality sensory.

The hazelnut packaged with PLA/RCE reached 2.5 on month 9. However, the aroma score of active film containing 5%nanofiller was 3.5 on month 9 (Fig. 5b). The flavor score in hazelnuts decreased with enhanced conservation time, according to Vera *et al*. (2022). They showed that the aroma score (after 42 days) of hazelnuts are 4 and 3 in laminate containing nano-selenium and pure specimen, respectively, and the laminate with nanofiller indicates higher sensory assay compared with the laminate without nanofiller. In another investigation, sensory quality represented significant difference between neat specimens and pistachio nuts packaged with cinnamon essential oil/Nano Zno (Kazemi *et al*., 2020).

**Conclusions**

The effects of PLA/RCE film containing ZnO-N were studied on sensory quality, mold and bacterial contamination, and chemical properties of walnut kernels. Moisture content loss and PV of specimens decreased significantly ($P < 0.05$) as a finding of SA PLA/RCE film with nano-ZnO. The mold count reduced significantly ($P<0.05$) in packaged hazelnuts compared to the neat specimen. The total microbial count of the treatment sample was significantly ($P<0.05$) lower than the pure sample. Moreover, bionanocomposite film created a positive effect on the sensory quality of hazelnuts, and panelist's acceptances for packed hazelnuts were higher than in the neat specimen. Based on the obtained findings, hazelnuts coating with PLA/RCE/ZnO-N can provide a fine protective technique for preserving hazelnuts. Furthermore, analysis and study in terms of smart packaging and color characteristics recommend for future research.

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**Conflict of interests**

The authors declare that there is no conflict of interest.

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