

Impact of *Zataria multiflora* Essential Oil, Nisin, Potassium Sorbate and LDPE Packaging Containing Nano-ZnO on Shelf Life of Caviar

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Caviar is one of the most luxury foods in the world. In addition to the traditional method (using salt and preservative with low temperature), there are various methods to extend the shelf life of caviar. The main objectives of this study were to evaluate the capabilities of *Zataria multiflora* essential oil (0.03%, 0.06%(W/W)), nisin (9, 18 mg/kg), potassium sorbate (500 and 1000 mg/kg) and LDPE package containing 0.4 and 1%(W/W) nano-ZnO on the shelf life, as a new approach in caviar. Total aerobic bacteria, molds & yeasts and color of samples were evaluated immediately after packaging and after 30, 60, 90, 120 and 150 days of storage. Results showed that LDPE package containing 1%(W/W) nano-ZnO have the most capability to control total aerobic bacteria. While for molds and yeasts count, 0.06%(W/W) *Zataria multiflora* essential oil had the most significant effect on the caviar samples ($p < 0.05$). For color changes analysis, there were significant differences between all of the treatments except 500 mg/kg potassium sorbate and 18 mg/kg nisin. Minimal color changes were observed in caviar samples packaged in LDPE film containing nano-ZnO. The results also showed a considerable Zn migration from package to caviar that its rate increased after 40 days.

Keywords: caviar, nanocomposite ZnO, nisin, potassium sorbate, shelf life, *Zataria multiflora* Boiss

Introduction

Caviar is an expensive delicacy with high nutritional factor full of vitamin A and complex vitamins B (Altug and Bayrak, 2003; Bledsoe *et al.*, 2003). The caviar from Russia and Iran, produced from roes of Sturgeon fish, is known as the original caviar. There are over 20 species of sturgeon, while the Caspian Sea has six commercially valuable sturgeon species, four of which produce 90% of the world's original caviar. They consist of *Acipenser Huso huso* (Beluga sturgeon), *Acipenser gueldenstaedtii* (Ossetra or Russian sturgeon), *Acipenser persicus* (Persian sturgeon), and *Acipenser stellatus* (Sevruga sturgeon) (Hosseini *et al.*, 2008). Iran is the most important exporter of sturgeon caviar in the world (Jelodar and Safari, 2006). In the conventional processing, caviar contains 4 – 8% salt, which is added after

sieving the eggs from the ovaries, with the better varieties generally containing less salt (Wang *et al.*, 2008). Although the caviar production is carried out with the highest hygiene level, it is still possible to observe microbial contaminations. Altug and Bayrak (2003) analyzed microbiologically the caviar samples from Russia and Iran. They detected coliforms, *Escherichia coli* and yeast in the samples and reported that the standard plate count and yeast counts were beyond the desired levels (6 log cfu/gr). Because high levels of salt and sodium borate (borax) used in caviar processing and the possibility of contamination during conventional process, it is necessary to replace them with other safe preservatives. *Zataria multiflora* Boiss. (Persian thyme), a native Iranian plant (Amin, 1991), is used traditionally as a flavoring agent (with a strong and pleasant aroma) (Mahboubi *et al.*, 2010; Zargari, 1990). The main components of *Z. multiflora* oil are phenolic compounds such as thymol and carvacrol (Mahboubi *et al.*, 2008; Mahboubi and Ghazian Bidgoli, 2010). Phenolic

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components have been known to possess antimicrobial activity and some are classified as Generally Recognized as Safe (GRAS) substances and therefore could be used to prevent the growth of native and contaminant bacteria (Sharififar *et al.*, 2007; Singh *et al.*, 2001). Basti *et al.* (2007) reported that the *Z. multiflora* Boiss. essential oil had inhibitory effect on *Salmonella typhimurium* and *Staphylococcus aureus* in brain heart infusion (BHI) broth.

Nisin, the natural antimicrobial peptide (produced by *Lactococcus lactis*) was discovered in 1928 (Hurst, 1967) and belongs to lantibiotics. It shows the antimicrobial activity against a broad spectrum of Gram-positive bacteria because of its cationic and hydrophobic peptides (Cleveland *et al.*, 2001; Driessen *et al.*, 1995). Gram-negative bacteria are generally resistant to nisin (Lee *et al.*, 2003). Nisin has been used in the food industry as a natural preservative in over 40 countries for more than 50 years. It has a GRAS application to inhibit *C. botulinum* spore outgrowth and toxin formation in certain pasteurized cheese spreads (Federal Register, 1988). The application of nisin, in food systems has been reviewed by different researchers (Abee *et al.*, 1995; Wessels *et al.*, 1998).

Organic acids and their salts are widely used in the food industry for their strong antimicrobial activity and are GRAS approved as food additives by E.C., FAO/WHO and FDA (González-Fandos and Dominguez, 2007; Surekha and Reddy, 2000). Sorbic acid and its salts initially thought to have only antimycotic activity, but they are now known to inhibit a wide range of bacteria, particularly aerobic catalase-positive organisms (Thomas, 2000). Potassium salt is commonly used because of its stability. In food, the effective concentration range (that does not affect sensory characteristics of food) is 0.05 – 0.3 g/100 g by weight (Yoshida *et al.*, 2010). The ability of potassium sorbate to inhibit *L. Monocytogenes*, *Salmonella* or *Staphylococcus aureus* has been studied in laboratory media and in some foods such as cheese, meat products or fish (González-Fandos and Dominguez, 2007; Moir and Eyles, 1992).

