JCHR (2025) 15(2), 433-445

Journal of Chemical Health Risks

sanad.iau.ir/journal/jchr

ORIGINAL ARTICLE

Enhancing Shelf-life and Quality of Ready-to-Eat Pomegranate Arils with Nanocomposite Film: A PLA/ZnO nanoparticle/Zataria Multiflora Essential Oil Innovation

Mojtaba Heydari-Majd[1,2], Leila Monjazeb-Marvdashti[*3], Amra Bratovcic[4], Anna Abdolshahi[5], Marzieh Yazdani-Dehnavi[6], Fahimeh Ebrahimi Tirtashi[7]

[1]*Department of Nutrition, Research Center for Clinical Immunology, Zahedan University of Medical Sciences, Zahedan, Iran*

[2]*Department of Nutrition, Varastegan institute for Medical Sciences, Mashhad, Iran*

[3]*Food Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*

[4]*Department of Physical Chemistry and Electrochemistry, Faculty of Technology, University of Tuzla, Tuzla, Bosnia and Herzegovina*

[5]*Food Safety Research Center (salt), School of Nutrition and Food Sciences, Semnan University of Medical Sciences, Semnan, Iran*

[6]*Department of Food Science and Technology, Faculty of Agriculture, University of Shiraz, Shiraz, Iran*

[7]*Department of Food Science and Technology, Faculty of Nutrition Sciences & Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

(Received: 19 September 2023 Accepted: 16 March 2024)

KEYWORDS

Poly(L-lactide);
ZnO nanoparticle;
Zataria multiflora essential oils;
Pomegranate arils;
Active packaging

ABSTRACT: The dried pomegranate (*Punica granatum L.*) arils have several health benefits and are in demand worldwide. Beside this they are highly perishable due to their high water content and susceptibility to microbial growth, resulting in shortened shelf-life and compromised quality attributes. Therefore, in the current study, the wrapping effects of Poly L-lactide (PLA) activated with zinc oxide nanoparticles (ZnO NPs, 1.5% w/w) and Zataria multiflora Boiss. essential oil (ZEO, 1 and 1.5% w/w) were studied on pomegranate arils' storage-life extension in cold storage. The arils were periodically analyzed for physiochemical properties (weight loss, pH, total soluble solids, color measurement, antioxidant activity, and sensory attributes) and microbial properties (total aerobic bacteria and total fungi counts). Results showed that ZEO caused a decrease in pH during storage, while ZnO caused an increase. The active films were more effective than the control film in maintaining TTS and preventing weight loss of the arils. The PLA/ZnO/1.5% ZEO sample had the highest phenolic content (~520 mg 100 ml^{-1} in juice) and antioxidant activity (~90%) on day 15. However, ZEO had a greater effect on the color properties of the arils compared to ZnO, with 0.5% and 1.5% ZEO-PLA showing the highest a* (55.14) and b* (31.30) values with fresh arils, respectively. The microbial test results showed that ZEO was more effective in controlling bacterial growth than ZnO. Based on atomic absorption spectrophotometer measurement, minimal amounts (~29 mg Kg^{-1} sample) of Zn2+ ions were observed to be released into the arils. Overall, the active PLA could be used as a safe and effective way to prolong the storage life of arils in refrigerated conditions.

*Corresponding author: leilamonjazeb@yahoo.com. (L. Monjazeb-Marvdashti)
DOI: 10.60829/jchr.2024.1823

INTRODUCTION

The pomegranate (*Punica granatum L*), a fruit of *Punicaceae* family, is cultivated throughout the Middle East. It is a predominant fruit with desired organoleptic properties and health benefits. and dietary properties [1]. Pomegranate seeds (arils) extract contains about 85% water, 10% sugar (mainly fructose and glucose), pectin, and ascorbic acid [2]. Arils are a wealthy supply of polyphenolic antioxidants, tannins, and anthocyanins. In addition to nutritional values, pomegranate has health effects such as arteries protective, anti-inflammatory, anti-allergic, anti-cancer, and anti-influenza. In some researches examined the toxicological effects of single and combined exposure to cerium oxide (CeO_2) and zinc oxide (ZnO) nanoparticles on the liver and kidney of male Wistar rats. Both CeO_2 and ZnO nanoparticles induced oxidative injury and inflammation in these organs [3]. The findings indicate that nanoparticles can cause hepatic and renal toxicity through mechanisms such as Reactive oxygen species (ROS) generation, inflammation, and apoptosis [4]. Pretreatment with conjugated linolenic acids (CLAs) showed protective effects against renal tissue damage and oxidative stress by regulating apoptotic signaling pathways. The trans-10, cis-12 and cis-9, trans-11 isomers of CLAs are commercially available as food supplements [5, 6]. Pomegranate extract, known for its antioxidant properties, may also inhibit the renin-angiotensin system and potentially mitigate the detrimental effects of ZnO nanoparticles while preserving product stability[7, 8].

Some fruits, such as pomegranates, have a very short ripening and consumption period and, therefore, the establishment of processing industries and the production of preservable products is a fundamental necessity [1]. The pomegranate fruit is susceptible to sunburn, splitting, hitting, damaging during storage [9]. Furthermore, removing the arils from the leather shell causes overall quality loses during cold storage and makes it prone to cold damage, weight loss, microbial spoilage, biological browning, and reduced commercial markets [10]. Therefore the production of processed ready to eat pomegranate arils that could be safely preserved is a consumer willing and need a way for extending the shelf-life.

