

RESEARCH ARTICLE

## Preparation of new nanocomposite film for controlling *Listeria monocytogenes* and *Staphylococcus aureus* in raw rainbow trout fillet

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### ABSTRACT

**Objective(s):** This study was aimed to evaluate the effect of chitosan-zinc oxide (CH-ZnO) film containing pomegranate peel extract (PPE; 0.5, 1 and 1.5%) on survival of *Listeria monocytogenes* and *Staphylococcus aureus* in raw rainbow trout fillets during refrigerated storage for 12 days.

**Methods:** Total polyphenolic contents of CH-ZnO films containing different concentrations of methanolic PPE were determined with Folin-Ciocalteu reagent. In order to enumerate the inoculated pathogenic bacteria in raw rainbow trout fillets, Palcam *Listeria* selective agar (*L. monocytogenes*, incubated at 30 °C for 48 h) and Baird Parker agar (*S. aureus*, incubated at 37 °C for 48 h) were used.

**Results:** Total phenolic content of CH-ZnO enriched with PPE was recorded to be 72-139 mg gallic acid/g film. For un-treated samples, the initially recorded population of 5 log CFU/g of *L. monocytogenes* and *S. aureus* were reached 5.36 and 3.03 log CFU/g at the end of designated study period, respectively. There were significant differences between samples packed with CH-ZnO films enriched with different concentrations of PPE (0.5, 1 and 1.5%) and untreated ones ( $P < 0.05$ ). In both samples treated with 1 and 1.5% PPE, the final bacterial population were reached below 1 log CFU/g at the end of storage period.

**Conclusions:** The results of the present study indicate a potential use of CH-ZnO film enriched with PPE as an effective type of antimicrobial packaging to inhibit the growth of *L. monocytogenes* and *S. aureus* in raw rainbow trout fillets.

### How to cite this article

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## INTRODUCTION

Fish and seafood are valuable sources of important nutrients including amino acids, vitamins, unsaturated fatty acids, minerals (iron, calcium, copper, zinc and iodine) and other nutrients, which are play important roles in human health [1]. Fishery products are extremely perishable foods compared with other fresh foodstuffs due to 65-80% water content, pH 6-7 and availability of important nutrients on the product surface [2]. Previous studies reported that spoilage of fresh and minimally processed fish during refrigerated storage is dominated not only

by microbial activities including aerobic mesophilic bacteria, psychrotrophic bacteria and coliforms but also is deeply influenced by endogenous enzymatic activity [1-3]. The microbial and chemical quality factors of untreated fresh fish including spoilage microorganism's count as well as lipid and protein oxidation have been previously found to decline during chilled condition [1, 4], which can result in sensorial quality change of the product, and subsequently related to both economic losses and food-borne diseases [3]. On the other hand, food-borne pathogens are causing a great number of diseases with remarkable effects on human health [5]. Among them, *Listeria monocytogenes* and

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*Staphylococcus aureus* have been frequently isolated from various foodstuffs [6].

Shelf life extension and safety improvement of fresh fish and seafood may be performed using various preservation techniques such as active and modified atmosphere packaging (MAP), direct addition of synthetic compounds, biotechnology products and natural antimicrobials are used [3, 7-9]. Among packaging materials, chitosan (CH) has been widely studied for the development of biocompatible packaging material due to excellent properties such as intrinsic antibacterial and antifungal activities and also excellent physic-mechanical properties [10]. CH, a linear polysaccharide, consist of  $\beta$ -(1-4)-2-acetamido-D-glucose and  $\beta$ -(1-4)-2-amino-D-glucose units [1]. It has unique combination properties such as non-toxicity, biocompatibility, metal complexation biodegradability, good film-forming ability, stability and flexibility [11]. Therefore, it is recognized as an eco-friendly polymer food packaging [12-14]. Some studies indicated that the application of plant essential oils and natural extracts from rosemary [15], cinnamon [1], thyme [8], oregano [16] and pomegranate peel [17] could improve the shelf life and safety of raw foods during storage at chilled condition. Different parts of pomegranate fruit (*Punica granatum*) such as arils and rinds have valuable bioactive compounds (hydrolysable tannins, anthocyanins and flavonoids) and their functional and medicinal impacts such as anti-inflammatory, anti-diabetic, anti-bacterial and anti-viral properties have been approved [18-21]. Previous studies have been evaluated the effect of pomegranate peel extract (PPE) to increasing microbial, chemical and sensory quality factors of raw, cooked and ready-to-eat food stuffs [17, 18, 22-24].

Zinc oxide nanoparticle (ZnO) is also considered as a novel compound for development of nanocomposite coatings and film materials [25]. A recent study has been reported that carboxymethyl cellulose-sodium alginate film containing ZnO could successfully extend shelf life of minced silver carp fillets under refrigerated condition and prevent the growth of *Listeria monocytogenes* in a food model [25]. There are no reported study on the application of nanocomposite film based on CH-ZnO containing PPE to control the growth of *L. monocytogenes* and *S. aureus* in fresh rainbow trout fillets. Hereto, the present study aimed to examine the impacts of CH-ZnO film enriched

with methanolic PPE (0.5, 1 and 1.5%) on growth controlling of *L. monocytogenes* and *S. aureus* in stored raw rainbow trout fillets under refrigerated condition for 12 days.

## MATERIALS AND METHODS

### Materials

Food-grade ZnO (< 35 nm diameter and purity > 99%) was purchased from the Iranian Nanomaterials Pioneers (Razavi Khorasan, Iran). CH powder (viscosity: 200-800 cP, 75-85% deacetylated and medium molecular weight: 190-310 kDa) was purchased from Sigma-Aldrich, UK. All chemicals and culture media were of analytical grade and purchased from Merck, Germany.