Nanotechnology has been recently introduced in the food packaging industry (Chaudhry *et al.*, 2008), because one of the many diverse characteristics existed in nanocomposites is bactericidal properties (Hu *et al.*, 2011). Indeed antimicrobially active packaging is based on metal nanocomposites, which are made by incorporating metal nanoparticles into polymer films (Chaudhry *et al.*, 2008; Emamifar *et al.*, 2010). Zinc oxide has strong antimicrobial effect on a broad spectrum of microorganisms (Jones *et al.*, 2008). Zinc oxide has attracted wide interest because of its good photocatalytic activity, high stability, antibacterial property and non-toxicity (Cohen, 2000; Li *et al.*, 2010). It was reported that

Zinc oxide exhibits antibacterial activity that increases with decreasing particle size (Yamamoto, 2001). However, the mechanism of toxicity is still only partially understood (Li *et al.*, 2008).

Several researches have been done on caviar with different subjects, such as determination of chemical composition (Duyar *et al.*, 2008), proteins (Al-Holy and Rasco, 2006), fatty acid and volatile compounds (Caprino *et al.*, 2008), organic and metal contaminants (Wang *et al.*, 2008), and microbiological aspects (Altug and Bayrak, 2003; Jelodar and Safari, 2006). Al-Holy *et al.* (2004) studied thermal inactivation of *Listeria innocua* in salmon caviar using conventional glass and novel aluminum thermal-death-time tubes. Also, they determined dielectric properties of salmon and sturgeon caviar for microwave pasteurization (Al-Holy *et al.*, 2005). However, little research has been published on the shelf life (Al-Holy and Ruiters, 2004) and application of new preservatives in caviar processing (Al-Holy and Lin, 2005).

The main objective of this study was to evaluate the capabilities of *Zataria multiflora* essential oil, nisin, potassium sorbate and LDPE film containing nano-ZnO as a new approach in caviar processing on the shelf life extension.

Materials and Methods

Preparation and storage of caviar samples To assess the effects of *Zataria multiflora* Boiss. essential oil, nisin, potassium sorbate and nanocomposite ZnO packaging on the shelf life of caviar, two levels of essential oil (0.03% and 0.06%W/W), nisin (9 and 18 mg/kg), potassium sorbate (500 and 1000 mg/kg) and the LDPE package containing 0.4 and 1% W/W nano-ZnO were applied at this study. Control samples contained no additives.

Raw caviar was obtained from Iran Shilat Company. Nisin containing 2.5% active nisin was purchased from SIGMA-ALDRICH Inc. (United Kingdom, N5764-5g). A stock solution of 1 mg nisin/ml was prepared by suspending 0.4 g of nisin in 10 mL of deionized water. The stock solution was then sterilized through a 0.2 µm-pore-size filter and stored at 4°C until use. *Zataria multiflora* Boiss. essential oil was purchased from Zardband Inc. (Iran) and potassium sorbate from SIGMA-ALDRICH Inc. (United Kingdom, 85520-250g). The LDPE package produced by Emamifar (2010) with oxygen permeability $2.5 - 3 \times 10^{-10}$ in cm³.cm/(cm².s.cmHg), was applied for nanocomposite packaging.

The preparation of caviar samples was done by a series of stages that include roe extraction, salting (3.5% NaCl and preservative addition), packaging and storage at 4°C. For caviar treatments with *Zataria*, nisin or potassium sorbate, the preservative was added to caviar at the salting stage and then, the caviar packaged in the 50 mL conical bottom sterile

propylene tubes with oxygen permeability 1.1×10^{-10} in $\text{cm}^3 \cdot \text{cm}/(\text{cm}^2 \cdot \text{s} \cdot \text{cmHg})$. For nanocomposite ZnO caviar treatment, the caviar after salting with 3.5% NaCl and without preservative, was packed in LDPE package containing nano-ZnO.

The samples were evaluated in duplicate for their microbiological and physicochemical characteristics immediately after packaging and after 1, 2, 3, 4 and 5 months of storage.

Chemical and microbial analysis Chemical composition (moisture, proteins, lipid, and ash contents) of caviar samples were analyzed according to standard protocols established by the Association of Official Analytical Chemists (934.01, 960.52, 948.22 and 942.05 AOAC Official Methods). The chemical composition of the raw caviar on a wet basis was 57.08% moisture, 19.96% proteins, 21.57% lipid, and 1.39% ash.

To count the total aerobic bacteria in the caviar samples, 10 g of sample and 90ml peptone-water were homogenized and further 10-fold dilutions were made. One hundred microliters of the diluent were plated onto Plate Count Agar with incubation at 37°C for 24 – 48 h to enumerate total aerobic bacteria (Shin and Rasco, 2007).

To count the mold and yeast in caviar samples, 10 g of sample and 90 mL peptone-water were homogenized and further 10-fold dilutions were made. After incubation for 5 days in Dichloran Rose Bengal Chloramphenicol Agar at 25°C, molds and yeasts colonies were counted separately (Altug and Bayrak, 2003; Pit and Hocking, 1997).

Color measurement Color was measured using a digital imaging method applying a combination of a digital camera (Sony, Japan), a computer, and graphic software. A Petri dish containing 5 g caviar was placed into the lighting system that consisted of two CIE source D65 lamps 45.0 cm long, mounted on the two sides of a frame installed on either side of the Petri dish, 30.5 cm above and at an angle of 45° to the caviar sample plane. Images of the caviar were taken and saved using the digital camera with its lens facing downwards towards the caviar. Finally, the color was analyzed using the Photoshop software to obtain *L*, *a*, and *b* values and the total color difference ($\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$) was determined (Yam and Papadakis, 2004).