Active packaging/film/coating have been developed to promote the shelf-life quality of foods and was successfully utilized for fresh fruits [11, 12]. In this new approach, the harmful event could be inhibited [13]. Various synthetic polymers and natural biopolymers are used to produce active packaging. Among them, polylactid (PLA) thermoplastic polyester is a plant-based one made from cassava roots, corn-starch, and other types of starch. PLA is generally recognized as safe (GRAS) that made from lactic acid monomers [13] has been shown to have the ability to extend the shelf-life and quality of food products such as fish fillet [14], mango [15] and mushroom [16]. Furthermore, it has a significant ability to the controlled release of bioactive compounds.

One scheme to ameliorate the efficiency of a package is to activate with antimicrobial substances such as essential oils (EOs) that can extend the quality of fruits [14]. *Zataria multiflora L*. (ZEO) is an aromatic and medicinal plant belonging to the *Labiatae* family native to North Africa, Europe, Asia, and the Middle East. It is rich in phenolic compounds and reported good antioxidant, antimicrobial and antifungal activity [13]. ZEO has been used in several types of active films like corn starch-based film [17], PLA film [13], and carboxymethyl cellulose-coated polypropylene film [18].

Zinc oxide nanoparticles (ZnO NP) have been considered as a safe active substance approved by the United States Food and Drug Administration (USFDA) [13]. There are many reports about ZnO utilization in the production of biodegradable antimicrobial packaging [13].

The kinetic release of zinc ions from PLA and physicochemical properties of PLA/ZnO/essential oils nano-composite was investigated in our previous articles [13, 19]. The present study aimed to investigate PLA films containing EOs and ZnO NPs on arils fruits' physicochemical, sensory, and microbial properties.

MATERIALS AND METHODS

Chemicals and bacteria

Pomegranate cultivar Bajestani was procured from the Mashhad market. PLA (code PLA 2002 D) was

purchased in pellet form (Tianjin Jia Add Green Products Co., China). Nano-ZnO powder with almost spherical morphology, a diameter of 10-30 nm, and purity of over 99% was purchased from Tamad Kala Company (Tehran, Iran). Tween 80 and Glycerol (plasticizer) were from Merck (Darmstadt, Germany). ZEO (100% pure) was supplied by Niko Shimi Company (Tehran, Iran) and sealed and stored in the dark at 4°C.

Films preparation

Preparation of PLA-based films followed the procedure discussed by [13]. PLA powder (2 g) was dissolved in 100 mL chloroform by agitation for 4 h. The glycerol was added 20% w/w and defined amount of ZnO NPs dispersed in chloroform (1.5% w/w); and sonicated for 240 s. the PLA solution was mixed with ZnO NPs solution and homogenized (5 min). After that the EOs which emulsified by 20% w/w of Tween 80 were added previous solution at different concentration (1, and 1.5% w/w). PLA/ZnO nanocomposite films incorporated ZEO were stored as previously described.

Arils sample preparation

Healthy fruits without cracking were transferred from the refrigerator to the laboratory. First, the fruits were washed and dried with distilled water. Then, with the help of a sharp and sterile knife, they were cut in half from the central area. Arils were removed from the skin manually under isolated hygienic conditions and were immersed in 5% perchlorine solution for 5 min. 150 g polystyrene plastic containers were used to store ready-to-eat arils, and a transparent polyethylene wrapper with thermal sealing was used as the final packaging of the product. The produced active films were cut in 5×10 cm^2 dimensions and placed in the bottom of plastic containers. Arils in contact with pure active film and without contact film (control samples) were used. Then 100 g of arils were weighed and poured on the active films in the containers. After thermal sealing of the package, the samples were stored for 15 days (at five day intervals) at 4°C.

The juice required for the relevant tests was extracted manually using hand pressure and filtration through a clean net.

Weight loss determination

To measure weight loss of arils, the weight change of arils samples between the first and 15th day was calculated and expressed as a percentage [9].

pH and total soluble solids (TSS)

The pH and TSS of extracted fruit juice were straightly evaluated using a pH meter (pH-315i; WTW, Weilheim, Germany, standardized at pH 4 and 7) and refractometer (Bertuzzl Lattometro, expressed as ˚Brix at 20 ˚C), respectively [10, 20].

Color measurement

The color analysis of arils was performed by Hunter Lab (Minolta, CIE, Lab). Then, color factors on the 15th day with fresh arils on the first day of the test were expressed in CIELAB, L^* (lightness), a^* (denotes the red/green value), and b^* (the yellow/blue value).[10].

Antioxidant activity of arils

Total polyphenols content (TPC) of extracted fruit juice was measured by the Folin–Ciocalteu method using gallic acid (Salarbashi, et al. [13]. The results of TPC were expressed as milligrams of gallic acid equivalents per 100 g of arils.

Free radical scavenging power in pomegranate juice was measured by its ability to decolorize methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Extracted fruit juice (0.5 ml) was added to 5 ml of methanolic solution of 0.004% DPPH. After being left to stand for 30 min, the absorbance of samples at 517 nm was read by spectrophotometer (Unico 2100, China) compared to the control sample. The antioxidant activity was calculated as a percentage as follows:

$$\text{DPPH scavenging activity} = \frac{A_{blank} - A_{sample}}{A_{sample}} \times 100$$

Where; A_{blank} is controlled absorption rate (methanol) and A_{sample} is the sample's absorption.