### Preparation of methanolic pomegranate peel extract

Pomegranates (*Punica granatum* L.) were purchased from a local garden in the Saveh, Tehran, Iran. The peels of the fruits cut into small pieces, cleaned, washed and dried in a dark place at ambient temperature for approximately two weeks. The extraction of methanolic PPE was performed using the previous procedures by Basiri *et al.* [12] as well as Mohebi and Shahbazi [17].

### Preparation of bacterial pathogens

*S. aureus* ATCC 6538 and *L. monocytogenes* ATCC 19118 were purchased from the Iranian Research Organization for Science and Technology Institute (Tehran, Iran). For long time maintenance, the corresponding pathogens were kept in Brain Heart Infusion broth containing 20% glycerol at -18 °C. Working cultures were stored on BHI agar slants at chilled temperature ( $4 \pm 1^\circ\text{C}$ ) and sub-cultured every week on the same agar. For inoculation, a single colony for each bacterium was cultured on BHI broth and incubated at 37 °C for 18 h. Then, the bacteria were diluted to 5 log CFU/ml using 0.1% peptone water before inoculation in meat samples [26].

### Preparation of active chitosan films

CH solution (final concentration = 2%) was made using an appropriate amount of CH powder in 1% (v/v) glacial acetic acid and constantly agitated for approximately 3 h at 37 °C on a magnetic stirrer to ensure complete dissolution. The mixture was filtered through Whatman No. 3 filter paper. Then, glycerol as a plasticizer was incorporated to a level of 0.75 ml/g CH and the solution was stirred for 30 min and Tween 80 at level of 0.2% of extract as an

emulsifier was added. After 1 h of stirring, PPE (0, 0.5, 1 and 1.5%) and ZnO (0.5%) were added to CH solution. Then, 50 ml of the film forming solutions were poured on the glass petri dish and dried for 48h at ambient temperature conditions ( $25 \pm 1^\circ\text{C}$ ) [27].

#### Determination of Minimum Inhibitory Concentration (MIC) of active films

The Minimum Inhibitory Concentration (MIC) of CH-ZnO films containing different concentrations of methanolic PPE (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 0.9 and 1%) was determined according to the previously published method by other authors [28].

#### Total polyphenolic content of active films

Total polyphenolic contents of CH-ZnO films containing methanolic PPE 0.5, 1 and 1.5% were determined with Folin-Ciocalteu reagent according to the published procedures by Shahbazi [31] using gallic acid as a standard.

#### Treatment of fish fillets

Fresh rainbow trout (*Oncorhynchus mykiss*) averaging from 900 to 980 g were purchased from a local fishery plant (Kermanshah, Iran) and immediately transported to the Food Hygiene laboratory. Upon arrival, the fishes were manually killed and filleted in aseptic condition. Then, the fillet samples dipped in beakers containing  $5 \log$  CFU/ml of *S. aureus* and *L. monocytogenes* for 30 min at room temperature. The samples were air dried for 30 min to allow attachment of bacterial pathogens onto fish fillets. After this step, fish fillets were packed in CH-ZnO films containing different concentrations of methanolic PPE (0.5, 1 and 1.5%), put in sterile bags (Interscience, France) and kept under chilled temperature ( $4 \pm 1^\circ\text{C}$ ) for 12 days. The experiment was conducted in triplicate.

#### Microbial evaluation

In order to enumerate the inoculated pathogenic bacteria in raw rainbow trout fillets, Palcam *Listeria* selective agar (*L. monocytogenes*, incubated at  $30^\circ\text{C}$  for 48 h) and Baird Parker agar (*S. aureus*, incubated at  $37^\circ\text{C}$  for 48 h) were used [5].

#### Sensory evaluation

Sensorial properties of un-inoculated samples including color, odor and overall acceptability was evaluated by a group of ten-members from the laboratory staff. For this purpose, ten descriptive hedonic scale (1 = extremely unacceptable and 10 = extremely acceptable) was used [29].

#### Film properties

Surface morphology of films was observed using a TeScan MIRA3 SEM. Film thickness was assessed using digital micrometer (Mitutoyo, Mitutoyo Corporation, Japan) to the nearest 0.001 mm. The interaction between the components and incorporation confirming was evaluated using Fourier Transform Infrared Spectroscopy (FTIR; Bruker, model ALPHA, Germany) within the wave number range of  $400\text{--}4000 \text{ cm}^{-1}$ . The color of film samples in terms of lightness ( $L$ ), redness ( $a$ ) and yellowness ( $b$ ) was determined using Minolta Chroma Meter Model CR-400 (Minolta Co Ltd, Osaka, Japan). Hence, the film pieces (20 mm in diameter) were placed over the standard white plate ( $L^* = 93.49$ ,  $a^* = -0.25$  and  $b^* = -0.09$ ). Color differences (DE) were calculated by the following equation [27]:

$$\sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$

Where  $L^*$ ,  $a^*$  and  $b^*$  are the color parameter values of the standard and  $L$ ,  $a$  and  $b$  are the color parameter values of the sample.

#### Statistical analysis

All experiments were conducted in triplicate. The microbiological data were expressed as  $\log$  CFU/g. Analysis of variance (ANOVA) and Duncan's multiple range test using SPSS 16 were performed for evaluation of significant differences ( $P < 0.05$ ) among designated groups.

## RESULTS AND DISCUSSION

#### Film properties

The effects of incorporating PPE in the thickness of CH-ZnO films on the performance of the nanocomposite is shown in Table 1. Based on our

Table 1. Thickness property of chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE)

Film	Inhibition zone (mm)			
	Control	PPE 0.5%	PPE 1%	PPE 1.5%
Thickness (mm)	0.082±0.001 <sup>a</sup>	0.084±0.003 <sup>a</sup>	0.097±0.00 <sup>a</sup>	0.098±0.001 <sup>a</sup>

Values are expressed as mean ± standard deviation. Different letters in the same raw indicate significant differences ( $P < 0.05$ ).

findings, the film thickness was in the ranges of 0.082 and 0.098 mm. There were no significant differences among control and films incorporated with different concentrations of PPE ( $P > 0.05$ ). Similarly, Moradi *et al.* reported that addition of *Zataria multiflora* Boiss essential oil and grape seed extract had no significant effects on the thickness property of CH film [30].