Zinc ion release measurement The release of zinc ion into tow caviar samples was determined by a graphic furnace atomic absorption spectrometer (PG990) operated at 213.9 nm wavelengths based on the standard method (A.O.A.C. 974.27, 2002) slightly modified by Bings *et al.* (2006).

Statistical analysis The experimental design was a split-plot (8 × 6) arranged in randomized completely block design with two replications. Analysis of variance was carried out using the SAS statistical software release 6.12 (SAS Institute, Cray, NC). Plate count and molds and yeasts data

were converted to logarithms prior to their statistical treatment. Duncan's multiple range test (DMRT) was performed on the treatments means data. Significant differences among the data were represented as $p < 0.05$.

Results and Discussion

Microbiological analysis The mean initial population of the caviar samples immediately after treatment by potassium sorbate (500 and 1000 mg/kg), *Zataria multiflora* essential oil (0.03% and 0.06%(W/W)), nisin (9 and 18 mg/kg), packaging in the LDPE package containing 0.4 and 1%(W/W) nano-ZnO, and/or control sample was between $1.18\text{E}+03$ to $1.31\text{E}+03$ cfu/g for total aerobic bacteria and $1.10\text{E}+03$ to $1.25\text{E}+03$ cfu/g for yeasts and molds.

Figures 1 and 2 compare the effect of two different concentrations of applied treatments on the total count and fungi population (log cfu/g) of caviar samples during 150 days of storage at 4°C.

In all the treatments, it can be seen that the microbial population increased with increasing storage time to 60 days and then decreased during remaining storage time. Also, it can be indicated that the antimicrobial activity of the treatments increased by increasing the concentration of applied treatments (Table 1 and Figures 1 and 2). This result is in common with the results of several studies (Al-Holy and Lin, 2005; Fazeli *et al.*, 2007; González-Fandos and Dominguez, 2007; Emamifar, *et al.*, 2010)).

Total aerobic bacteria and fungi population changes influenced by high concentration of the applied treatments are presented in Figures 3 and 4, respectively. As can be indicated, the LDPE package containing 1%(W/W) nano-ZnO has a slightly slower ascending phase in comparison with the other treatments that has continued to 60 days of storage (Fig. 3). As shown in Table 1, there were significant differences between all treatments with different concentrations on the total aerobic bacteria population, but minimal microbial growth was observed in caviar samples packaged in LDPE film containing 1%(W/W) nano-ZnO. After that, 1000 mg/kg potassium sorbate and LDPE package containing 0.4%(W/W) nano-ZnO had more significant effect for decreasing total count in the caviar samples. It seems that packaging in LDPE film containing 1%(W/W) nano-ZnO has more capability to control total aerobic bacteria than other treatments (Figure 1). This is contrary to the result reported by Emamifar *et al.* (2011). They reported the higher total aerobic bacteria population for 1%(W/W) nano-ZnO than lower concentrations. Antimicrobial effects of nano-ZnO may be caused by 3 mechanisms: 1) induction of oxidative stress (Adams *et al.*, 2006); 2) membrane disorganization (Brayner *et al.*, 2006); 3) release of Zn ions and binding to the membrane of microor-