Microbial analyses of arils

The samples were microbiologically assessed after 15 days of storage. In this regard, 10 g of samples was

poured into 90 mL of sterile physiological solution in a stomacher (Lab-Blender 400, Steward Medical, London, UK). Appropriate dilutions were cultured on Plate Count Agar for total aerobic bacteria and Potato Dextrose Agar for total fungi count [12].

Sensory evaluation

A five-point hedonic scale test was used for sensory evaluation of the samples [12]. For this purpose, 12 trained panelists evaluate the samples based on color, odor, texture, freshness, and product acceptability. Considering the sum of these sensory features, the overall product acceptability of the samples was evaluated with ranging from1 (corresponded to extremely dislike) to 5 (corresponded to extremely like). Scores from 2.5 to 5 were considered acceptable.

Release of ZnO NP to arils

To estimate the release of ZnO NP to the arils samples, atomic absorption spectrophotometer (AAS) (AA-7003 model, Chin) was used to analyze arils samples according to Asadi et al., (2019) method [21].

Statistical analysis

The Statistical tests were performed using a completely randomized factorial with three replications, and the results were calculated as "mean ± standard deviation". A significant difference (P <0.05) and differences among the mean values were analyzed by analysis of variance (ANOVA) and Duncan test, respectively. Evaluations were performed using SPSS software version 21, and graphs were drawn in Excel 2013 software.

RESULTS AND DISCUSSION

Weight loss, pH, and total soluble solids (TSS)

Weight loss could influence the quality and appearance of pomegranate arils. Figure 1 shows that arils, like other crops, have lost their weight overtime during storage in cold conditions, and the effect of time on weight loss of arils in all treatments is significant. According to Figure 1, the highest weight loss (4.1%) was in control on the 15th day, and the lowest (0.85%) was in treated arils on the 5th day of storage. According to the results (Figure 1), it was found that the combined treatment used significantly slowed down the steep weight loss slope in the control arils. The main influential factor on weight loss is respiration, water evaporation from the fruit, and oxidation activities. The mechanism is related to water vapor pressure gradient in different parts of the arils [22]. As observed, pure PLA film caused a reduction in weight loss during storage. The PLA film can act as a protective barrier via the formation of a semi-permeable and smooth layer on the arils' surface and thus reduce the respiration and transpiration of product surfaces [23].

Compared to hydrocolloid edible films, PLA film has better water vapor barrier properties (WVP) due to its hydrophobic quiddity [13]. On the other hand, the presence of ZnO NP also caused a significant ($p < 0.05$) reduction in weight loss. The existence of ZnO NP in the PLA matrix could enhance the WVP [13]. The results of a positive effect of ZnO NP on weight loss reduction of fruit during cold storage is consistent with the results of Zhao, et al. [24], in connection with apricots [22], Meng, et al. [25] in connection with fresh-cut kiwifruit, and Sogvar, et al. [26] in connection with strawberries. In arils treated with active ingredients, samples containing 0.5 and 1.5% of ZEO, respectively, showed the lowest weight loss on other treatments on the 15th day of cold storage. The ZEO has a notable impact on reducing weight loss. Studies show that the hydrophobic compounds in film structure improve the barrier properties against WVP and moisture.

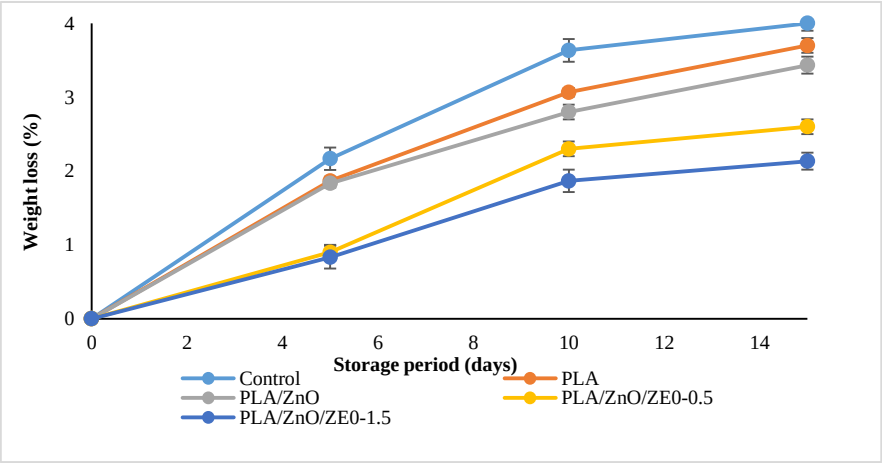

Figure 1. Weight loss (%) of arils during cold storage. Data are shown as mean ± SD of three independent measurements.

The result of cold storage time on the pH changes is presented in Table 1. The primary (day 0) pH of the arils was ~3.59. Similarly, the initial pH of ~3.86 and 3.84 was reported for arils in Özdemir and Gökmen [10] and Palma, et al. [27] studily. According to Table 1, the pH level decreases during the storage period in all samples except for varying concentrations of ZEO-containing samples, which could maintain the pH of the product without significant change ($p < 0.05$). This control of pH was continued until the 10th day by films containing ZEO EOs. But on the 15th day, there was no significant ($p < 0.5$) difference between different treatments (Table 1.). In general, ZnO NP decreased pH, and ZEO increased pH. Various factors affect the pH changes of arils during storage. These influential factors include changes in the biochemical conditions of the products, microbial activity, slower respiration rate, and metabolic activity, consumption of acids during respiration, and production of acidic metabolites [28]. Therefore, it is difficult to predict a decrease or increase of pH in fruits such as pomegranate during the storage period. A reduced pH from 3.30 to 2.27 during storage was reported in Ayhan and Eştürk [29]. However, pH increases have been reported in many arils packaging studies.(Ayhan and Eştürk [29], Palma, et al. [27] and Özdemir and Gökmen [10]. Varying concentrations of ZEO with potent antimicrobial compounds (such as Carvacrol, O-Cymene, Thymol, and Myrcene) [14] reduces the growth of microorganisms and, as a result, prevents the production of acidic metabolites resulting from the growth of microorganisms. As a result, the consumption of organic acids by respiration is more than the production of acidic metabolites due to microbial activity, and therefore, the pH increases. However, due to the less practical effect of ZnO NPs on microbial growth, the production of acidic metabolites and lowering the pH in these samples was higher.