Based on the results of the color property (Table 2), yellowness ( $b^*$  value) reduced significantly with incorporation of the PPE, whereas control CH film had the most positive value ( $29.11 \pm 0.2$ ). The  $a^*$  and  $L^*$  values were higher and lower in films

incorporated with PPE compared to control group, which is could be related to internal interaction between film compounds and phenolic constituents of PPE during film drying [31]. Previous study reported that the droplet size of film compounds had the important effect on the color properties of films made from them. Similar trend was found by incorporation of plant extracts in soy protein [32] and pea starch [33].

Through comparison of Figs. 1a-c which show the surfaces of CH films without or with ZnO and PPE, homogenous structure (without any insoluble particles) was observed in the CH film.

Table 2. Color of chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE)

Film	$L^*$	$a^*$	$b^*$	$\Delta E$
Control	$84.10 \pm 0.5^a$	$-1.76 \pm 0.1^a$	$29.11 \pm 0.2^a$	$26.73 \pm 0.4^a$
PPE 0.5%	$55.12 \pm 0.8^b$	$18.05 \pm 0.5^b$	$28.74 \pm 0.1^a$	$48.94 \pm 0.3^b$
PPE 1%	$47.21 \pm 0.3^c$	$19.20 \pm 0.4^b$	$27.20 \pm 0.1^a$	$55.94 \pm 0.2^c$
PPE 1.5%	$42.33 \pm 0.2^c$	$19.80 \pm 0.4^b$	$27.21 \pm 0.1^a$	$60.31 \pm 0.3^d$

Values are expressed as mean  $\pm$  standard deviation. Different letters in the same column indicate significant differences ( $P < 0.05$ ).

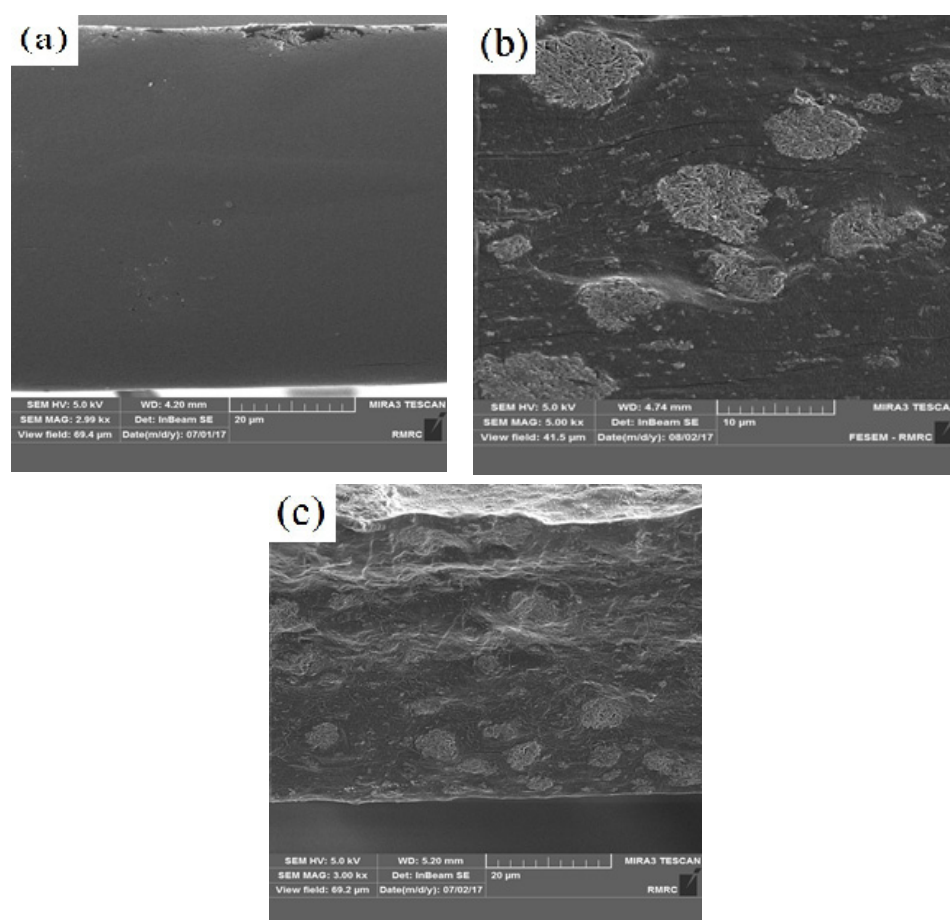


Fig. 1. SEM Images of pure chitosan (a), chitosan-ZnO (b) and chitosan-ZnO containing pomegranate peel extract 0.5% (c)

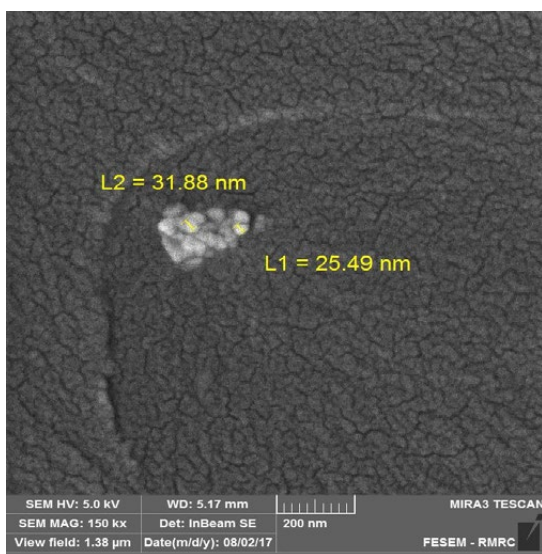


Fig. 2. Size of ZnO in chitosan film

From Fig. 2 SEM analysis, it was observed that the ZnO size in the nanocomposite films was lower than 35 nm. As it can be seen, high degree of ZnO agglomeration was observed in the nanocomposite film, which is in consistent with the findings of

Rezaei and Shahbazi [25] and Rahman *et al.* [34].