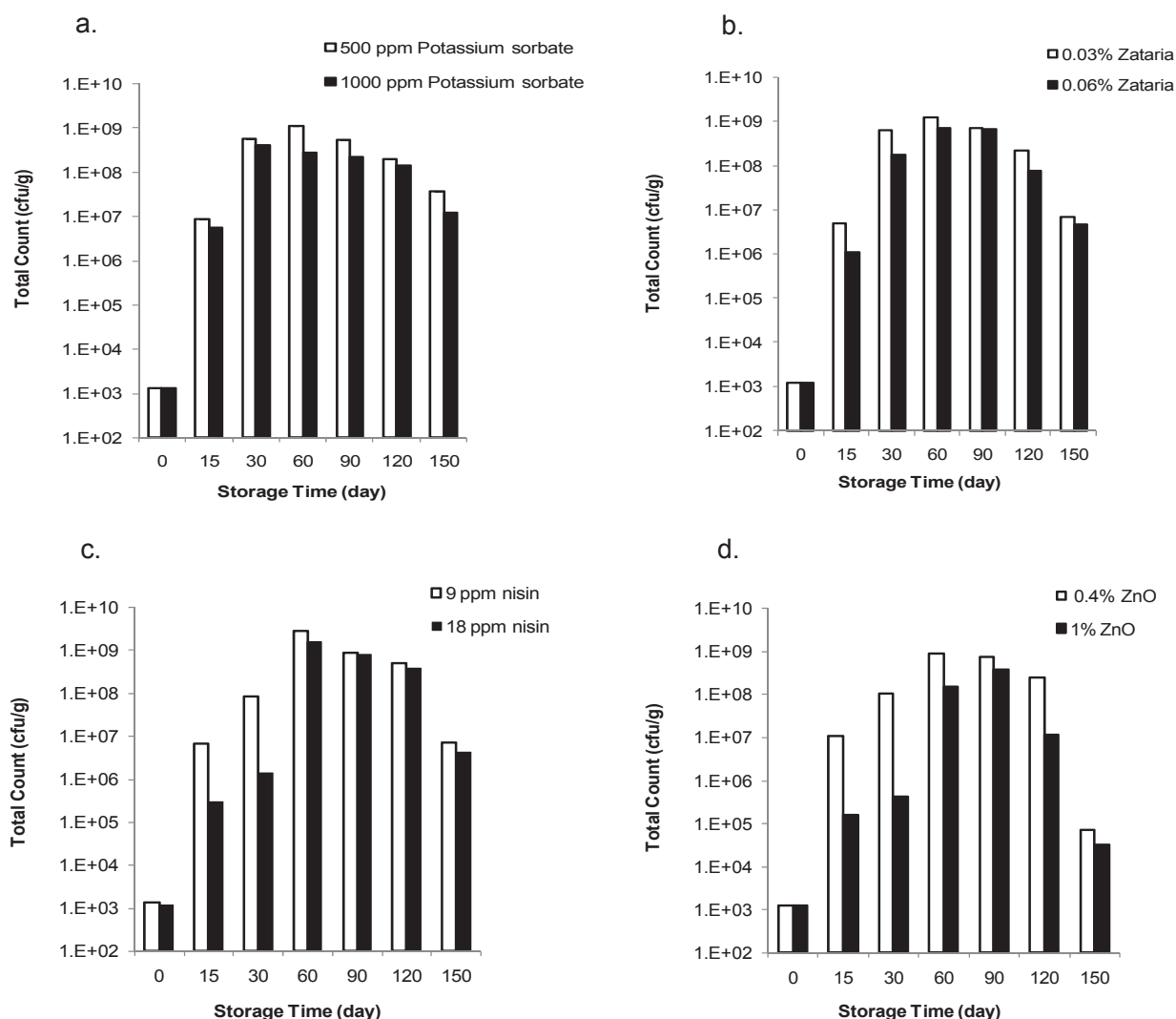


Fig. 1. Effect of two different concentrations of applied treatments: a) potassium sorbate, b) *Zataria multiflora* essential oil, c) nisin, and d) packaging containing nano-ZnO on total count (log cfu/g) of caviar samples during 150 days of storage at 4°C.

ganisms (Gajjar *et al.*, 2009).

Figure 5 shows the evolution of Zn ion quantity in caviar packed in LDPE film containing nano-ZnO during storage for 60 days at 4°C. As can be seen, there is a considerable rate of Zn migration from package to caviar, but as Zinc is classified as a GRAS compound for food applications, its final concentration is still in the acceptable level for food consumptions. It seems that the Zn migration rate from packaging to caviar increases after 40 days. The initial amount of Zn in raw caviar was 8.126 mg/kg that approximately is in compromise with results of Sadeghi Rad *et al.* (2003).

Table 1 shows the significant differences between all treatments on the molds and yeasts count. Among them, 0.06%(W/W) *Zataria multiflora* essential oil showed the most significant effect in the caviar samples. After that, LDPE package containing 1 and 0.4%(W/W) nano-ZnO and 0.03%(W/W) *Zataria multiflora* essential oil had more significant effect for decreasing fungi count (Table 1 and Fig. 4).

Mahboubi *et al.* (2008) reported the high antifungal activity of *Zataria multiflora* essential oil, while Sawai and Yoshikawa (2004) have concluded that ZnO powder has a poor antimicrobial effect on *Saccharomyces cerevisiae* and other yeasts and molds compared with bacteria. In agreement with these studies, it appears that antifungal activity of *Zataria multiflora* essential oil is significantly ($p < 0.05$) higher than nanocomposites containing nano-ZnO. Treatment with 18 mg/kg nisin showed the minimal capability to decrease the population of total aerobic bacteria and fungi. Misaghi and Akhondzadeh Basti (2007) reported that *Zataria multiflora* essential oil in comparison with nisin had the better effect on *Bacillus cereus* ATCC 11778.

The shelf life of caviar is defined as the time to reach a microbial population of 6 log cfu/gr (ISIRI, 186). The mean population of total aerobic bacteria increased up to 6 log cfu/gr after 30 days of storage in all the treatments except for the LDPE +1%(W/W) nano-ZnO. The study revealed that to