Most TSS in fruits contains sugars, and a small percentage also includes amino acids, organic acids, vitamins, and minerals. TSS has a significant effect on the taste of the fruit and is considered as a chemical indicator. TSS usually increases as the fruit ripens and its moisture content decreases. The TSS content of arils packaged without and with active films during cold storage for 15 days is presented in Table 1. At the beginning of the fresh aril's refrigeration, TSS content was ~16.1% (Table 1). According to Table 1, the TSS content of control samples show a gradual non-significant ($p < 0.05$) increase until the 5th day of cold storage, and after that, a declining trend occurred until the end of storage. Table 1 showed the most significant difference between storage days in the control sample and then samples containing pure film, respectively. In contrast, active PLA films were able to preserve the TSS of arils over time. The increase in TSS content in the initial stage of cold storage contributed to the progression of aging. Cell-wall polysaccharides were digested during this process, and TSS was increased. The reduced TSS content may be related to the aging and metabolism of the fruit [30]. The results obtained in this study did not change over time in samples containing active PLA film, which may be related to the inhibitory effect of active compounds (ZEO and ZnO NPs) on respiration. ZEO reduces the respiratory activity of arils by saturating the closed atmosphere with volatile phenolic compounds. The photocatalytic properties of ZnO NPs also may be

effective in lowering respiration by removing ethylene produced from the fruit and thus preventing the increase of TSS during storage. The results of this part of the research on preventing the rise of TSS of arils due to ZnO NPs are following the results of Emamifar and Mohammadizadeh [31] on strawberries packaging.

Table 1. pH and TSS content of arils subjected to different packaging treatments and cold storage.

	Treatment	Day 0	Day 5	Day 10	Day 15
pH	Control (Neat arils)	3.59 ±0.01^{a}	3.50±0.02^{c}	3.46±0.01^{d}	3.38±0.03^{cd}
	PLA	3.59 ±0.02^{a}	3.47±0.01^{c}	3.44±0.02^{d}	3.35±0.04^{d}
	PLA/ZnO	3.58 ±0.01^{a}	3.48±0.01^{c}	3.52±0.02^{c}	3.45±0.03^{bc}
	PLA/ZnO/ZEO-0.5	3.59 ±0.03^{a}	3.71±0.01^{b}	3.65±0.02^{b}	3.52±0.01^{ab}
	PLA/ZnO/ZEO-1.5	3.60 ±0.01^{a}	3.75±0.01^{a}	3.7±0.02^{a}	3.55±0.02^{a}
TSS (°Brix)	Control (Neat arils)	16.23±0.15^{a}	16.30±0.10^{a}	15.60±0.24^{b}	14.78±0.17^{b}
	PLA	16.0±0.10^{a}	15.90±0.10^{b}	15.38±0.12^{b}	14.63±0.22^{b}
	PLA/ZnO	16.1±0.30^{a}	16.34±0.10^{a}	16.18±0.17^{a}	15.66±0.25^{a}
	PLA/ZnO/ZEO-0.5	16.16±0.40^{a}	16.40±0.10^{a}	16.21±0.11^{a}	15.76±0.30^{a}
	PLA/ZnO/ZEO-1.5	16.0±0.40^{a}	16.45±0.10^{a}	16.24±0.21^{a}	15.82±0.13^{a}

Values are presented as mean ± standard deviation of three independent measurement; Different letters in the same column indicate significant differences ($p < 0.05$).

Color attributes

The color characteristics of arils are one of the critical, influential parameters in the acceptance of this product. The attractive color of arils contributes to anthocyanin pigments, influencing consumer satisfaction. The color attributes and visual qualities of arils are shown in Table 2 and Figure 2, respectively. The initial L^*, a^*, and b^* in the arils (control) were ~ 41.03, 46.6, and 23.0, respectively (Table 2). Higher values of a^* index than other parameters (L^* and b^*) indicate a higher degree of redness and predominance of red anthocyanin in this fruit. Various packaging treatments and storage duration had significant effects ($P \leq 0.05$). The result indicated that active PLA film containing ZnO and multiple concentrations of EOs (0.5 and 1.5% v/w) were the most successful factors in the red colorfastness of arils. The samples in contact with the films containing 1 and 1.5% v/w ZEO showed the highest brightness (L^*) and redness (a^*) on the 15th day. It could be verified by the visual appearance of the samples given in Figure 2. This can be attributed to the volatility of effective compounds of ZEO compared to ZnO NPs. EOs compounds reduce the reactivity of anthocyanins with oxygen by saturating the closed interior and placing them on arils, but ZnO NPs cannot form such a protective layer. Regarding the positive effect of packaging with active compounds and various biopolymers in preserving the color properties of arils, numerous studies have been reported that confirm the effect of EOs [10, 12, 32].