The FTIR spectra of ZnO, CH, CH + ZnO and CH + ZnO + PPE 0.5% films are presented in Fig. 3. The most important peaks of pure CH film were as follow: 3428.59  $\text{cm}^{-1}$  (stretching vibrations of O-H and N-H), 2927.26  $\text{cm}^{-1}$  (aliphatic C-H stretching vibration), 3068.88  $\text{cm}^{-1}$  ( $\text{CH}_2$  stretching vibration), 3027.81  $\text{cm}^{-1}$  ( $\text{CH}_3$  stretching vibration), 1702.25  $\text{cm}^{-1}$  (C=O stretching of amide I), 1664.02  $\text{cm}^{-1}$  (NH of amide II stretching vibration), 1110.29  $\text{cm}^{-1}$  (C3-OH stretching vibration) and 1039.48  $\text{cm}^{-1}$  (C6-OH stretching vibration) [35]. For the FTIR of ZnO, two peaks including 548  $\text{cm}^{-1}$  (Zn-O stretching) and 3254  $\text{cm}^{-1}$  (OH group) were observed [36]. Based on our findings, there were no new bands for designated nanocomposite film without PPE, suggesting ZnO physically included to the film matrix. The result of the present study is in consistent with those reported for polyvinyl chloride-ZnO [36] and fish protein isolate-fish skin gelatin-ZnO [37] based films. For film containing PPE, peaks around 1000-1800  $\text{cm}^{-1}$  might be attributed to the stretching of C=O, -C=C-C=O, -C=C- [(in-ring) aromatic] and -C-C- [(in-ring) aromatic] found in the phenolic components of PPE [25].

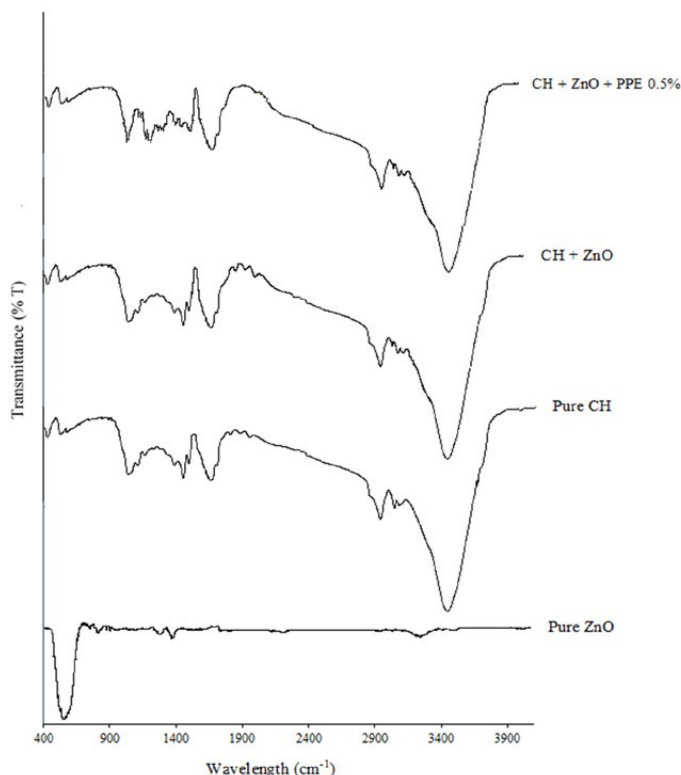


Fig. 3. FTIR spectra of ZnO, chitosan (CH), CH + ZnO and CH + ZnO + PPE 0.5%



*Total phenolic content of active films*

In the current work (Fig. 4), total phenolic contents of CH-ZnO films containing PPE (0.5, 1 and 1.5%) was recorded to be 72-139 mg gallic acid/g film. Based on our findings, pure CH had a total phenolic contents of 8 mg gallic acid/g film. It might be related to the production of chromogens during the experiment, because of the reaction of Folin-Ciocalteu reagent with other reducing constituents which determined spectrophotometrically [38]. According to the previous studies [18, 20, 21, 39, 40], phenolic compounds are the main constituents

in PPE. Berizi *et al.* [15] found that the total phenolic content of pomegranate extract obtained from Shiraz, Iran (Rabab variety) was 70.83 mg tannic acid/g PPE. Turgut *et al.* [13] reported total phenolic content of 165.4 mg gallic acid /g PPE for pomegranate obtained from Isparta, Turkey. Özdemir *et al.* [16] indicated that phenolic content of aqueous pomegranate extract was 140 mg catechin/g and 158.5 mg gallic acid/g, respectively. In another studies [39, 40], the total phenolic contents of PPE were also reported 258.2 mg/g and 508.8 mg/g PPE, respectively. It has been generally