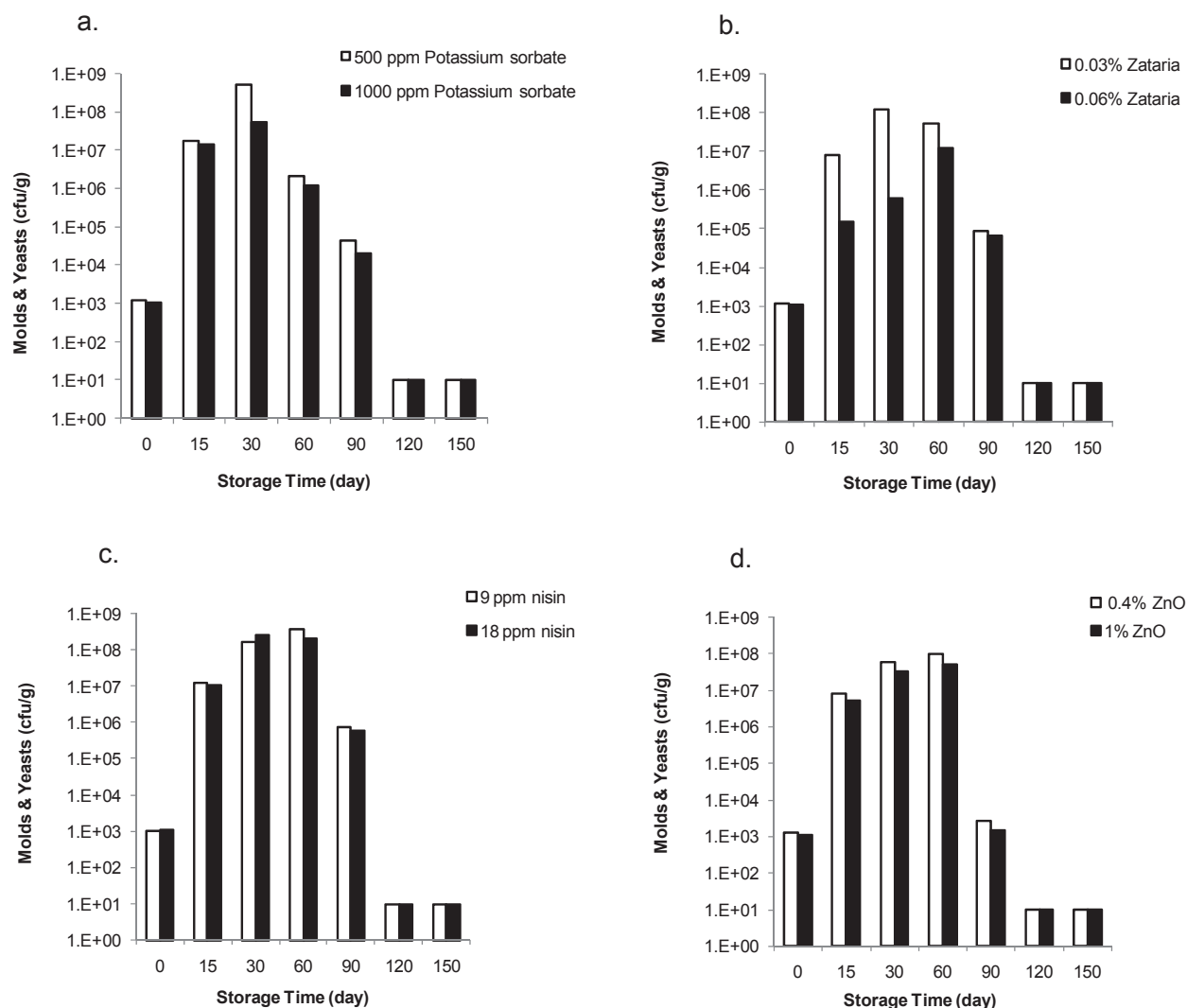


Fig. 2. Effect of two different concentrations of applied treatments: a) potassium sorbate, b) *Zataria multiflora* essential oil, c) nisin, and d) packaging containing nano-ZnO on the molds and yeasts count (log cfu/g) of caviar samples during 150 days of storage at 4°C.

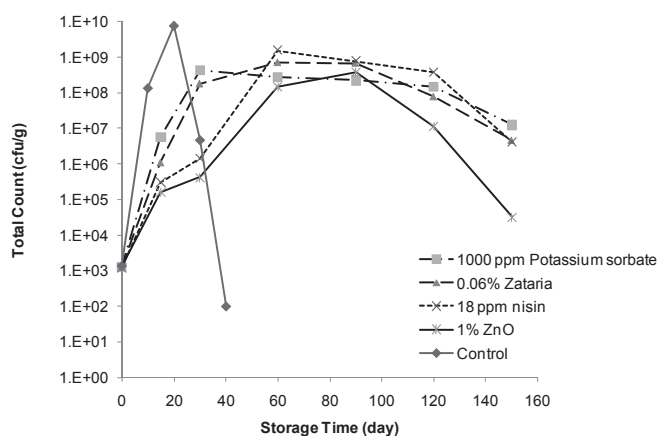


Fig. 3. Total count (log cfu/g) of the caviar samples treated with potassium sorbate, *Zataria multiflora* essential oil, nisin, and packaging containing nano-ZnO during 150 days of storage at 4°C.

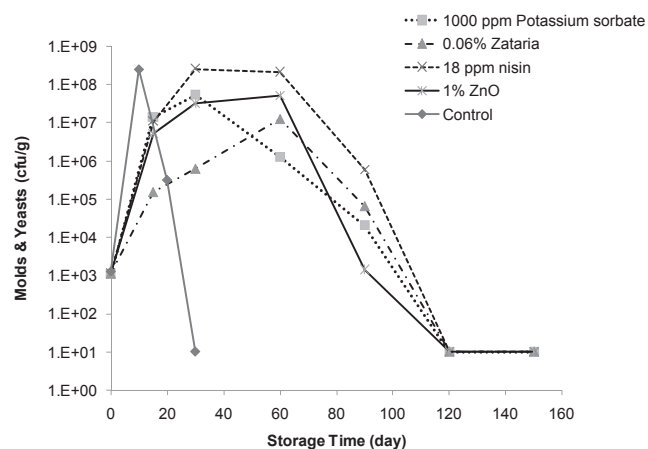


Fig. 4. Molds & yeasts count (log cfu/g) of the caviar samples treated with potassium sorbate, *Zataria multiflora* essential oil, nisin, and packaging containing nano-ZnO during 150 days of storage at 4°C.