Table 2. Color parameters (L^*, a^* and b^*) of arils on the 15th day of storage.

Treatments	L^*	a^*	b^*
Control (Day 0)	41.03±0.40^{d}	47.25±0.57^{b}	23.0±0.50^{b}
Control (Day 15)	37.66±0.64^{e}	21.00±0.85^{d}	19.48±0.58^{c}
PLA (Day 15)	37.10±0.29^{e}	25.20±0.94^{c}	17.58±0.37^{c}
PLA/ZnO(Day 15)	65.06±0.39^{a}	11.48±0.32^{e}	19.46±0.90^{c}
PLA/ZnO/ZEO-0.5(Day 15)	51.16±0.50^{c}	55.14±1.13^{a}	29.55±0.87^{a}
PLA/ZnO/ZEO-1.5(Day 15)	47.44±0.58^{b}	53.48±0.32^{a}	31.30±1.12^{a}

Values are presented as mean ± standard deviation of three independent measurement; Different letters in the same column indicate significant differences ($p < 0.05$).

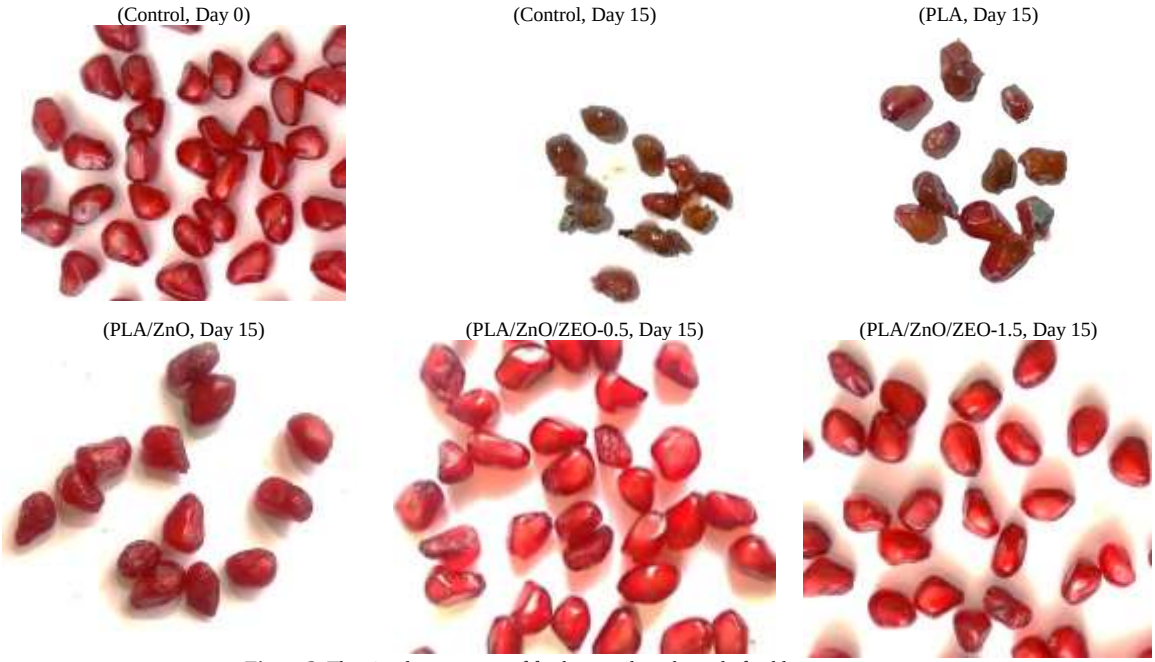

Figure 2. The visual appearance of fresh-cut arils at the end of cold storage.

Antioxidant activity

TPC is an index of antioxidant activity. The antioxidant activities of arils are mainly due to the redox properties of phenolic compounds, including hydrogen-donating, reducing agents, and singlet oxygen-quenching. The effect of phenolic compounds on free-radical scavenging may depend on structural factors, such as flavone hydroxyl, free carboxylic group, keto groups, the number of phenolic methoxyl groups, and other structural features. Packaging treatments and cold storage days had significant ($P \leq 0.05$) effects on the total phenol content. At the initial (day 0), the TPC of the arils was ~350 mg 100 ml^{-1} in juice (Figure 3a). According to a report by Tehranifar, et al. [33], the range of TPC in several Iranian pomegranate cultivars was ranged from 295.79 to 985.37 mg. 100^{-1} ml. A low increase of TPC was obtained on the fifth day of storing, and then it remained somewhat stable until the 10th day and finally decreased to the end time of cold storage time. The use of PLA-film containing ZnO and ZEO reduced the declining trend of TPC compared to the control sample. Different treatment concentrations (0.5 and 1.5% v/w) of ZEO compared to constant concentration ZnO NPs had a more significant effect on TPC preservation. Ellagic acid, hydrolyzable tannins, glycoside, and anthocyanin pigments are the main phenolic compounds in arils juice [2]. The variation of TPC in storage time was related to the TSS content of arils that influenced antioxidant activity and anthocyanin concentration [2]. However, the TPC of arils was diminished during cold storage due to phenolic oxidation and later structural degradation [34].

Arils have many antioxidant activities among fruits due to their flavonoids, phenolic, and anthocyanin sources [2]. These compounds have a therapeutic effect on cardiovascular disease and cancers. The results of the antioxidant activity of arils are shown in Figure 3b. The antioxidant activity of fresh arils (control sample, day zero) was 78.5%. The results showed that storage time and type of packaging were adequate on antioxidant activity. A significant ($P \leq 0.05$) increase was observed in antioxidant activity in all samples up to the 10th day of cold storage and then showed a downward trend from the 10th day to the end of the cold storage.