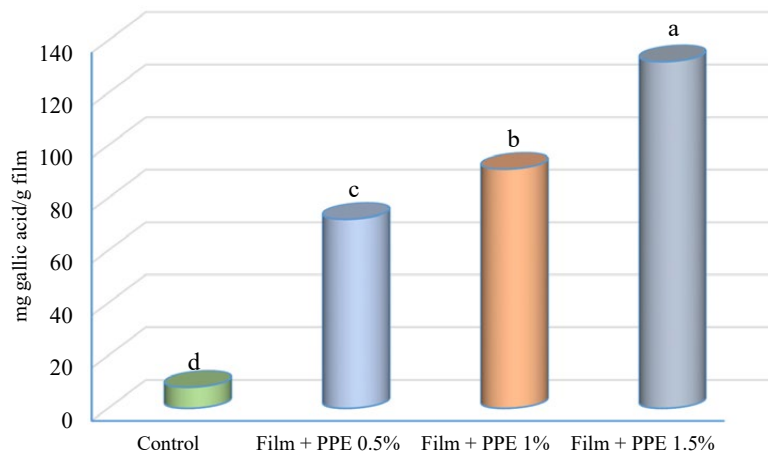


Fig. 4. Total phenolic contents of chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE; 0.5, 1 and 1.5%). Values are expressed as mean  $\pm$  standard deviation. Different letters indicate significant differences ( $P < 0.05$ )

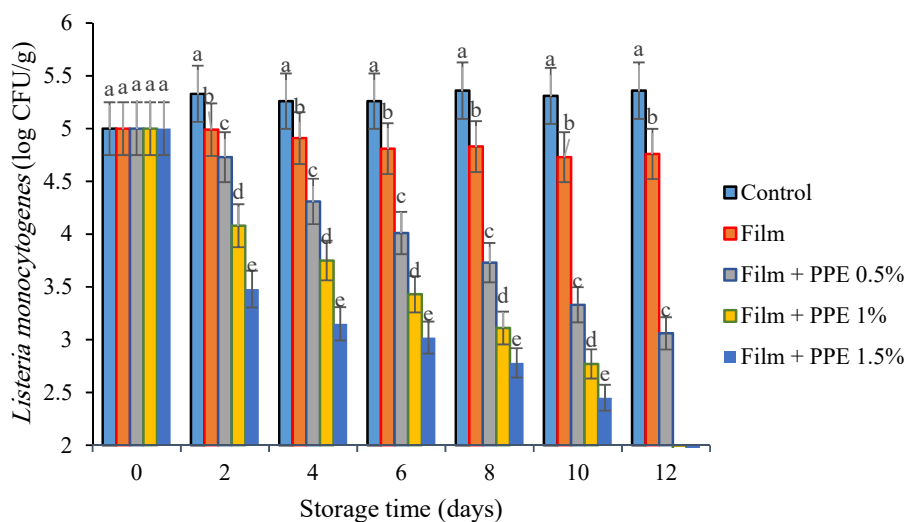


Fig. 5. Changes in *Listeria monocytogenes* of raw rainbow trout fillets packed in chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE) during refrigerated storage. Each number is the mean  $\pm$  standard error taken from different experiments. <sup>a-e</sup> For each day, columns representing different bacterial population are labeled with different letters ( $P < 0.05$ )

accepted that the variability of total phenolic contents of fruit extracts in different studies are greatly depends on growth stage and strain of fruits and also climate change, seasonal variation and geographical condition [39].

*Antibacterial effect of active films*

Based on the findings of the current study, the MICs of active films containing PPE for both *S. aureus* and *L. monocytogenes* were found to be 0.5%.

As it can be seen in Fig. 5, for untreated samples, the initially recorded population of 5 log CFU/g of *L. monocytogenes* reached 5.36 log CFU/g at the end of designated study period. There were significant differences between samples packed with CH-ZnO films enriched with PPE at concentrations of 0.5, 1 and 1.5% and untreated one ( $P < 0.05$ ). In both samples packed with 1 and 1.5% PPE, the final population of *L. monocytogenes* were reached below 1 log CFU/g on days 12 of storage, while the final count of treated samples with 0.5% PPE was found as 3.06 log CFU/g at the end of refrigerated storage.

The changes of *S. aureus* in untreated and treated raw trout fillets with designated films enriched with PPE at concentrations of 0.5, 1 and 1.5% are shown in Fig. 6. In control group, the initial bacterial count of 5 log CFU/g decreased up to 3.03 log CFU/g after 12 days of refrigerated storage. All samples packed with CH-ZnO films incorporated

with PPE significantly prevented *S. aureus* growth during refrigerated period as compared to control group ( $P < 0.05$ ). The group treated with CH-ZnO + PPE 1.5% showed the maximum antibacterial impact against the microorganism ( $P < 0.05$ ). Like *L. monocytogenes*, CH-ZnO + PPE 0.5% could not inhibit the growth of bacterial strain; as the final bacterial population in treated sample with PPE 0.5% was found to be 2 log CFU/g at the end of storage day.

Control CH-ZnO film exhibited antibacterial effect against both food-borne pathogens, however, prevention of their growth was significantly lower than designated films enriched with PPE. In general, the positively charged amino group of CH is interacted with negatively charged bacterial cell membranes and it is considered as the most important reason of antibacterial effect of CH film. This phenomena cause the leakage of intracellular essential constituents of the bacterial pathogens and ultimately killed them [27, 41]. These results are similar with those published by Ojagh *et al.* [1] and Mohebi and Shahbazi [22] who indicated that CH film was success in delaying growth of bacteria in food models compared to untreated ones. Moreover, previous studies reported that  $Zn^{+2}$  ions from the powder could react with the microorganism surfaces and interior constituents, irreversibly damage the cell membrane and cause leakage of essential process in the bacterial cell [25, 34]. Previous studies have been reported that