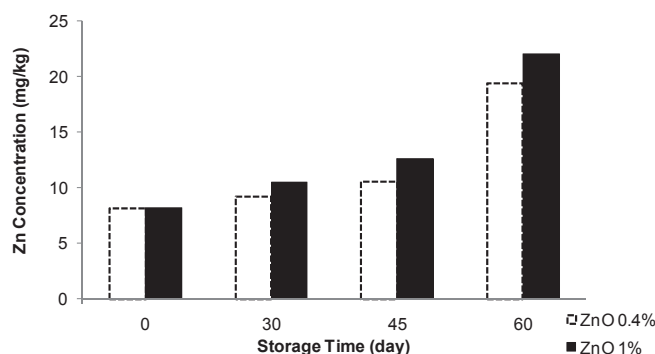


Fig. 5. Evolution of the Zn ion quantity of caviar packed in package containing Nano-ZnO during storage at 4°C.

prolong shelf life of caviar, other treatments or combination of these treatments are necessary.

Color analysis Caviar color is mainly due to the presence of carotenoid pigments and is influenced by caviar type, processing treatments and storage conditions (Keyvan, 2005). Figures 6, 7, 8 and 9 show the evolution of '*L*', '*a*', '*b*' and ΔE values of caviar samples treated by different treatments during storage at 4°C, respectively. In color analysis, '*L*' value represents the lightness, '*a*' value greenness to redness and '*b*' value blueness to yellowness of samples; while ΔE shows total color difference compared to initial color (Yam and Papadakis, 2004).

As can be seen in Figure 6, the '*L*' value decreases with increasing the storage time. Despite significant differences between the effects of some treatments on the lightness, the

distinction between them is not very clear (Table 1). In contrast, the '*a*' values of caviar samples increase during storage (from $a = -13$ to $a = 0$) (Fig. 7).

It indicates that the greenness of caviar color has been decreased and the color has been closed to gray. As shown in Table 1, there were significant differences between all treatments with different levels on "*a*" values except 500 and 1000 mg/kg potassium sorbate, but minimal changes were observed in caviar samples packaged in LDPE film containing 1 and 4% (W/W) nano-ZnO, respectively. It can be seen that the '*b*' values of caviar samples decrease during storage (Fig. 8). It indicates that the blueness of caviar samples has been decreased and their grayness has been increased. In correlation to the '*a*' values, there were significant differences between all treatments on "*b*" values except 9 and 18 mg/kg nisin and minimal changes were observed in caviar samples packaged in LDPE film containing ZnO (Table 1).

As a result of '*L*', '*a*', '*b*' values changes, ΔE change is also predictable. Figure 9 shows the changes in total color differences for all the treatments during storage compared to initial caviar color. Similar to the '*L*', '*a*', '*b*' values of caviar samples, the ΔE changes had a high slope in the beginning and a stationary state at the end of storage. Statistical results show significant differences between all of the treatments except 500 mg/kg potassium sorbate and 18 mg/kg nisin (Table 1).

It can be concluded that the storage time was an important factor influencing color during caviar storage and minimal color changes were observed in caviar samples packaged in LDPE film containing nano-ZnO.

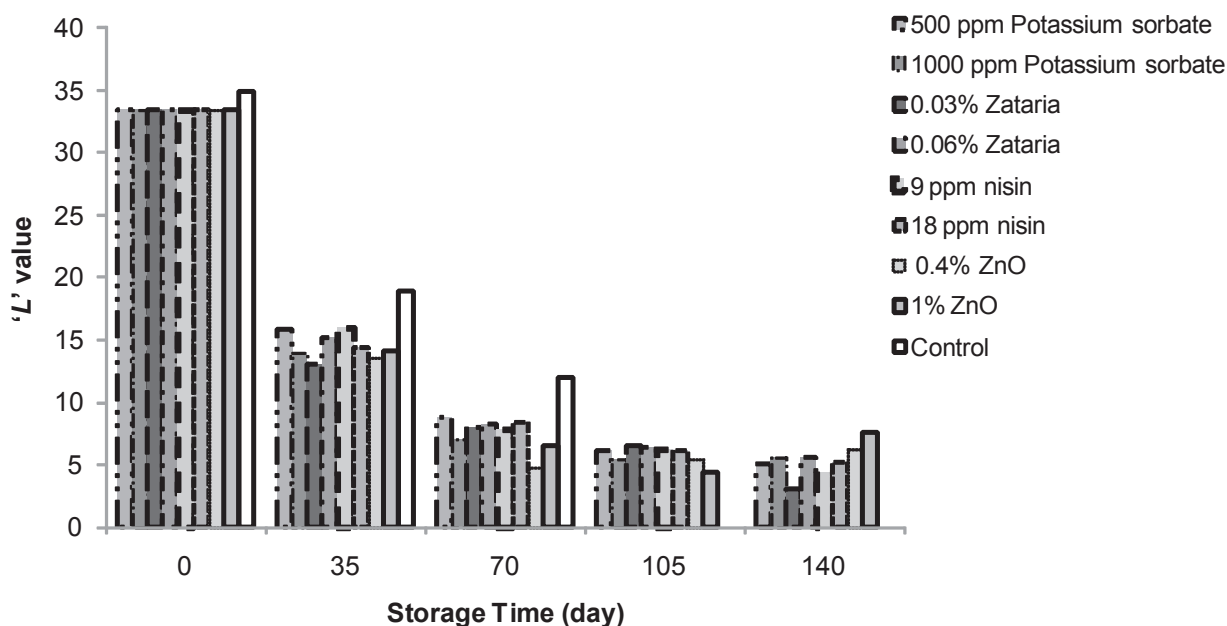


Fig. 6. '*L*' values in caviar samples treated with potassium sorbate, *Zataria multiflora* Boiss. essential oil, nisin, and packaging containing nano-ZnO during storage at 4°C.