Similarly, Soloklui, et al. [2] showed an increase in antioxidant activity of arils until the 7th day of storage and then decreased. According to these researchers, the higher amount of free radical scavenging could be due to the exposure of packaged fruits to an enriched O_2 atmosphere and a biochemical response to damaged arils during minimal processing. The increase in free radical scavenging until the 5th day in this study can be attributed to water loss and consequently increasing the content of phenolic compounds in arils.

The effect of ZEO in maintaining antioxidant power was more remarkable than ZnO NPs, and with increasing the concentration of ZEO, this property was preserved to a greater extent. The onset of degradation of phenolic compounds from the 10th day onwards also indicates that the breakdown of these compounds and their reduction is slower than other metabolic activities in arils and begins with a slight delay. Active films slow down the process of metabolic changes by changing the atmosphere inside the packaging and controlling microbial growth, preventing the breakdown of antioxidant compounds. For this reason, when using active films, there is no decrease in the antioxidant activity of arils. In a similar study, Palma, et al. [27] reported that the arils packaged in polypropylene film under a modified atmosphere did not show a significant ($P \leq 0.05$) change in antioxidant activity throughout 30 days of cold storage.

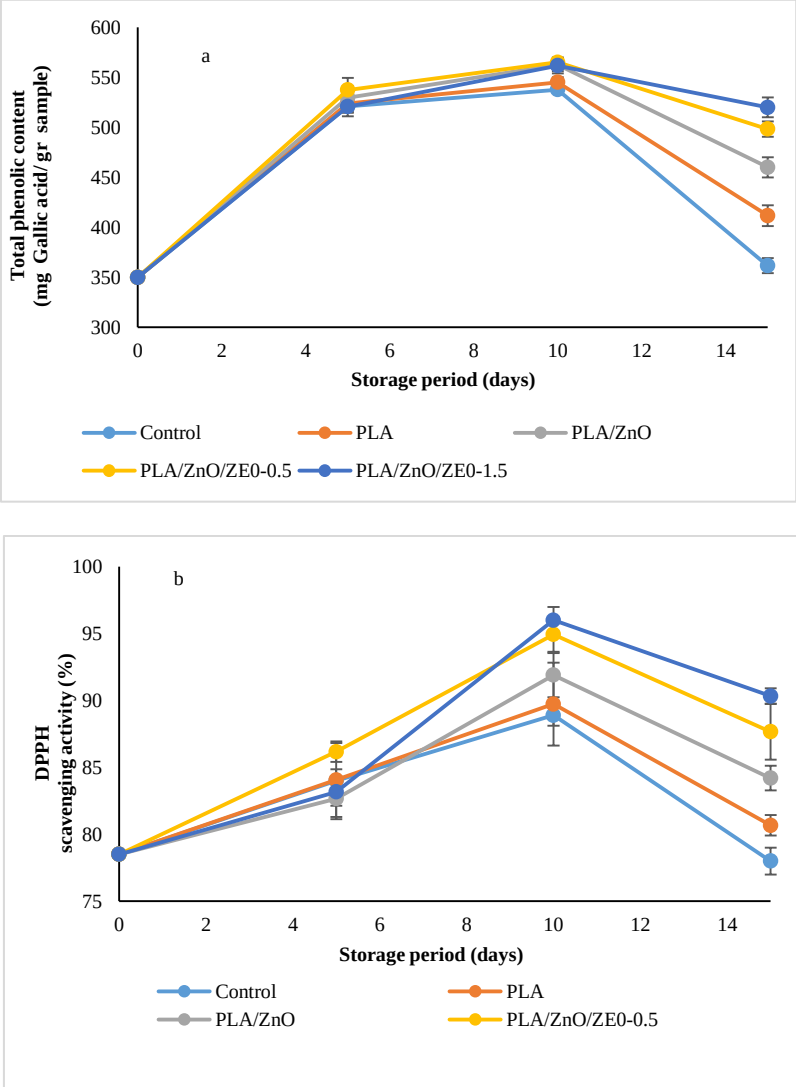

Figure 3. The effect of packaging on (a) total phenolic content and (b) antioxidant activity of arils during refrigerated storage.

Microbial analysis

The arils' initial (day 0) TBC and TFC were ~1.32 and ~0 log CFU/g, respectively, indicating that the product was hygienic (Figure 4). besides, low initial TBC and TFC of ~1.66 and < 1 log CFU/g were reported for arils, respectively [9]. In general, molds grow slower than bacteria, so if the conditions are right for bacteria to grow, molds usually grow and multiply less. As shown in Figure 4a and b, the microbial count of all samples

increased significantly over time, and the highest increase was related to the control sample (~9.66 and ~2.28 log CFU/g for TBC and TFC at day of 15). This indicates the increased susceptibility of arils to microbial spoilage during storage. However, this upward trend was slower in samples in contact with active films. The film containing 1.5% ZEO showed the lowest TBC (~3.13 log CFU/g) and fungal growth (~1.05 log CFU/g) on the 15th day. According to the Spanish legislation for foods [35], the TBC limit for fresh arils was 7 log CFU/g. In our study, TBC remained below detection limits in aril-active packaged film until day 15 of storage at 4 °C. The release of an active agent from active films resulted in reducing the microbial load until the end of refrigerated storage. An increase in TFC was observed until 10th day in all samples and then decreased to the end of storage. From the 10th day, due to the production of secondary metabolites by molds and other microorganisms themselves, the conditions for the growth of these organisms are not provided. The point to consider about microbial load changes is that the active films were only in contact with the bottom and top layers of arils, but arils were sampled for testing from all parts of the pomegranate seeds. This suggests that mere physical connection between active films and arils is not necessary to play a protective role. The active ingredients in ZEO and ZnO NPs help microbial control growth by altering the atmosphere. ZEO shows its antimicrobial position by evaporating phenolic compounds and saturating the closed interior space. ZnO NPs also help kill bacteria by absorbing oxygen and producing reactive oxygen species. Li, et al. [36] reported that active PLA/ZnO film reduced the microbial load of fresh-cut apples during storage.