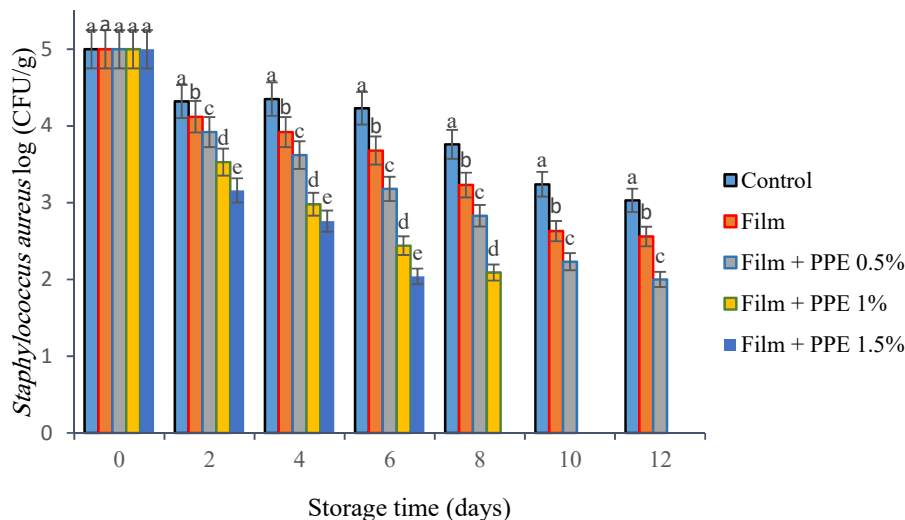


Fig. 6. Changes in *Staphylococcus aureus* of raw rainbow trout fillets packed in chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE) during refrigerated storage. Each number is the mean ± standard error taken from different experiments. <sup>a-c</sup> For each day, columns representing different bacterial population are labeled with different letters ( $P < 0.05$ )

antimicrobial activities and many other benefits of essential oils and natural extracts are related to their phenolic compounds [42]. It has been demonstrated that PPE has excellent antibacterial effects against some pathogenic bacteria including *Escherichia coli*, *Pseudomonas aeruginosa* and *S. aureus* [43-46]. A previous study [47], reported that MIC of methanolic PPE against *L. monocytogenes* was 0.5%. They also reported that PPE was effective (MIC 2%) against two strains of *S. aureus* including *S. aureus* ATCC 6538 and methicillin-resistant *S. aureus*. Regarding the results of disk diffusion method, PPE was a good inhibitor for *L. monocytogenes*, *S. aureus*, *E. coli* O157:H7 and *Yersinia enterocolitica* by inhibition zones of 3.68, 14.42, 4.12 and 5.32 mm, respectively [47]. Variation in findings among studies were found on PPE with MIC determinations against *L. monocytogenes* and *S. aureus* [48-50]. The antibacterial effect differences of PPE might be described with differences of fruit species, bacterial inoculation level, culture media and the used methods for evaluation of antibacterial efficacy [38]. Our previous study [22] showed that CH and gelatin films containing *Ziziphora clinopodioides* essential oil (0 and 1%) alone and in combination with PPE (0 and 1%) were very success to increase shelf life of peeled shrimp during chilled storage. Moreover, we found that all shrimp groups treated with the aforementioned designated films contained 2-3 log CFU/g reduction of *L. monocytogenes* as compared to control group. In another study [17], treating the

peeled shrimp with PPE could significantly retard the microbial spoilage counts. Moreover, previous studies indicated that the incorporation of plant essential oils and natural extracts caused a decrease of 2-4 log CFU/g in the numbers of *L. monocytogenes* and *S. aureus* in different food models [9, 16, 51, 52]. Khan and Haneef [28] have also found the role of bioactive compounds of PPE such as anthocyanins, hydrolysable tannins, flavan-3-ols and flavonoids as the most active compounds against bacteria. According to their results, these compounds were also responsible for its antifungal and antioxidant properties.

The impact of CH-ZnO + PPE films on the general aspect of raw rainbow trout fillets after 12 days of storage during chilled condition is shown in Fig. 7. Our findings indicated that PPE 1 and 1.5% had no negative impacts on the sensorial characteristics of treated samples in comparison control samples. The reason of lower acceptability scores of untreated samples was higher microbial spoilage growth and chemical changes.

## CONCLUSIONS

The findings of the current work indicate a potential use of CH-ZnO films enriched with PPE as an excellent types of biodegradable compounds to inhibit the growth of *L. monocytogenes* and *S. aureus* in raw rainbow trout fillets. Both samples containing 1 and 1.5% of PPE had the highest scores of sensory evaluation until day 12 of storage.

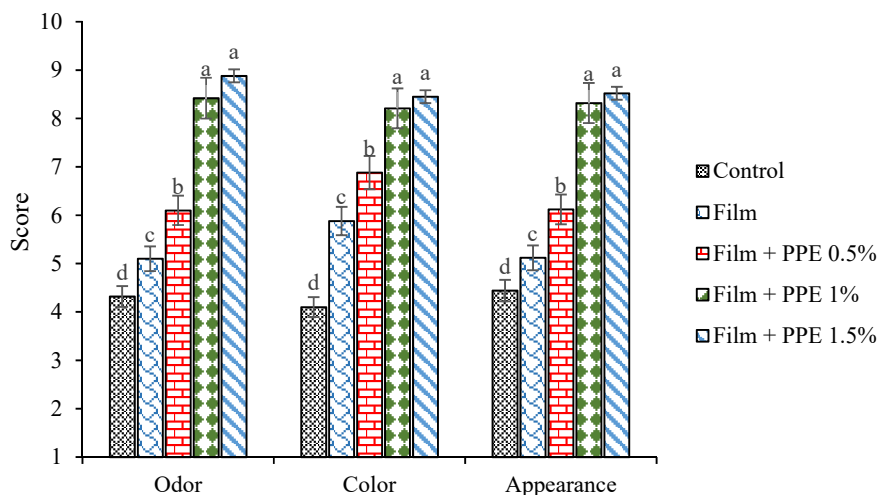


Fig. 7. Sensory attributes (odor, color and overall acceptability) of raw rainbow trout fillets packed in chitosan-ZnO films containing different concentrations of pomegranate peel extract (PPE) during refrigerated storage. Each number is the mean  $\pm$  standard error taken from different experiments. <sup>a-d</sup> For each attribute, columns representing different bacterial population are labeled with different letters ( $P < 0.05$ )



## CONFLICT OF INTERESTS

The author claims that there is no conflict of interest.