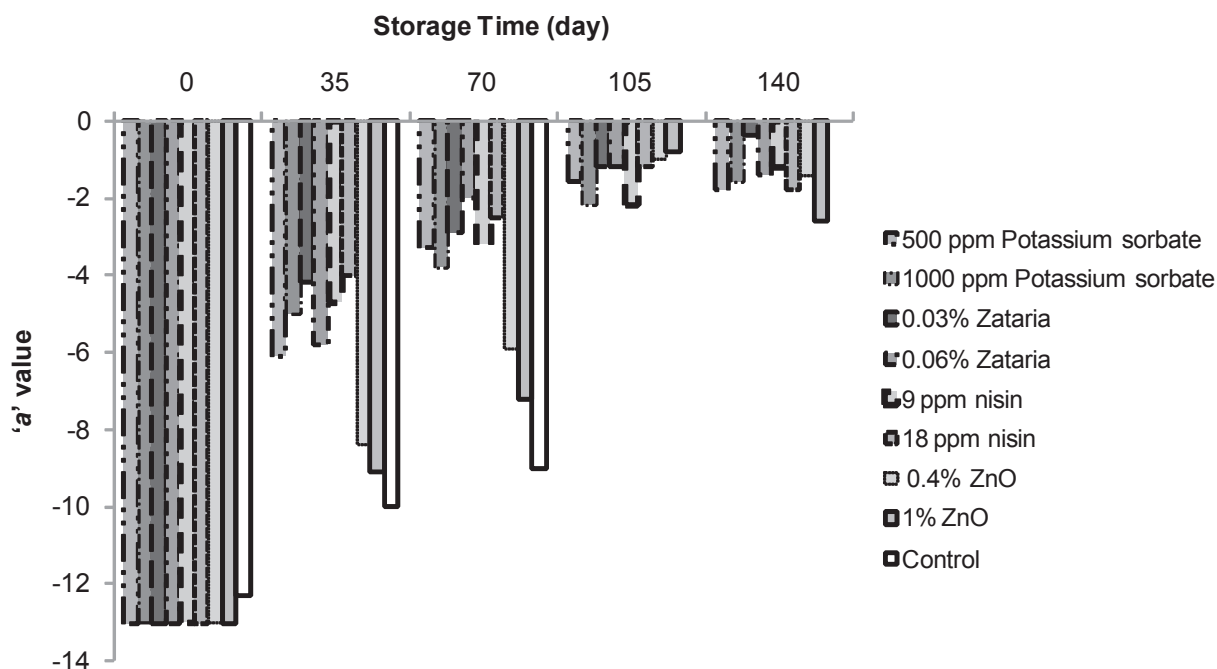


Fig. 7. 'a' values in caviar samples treated with potassium sorbate, *Zataria multiflora* Boiss. essential oil, nisin, and packaging containing nano-ZnO during storage at 4°C.

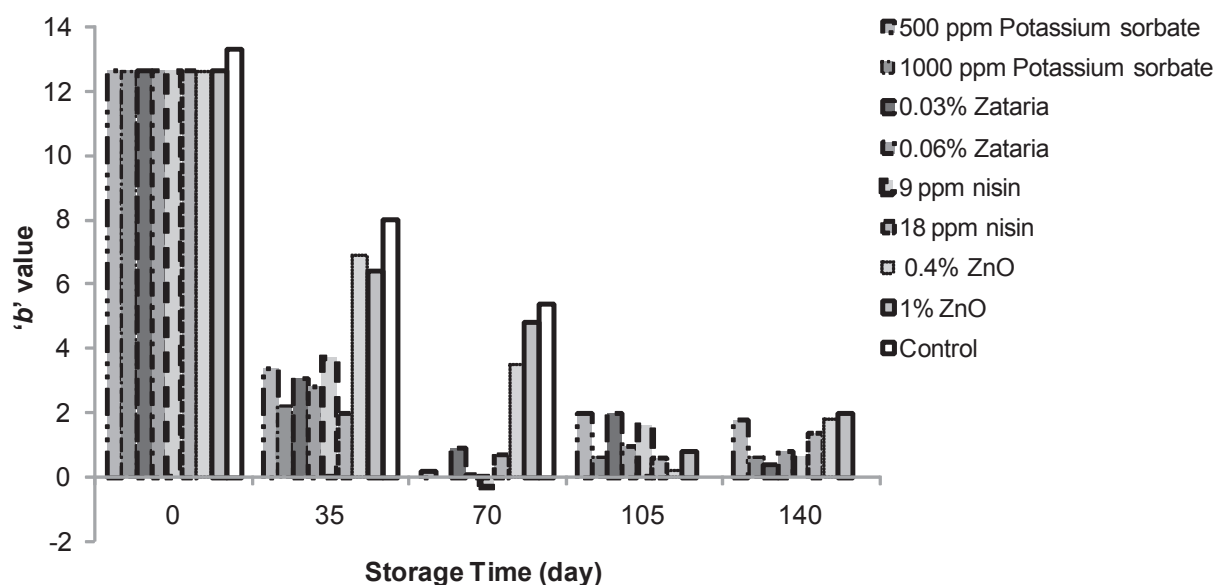


Fig. 8. 'b' values in caviar samples treated with potassium sorbate, *Zataria multiflora* Boiss. essential oil, nisin, and packaging containing nano-ZnO during storage at 4°C.