In this study, the packaging of arils with PLA/ZnO/ZEO films reduced TBC and TFC, and finally, TBC has remained below 7 log CFU/g even at the end of cold storage.

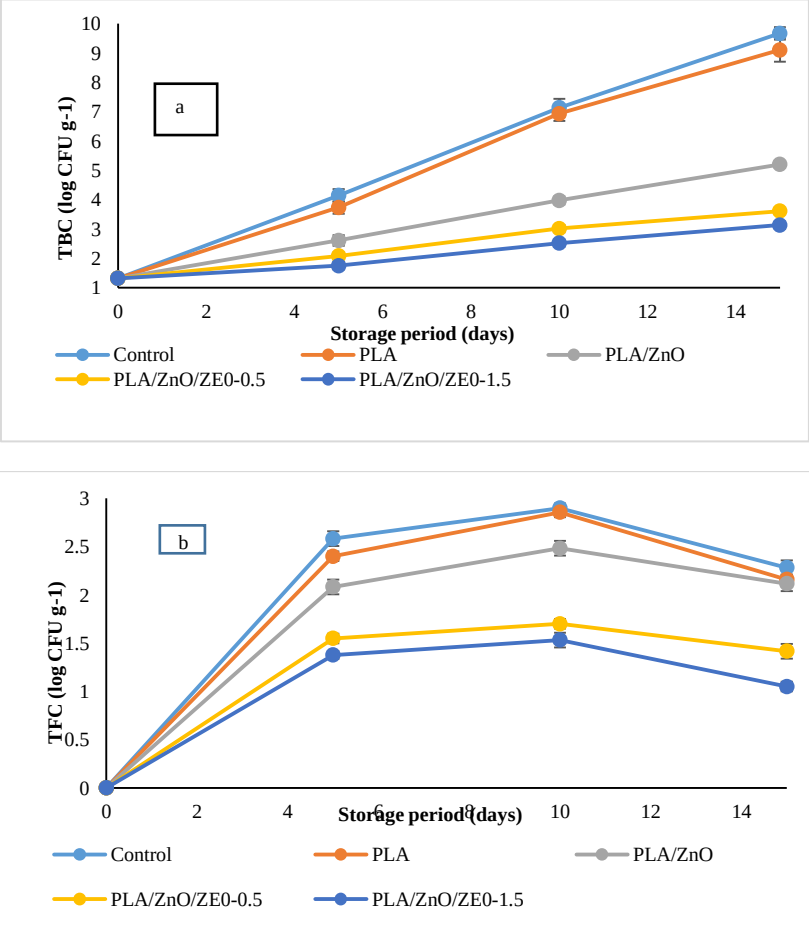

Figure 4. Changes in (a), TBC and (b), TFC values of arils during storage in the refrigerator. Data are shown as mean ± SD of three independent measurements.

Sensory evaluation

Sensory evaluation measures the degree of consumer acceptance of food products. The sensory attributes of arils are shown in Figure 5. The appearance and nutritional quality of the fruit (aroma, taste, and color) during storage are significantly reduced due to increased respiration and enzymatic activity of the fruit.

The results indicated that all control and neat PLA films had identical scores in all sensory attributes ($p > 0.05$) during the first five storage days. However, control and pure PLA film packaged arils were spoiled on the 15th day. In contrast, packaged samples had acceptable sensory properties by scores of higher than 2.5, regardless of the ZEO and ZnO concentration (Figure 5). Moreover, PLA film containing ZnO NP and 1.5% ZEO EOs maintained fruit characteristics, including color, taste, aroma, and overall acceptance, till the 15th storage day. Besides, the panelists did not realize any off-flavor in active packaged arils. This study's sensory evaluation results were also agreed with Özdemir and Gökmen [10] that no significant changes were found in sensory features of stored arils.