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## REFERENCES

1. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Food Chemistry*. 2010;120(1):193-8.
2. Jouki M, Yazdi FT, Mortazavi SA, Koocheki A, Khazaei N. Effect of quince seed mucilage edible films incorporated with oregano or thyme essential oil on shelf life extension of refrigerated rainbow trout fillets. *International Journal of Food Microbiology*. 2014;174:88-97.
3. Lu F, Ding Y, Ye X, Liu D. Cinnamon and nisin in alginate-calcium coating maintain quality of fresh northern snakehead fish fillets. *LWT - Food Science and Technology*. 2010;43(9):1331-5.
4. Volpe MG, Siano F, Paolucci M, Sacco A, Sorrentino A, Malinconico M, et al. Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchus mykiss*) fillets. *LWT - Food Science and Technology*. 2015;60(1):615-22.
5. Jay JM, Loessner MJ, Golden DA. *Modern Food Microbiology*, 7th Ed., New York, NY: Springer Science Business Media, Inc; 2005.
6. Lv F, Liang H, Yuan Q, Li C. In vitro antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms. *Food Research International*. 2011;44(9):3057-64.
7. Zouaghi F, Cantalejo MJ. Study of modified atmosphere packaging on the quality of ozonated freeze-dried chicken meat. *Meat Science*. 2016;119:123-31.
8. Solomakos N, Govaris A, Koidis P, Botsoglou N. The antimicrobial effect of thyme essential oil, nisin and their combination against *Escherichia coli* O157:H7 in minced beef during refrigerated storage. *Meat Science*. 2008;80(2):159-66.
9. Boziaris IS, Proestos C, Kapsokefalou M, Komaitis M. Antimicrobial effect of *Filipendula ulmaria* plant extract against selected foodborne pathogenic and spoilage bacteria in laboratory media, fish flesh and fish roe product. *Food Technology and Biotechnology*, 2011;49 (2):263-270.
10. Peng Y, Li Y. Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. *Food Hydrocolloids*. 2014;36:287-93.
11. Siripatrawan U, Harte BR. Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. *Food Hydrocolloids*. 2010;24(8):770-5.
12. Aider M. Chitosan application for active bio-based films production and potential in the food industry: Review. *LWT - Food Science and Technology*. 2010;43(6):837-42.
13. Siripatrawan U, Noipha S. Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. *Food Hydrocolloids*. 2012;27(1):102-8.
14. Du W-L, Niu S-S, Xu Y-L, Xu Z-R, Fan C-L. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydrate Polymers*. 2009;75(3):385-9.
15. Gao M, Feng L, Jiang T, Zhu J, Fu L, Yuan D, et al. The use of rosemary extract in combination with nisin to extend the shelf life of pompano (*Trachinotus ovatus*) fillet during chilled storage. *Food Control*. 2014;37:1-8.
16. Govaris A, Solomakos N, Pexara A, Chatzopoulou PS. The antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella Enteritidis* in minced sheep meat during refrigerated storage. *International Journal of Food Microbiology*. 2010;137(2-3):175-80.
17. Basiri S, Shekarforoush SS, Aminlari M, Akbari S. Corrigendum to "The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and quality of Pacific white shrimp (*Litopenaeus vannamei*) during refrigerated storage" [*LWT - Food Sci and Technol* 60 (2015) 1025–1033]. *LWT - Food Science and Technology*. 2015;63(1):798.
18. Turgut SS, Soyer A, Işıklı F. Effect of pomegranate peel extract on lipid and protein oxidation in beef meatballs during refrigerated storage. *Meat Science*. 2016;116:126-32.
19. Turgut SS, Işıklı F, Soyer A. Antioxidant activity of pomegranate peel extract on lipid and protein oxidation in beef meatballs during frozen storage. *Meat Science*. 2017;129:111-9.
20. Berizi E, Hosseinzadeh S, Shekarforoush SS, Barbieri G. Microbial, chemical, textural and sensory properties of coated rainbow trout by chitosan combined with pomegranate peel extract during frozen storage. *International Journal of Biological Macromolecules*. 2018;106:1004-13.
21. Özdemir H, Soyer A, Tağı Ş, Turan M. Nar kabuğu ekstraktının antimikrobiyel ve antioksidan aktivitesinin köfte kalitesine etkisi. *GIDA-Journal of Food*. 2014;39 (6):1-4.
22. Mohebi E, Shahbazi Y. Application of chitosan and gelatin based active packaging films for peeled shrimp preservation: A novel functional wrapping design. *LWT - Food Science and Technology*. 2017;76:108-16.
23. Kanatt SR, Chander R, Sharma A. Antioxidant and antimicrobial activity of pomegranate peel extract improves the shelf life of chicken products. *International Journal of Food Science & Technology*. 2010;45(2):216-22.
24. Özdemir H, Soyer A, Tağı Ş, Turan M. Effects of antimicrobial and antioxidant activity of pomegranate peel extract on the quality of beef meatballs. *GIDA-Journal of Food*. 2014;39 (6):355-362.
25. Rezaei F, Shahbazi Y. Shelf-life extension and quality attributes of sauced silver carp fillet: A comparison among direct addition, edible coating and biodegradable film. *LWT - Food Science and Technology*. 2018;87:122-33.
26. Khezrian A, Shahbazi Y. Application of nanocomposite chitosan and carboxymethyl cellulose films containing natural preservative compounds in minced camel's meat. *International Journal of Biological Macromolecules*. 2018;106:1146-58.
27. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry*. 2010;122(1):161-6.
28. Azizkhani M, Misaghi A, Basti AA, Gandomi H, Hosseini H. Corrigendum to "Effects of *Zataria multiflora* Boiss. Essential Oil on Growth and Gene Expression of Enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213." [*Int. J. Food*