Conclusion

In this research, the effect of different treatments by *Zataria multiflora* essential oil, nisin, potassium sorbate and LDPE packaging containing nano-ZnO as a new approach in the caviar processing was evaluated on its shelf life. Microbial counts (total aerobic bacteria and molds & yeasts) and color of caviar samples, and Zinc release from package to caviar were determined during storage. The caviar packaging in LDPE film containing 1%(W/W) nano-ZnO had more

capability to control total aerobic bacteria than other treatments. While in the case of molds and yeasts, 0.06%(W/W) *Zataria multiflora* essential oil. Minimal color changes were observed in caviar samples packaged in LDPE film containing nano-ZnO. The study revealed that to prolong shelf life of caviar, other treatments or combination of these treatments are necessary. Work will continue on the shelf life extension of the caviar by combination of the different hurdles.

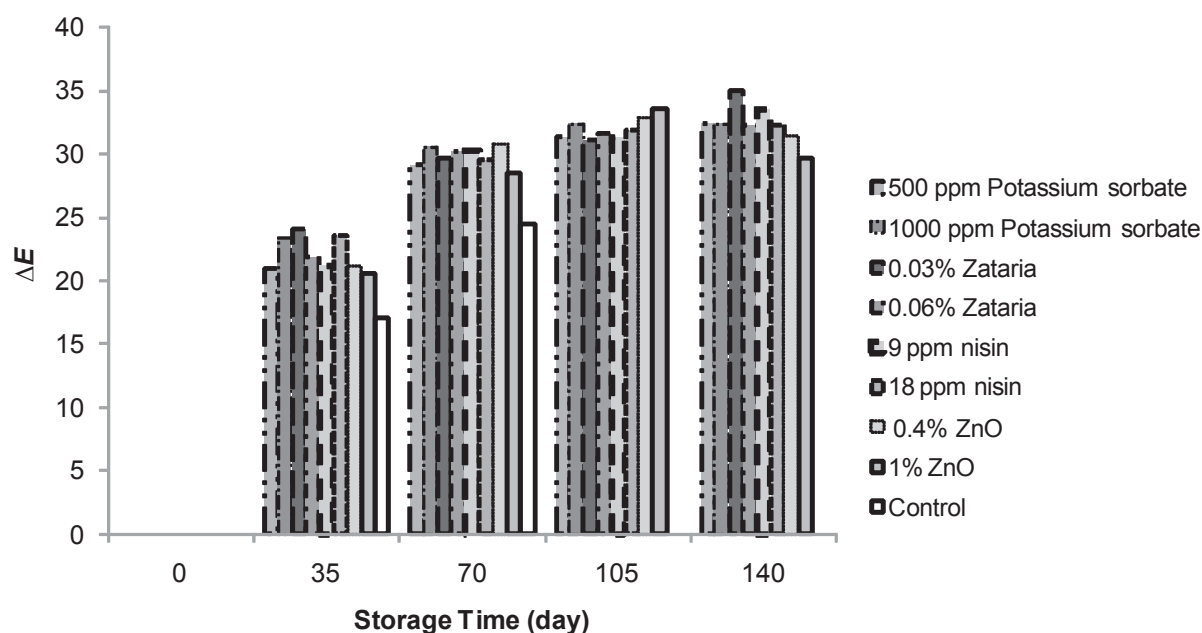


Fig. 9. ΔE values in caviar samples treated with potassium sorbate, *Zataria multiflora* Boiss. essential oil, nisin, and packaging containing nano-ZnO during storage at 4°C.

Table 1. Effect of treatment by potassium sorbate, *Zataria multiflora* Boiss. essential oil, nisin, and packaging containing nano-ZnO (Mean value) on the fungi and total aerobic bacteria population, standard *L*, *a* and *b* values, and total color differences (ΔE) of caviar during storage at 4°C.

Source of changes	Mean value				Total count	Molds & yeasts
	<i>L</i>	<i>a</i>	<i>b</i>	ΔE		
Treatment						
Potassium sorbate (500 ppm)	13.752 ^a	−5.140 ^c	3.959 ^c	20.011 ^d	3.97E+08 ^c	8.59E+07 ^b
Potassium sorbate (1000 ppm)	13.200 ^b	−5.144 ^c	3.223 ^g	19.387 ^f	1.91E+08 ^g	5.71E+07 ^d
<i>Zataria multiflora</i> (0.03%)	12.930 ^c	−4.251 ^a	3.822 ^d	19.627 ^c	3.82E+08 ^d	4.55E+07 ^c
<i>Zataria multiflora</i> (0.06%)	13.830 ^a	−4.689 ^c	3.657 ^c	19.310 ^g	3.51E+08 ^c	1.05E+07 ^h
Nisin (9 ppm)	13.630 ^a	−4.871 ^d	3.477 ^f	20.487 ^c	6.82E+08 ^a	9.25E+07 ^a
Nisin (18 ppm)	13.590 ^a	−4.474 ^b	3.475 ^f	20.014 ^d	4.62E+08 ^b	7.76E+07 ^c
ZnO nanocomposite (0.4%)	12.620 ^d	−5.944 ^f	5.026 ^b	23.281 ^a	3.25E+08 ^f	2.61E+07 ^f
ZnO nanocomposite (1%)	13.160 ^b	−6.547 ^g	5.365 ^a	22.483 ^b	8.85E+07 ^h	1.40E+07 ^g

^{a-h} Values in the same column not sharing the same superscript letter are significantly different ($p < 0.05$).

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