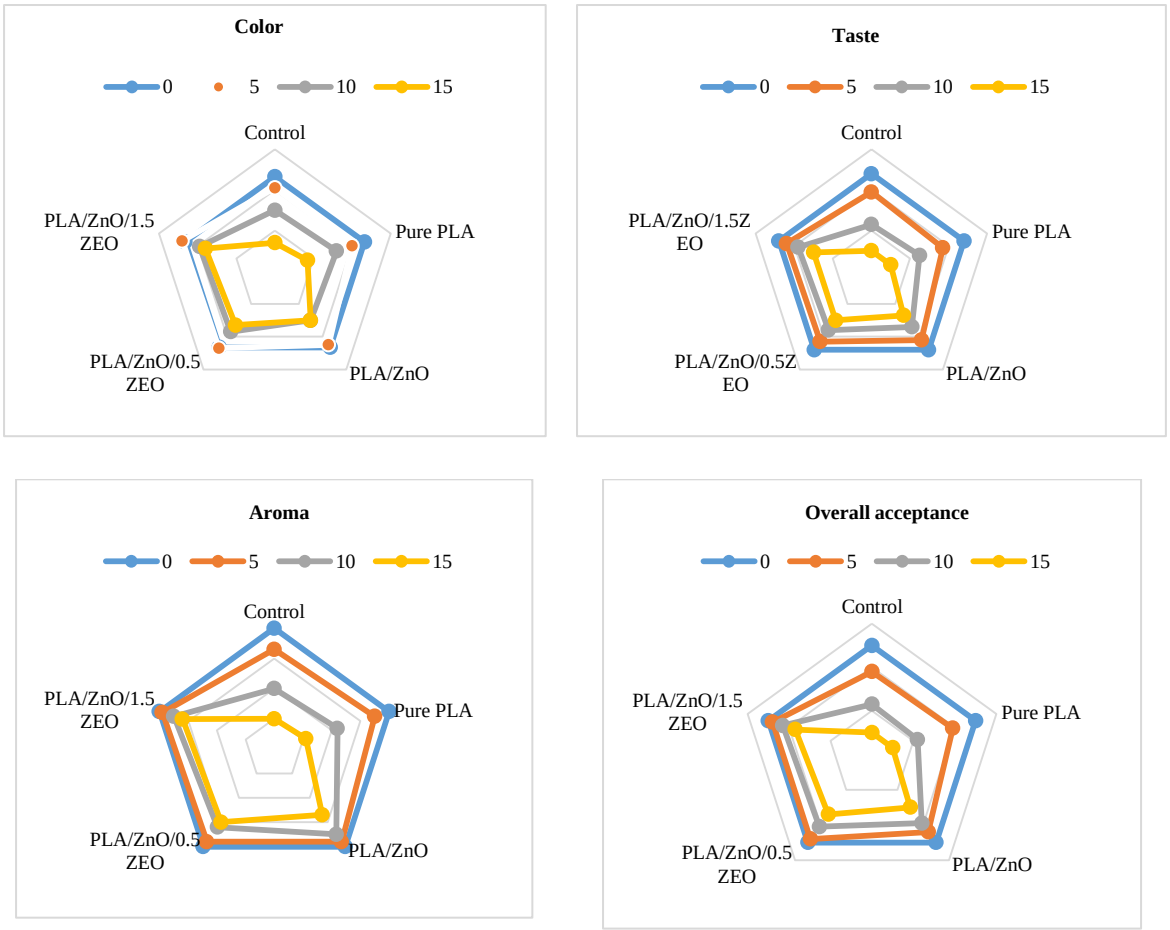

Figure 5. Sensory analysis of packaged arils.

ZnO NP release

The release of ZnO NP into the food matrix has been the main problem of active packaging approach. Therefore, the Zn^{2+} ions contents were routinely analyzed in the arils, before and after packaging to ensure compliance with regulations (Table 3). The Zn^{2+} ions amount in un-packaged arils was about 24.2 mg kg^{-1}. Similarly, a low initial Zn^{2+} ions amount of ~26.4 mg kg^{-1} arils was reported for arils [37]. Zinc is involved in regulating the metabolism of proteins, carbohydrates, and enzymes. [21]. While this element is essential, it can be toxic when is taken in excess. As shown in table 3, in the arils packaged with PLA/ZnO films with or without ZEO, the

amount of Zn^{2+} ions increased slightly after cold storage. This result indicated that the migration of ZnO NP from films was done successfully. Besides, it showed that the use of ZnO NP in active PLA film could safely applied for fruit-products due to the Zn2+ ions content transfer from the active film to arils being lower than the maximum tolerated level of 40 mg day^{-1} (https://ods.od.nih.gov/factsheets/Zinc-HealthProfessional/).

Table 3. The Zn^{2+} ions content of during 15 days cold storage

	Zn^{2+} (mg Kg^{-1} sample)	
Film	Day 0	Day 15
Control (Neat arils)	24.2±1.2	24.0±0.1
PLA	--	23.9±1.1
PLA/ZnO	--	29.8±1.2
PLA/ZnO/ZEO-0.5	--	27.2±0.8
PLA/ZnO/ZEO-1.5	--	26.5±0.9

CONCLUSIONS

The results of this study showed that the combined treatment of ZnO NPs and ZEO EOs with synergic effect on each other in PLA film increased the shelf life of freshly cut ready-to-eat arils and can be used in post-harvest management of this fruit. Most importantly, ZnO NPs and ZEO enhance the efficacy of the PLA packaging. Active packaging containing ZnO has a lower effect than ZEO on maintaining the quality characteristics of arils. Wrapping the ready-to-eat arils in the active films could extend the aril's shelf life up to 15 days. It is also evidenced that the maximum amount of Zn^{2+} ions migration from polymer to arils samples was much lower than the limit of zinc daily consumption. Taken together by PLA/ZnO/ZEO, a new ready-to-eat arils product can be introduced to the consumer market so that it retains its durability and quality characteristics during storage.

ACKNOWLEDGEMENTS

The authors would like to appreciate Food Health Research Center, Hormozgan University of Medical Sciences for supporting this research.

ETHICAL CONSIDERATION

The study project had approved by the research ethics committee of Food Health Research Center, Hormozgan University of Medical Sciences (Grant ID: 4020412, Research Ethics Certificate: IR.HUMS.REC.1402.333).

Conflict of interests

The authors declare no conflicts of interest.

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