- Microbiol. 163 (2013) 159–165]. International Journal of Food Microbiology. 2013;166(1):125.
29. Hui G, Liu W, Feng H, Li J, Gao Y. Effects of chitosan combined with nisin treatment on storage quality of large yellow croaker ( *Pseudosciaena crocea* ). Food Chemistry. 2016;203:276-82.
  30. Moradi M, Tajik H, Razavi Rohani SM, Oromiehie AR, Malekinejad H, Aliakbarlu J, et al. Characterization of antioxidant chitosan film incorporated with *Zataria multiflora* Boiss essential oil and grape seed extract. LWT - Food Science and Technology. 2012;46(2):477-84.
  31. Shahbazi Y. Application of carboxymethyl cellulose and chitosan coatings containing *Mentha spicata* essential oil in fresh strawberries. International Journal of Biological Macromolecules. 2018;112:264-72.
  32. Sivaroban T, Hettiarachchy NS, Johnson MG. Physical and antimicrobial properties of grape seed extract, nisin, and EDTA incorporated soy protein edible films. Food Research International. 2008;41(8):781-5.
  33. Corrales M, Han JH, Tauscher B. Antimicrobial properties of grape seed extracts and their effectiveness after incorporation into pea starch films. International Journal of Food Science & Technology. 2009;44(2):425-33.
  34. Rahman PM, Mujeeb VMA, Muraleedharan K. Flexible chitosan-nano ZnO antimicrobial pouches as a new material for extending the shelf life of raw meat. International Journal of Biological Macromolecules. 2017;97:382-91.
  35. Vanamudan A, Pamidimukkala P. Chitosan, nanoclay and chitosan-nanoclay composite as adsorbents for Rhodamine-6G and the resulting optical properties. International Journal of Biological Macromolecules. 2015;74:127-35.
  36. Zarrinkhameh M, Zandehnam A, Hosseini SM. Fabrication of polyvinylchloride based nanocomposite thin film filled with zinc oxide nanoparticles: Morphological, thermal and optical characteristics. Journal of Industrial and Engineering Chemistry. 2015;30:295-301.
  37. Arfat YA, Benjakul S, Prodpran T, Sumpavapol P, Songtipya P. Properties and antimicrobial activity of fish protein isolate/fish skin gelatin film containing basil leaf essential oil and zinc oxide nanoparticles. Food Hydrocolloids. 2014;41:265-73.
  38. Shahbazi Y. The properties of chitosan and gelatin films incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil as biodegradable materials for active food packaging. International Journal of Biological Macromolecules. 2017;99:746-53.
  39. Çam M, Hışıl Y. Pressurised water extraction of polyphenols from pomegranate peels. Food Chemistry. 2010;123(3):878-85.
  40. Zhang L, Yang X, Zhang Y, Wang L, Zhang R. In vitro antioxidant properties of different parts of pomegranate flowers. Food and Bioproducts Processing. 2011;89(3):234-40.
  41. Jeon Y-J, Kamil JYVA, Shahidi F. Chitosan as an Edible Invisible Film for Quality Preservation of Herring and Atlantic Cod. Journal of Agricultural and Food Chemistry. 2002;50(18):5167-78.
  42. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-53.
  43. Tesaki S, Tanabe S, Moriyama M, Fukushi E, Kawabata J, Watanabe M. Isolation and Identification of an Antibacterial Compound from Grape and Its Application to Foods. Journal of the agricultural chemical society of Japan. 1999;73(2):125-8.
  44. Duman A, Ozgen M, Dayisoylu K, Erbil N, Durgac C. Antimicrobial Activity of Six Pomegranate (*Punica granatum* L.) Varieties and Their Relation to Some of Their Pomological and Phytonutrient Characteristics. Molecules. 2009;14(5):1808-17.
  45. Khan A, Hane S. Antibacterial properties of *Punica granatum* peels. International Journal of Applied Biology and Pharmaceutical Technology, 2011;2 (3):23-27.
  46. Pai V, Chanu TR, Chakraborty R, Raju B, Lobo R, Ballal M. Evaluation of the antimicrobial activity of *Punica granatum* peel against the enteric pathogens: An invitro study. Asian Journal of Plant Science and Research, 2011;1 (2):57-62.
  47. Al-Zoreky NS. Antimicrobial activity of pomegranate (*Punica granatum* L.) fruit peels. International Journal of Food Microbiology. 2009;134(3):244-8.
  48. Shahbazi Y. Antibacterial and Antioxidant Properties of Methanolic Extracts of Apple (*Malus pumila*), Grape (*Vitis vinifera*), Pomegranate (*Punica granatum* L.) and Common Fig (*Ficus carica* L.) Fruits. Pharmaceutical Sciences. 2017;23(4):308-15.
  49. Machado TB, Pinto AV, Pinto MCFR, Leal ICR, Silva MG, Amaral ACE, et al. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. International Journal of Antimicrobial Agents. 2003;21(3):279-84.
  50. Prashanth D, Asha MK, Amit A. Antibacterial activity of *Punica granatum*. Fitoterapia. 2001;72(2):171-3.
  51. Shahbazi Y, Karami N, Shavisi N. Effect of *Ziziphora clinopodioides* essential oil on shelf life and fate of *Listeria monocytogenes* and *Staphylococcus aureus* in refrigerated chicken meatballs. Journal of Food Safety. 2017;38(1):e12394.
  52. Higginbotham KL, Burris KP, Zivanovic S, Davidson PM, Stewart CN. Aqueous extracts of *Hibiscus sabdariffa* calyces as an antimicrobial rinse on hot dogs against *Listeria monocytogenes* and methicillin-resistant *Staphylococcus aureus*. Food Control. 2014;40:274-7.