ORIGINAL ARTICLE

Veterinary Research Forum. 2022; 13 (4) 577 - 585
doi: 10.30466/vrf.2021.526224.3150
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Effect of chia seed mucilage coating containing zinc oxide nanoparticles on shelf life of chicken fillet

Fereshteh Akhavan Seiasipour Foumani[1], Mehdi Sharifi Soltani[2], Shahin Zomorodi[3]*, Sara Jafarian[1], Asghar Khosrowshahi Asl[4]

[1] Department of Food Science and Technology, Agriculture Faculty, Islamic Azad University, Nour Branch, Nour, Iran; [2] Department of Veterinary, Agriculture Faculty, Islamic Azad University, Chalous Branch, Chalous, Iran; [3] Department of Engineering Research, West Azerbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Urmia, Iran; [4] Department of Food Science and Technology, Agriculture Faculty, Urmia University, Urmia, Iran.

Article Info

Article history:

Received: 06 March 2021
Accepted: 15 June 2021
Available online: 15 December 2022

Keywords:

Chicken fillet
Chia seed mucilage
Shelf life
Zinc oxide nanoparticle

Abstract

Chicken fillet is a suitable medium for growth and activity of different types of microorganisms. The pH and nutrients content of fillets are the most important factors in their microbial spoilage and degradation during cold storage at the retail level. In this regard, the uses of edible coatings containing antimicrobial and antioxidant compounds are effective approaches to maintain the quality of fillets. In this study the inhibitory effect of edible coating materials based on chia seed mucilage containing 0.00%, 0.25% and 0.50% zinc oxide nanoparticles (ZnO-NPs) on microbial growth and chemical spoilage as well as enhancing shelf life of chicken fillets during refrigerated storage for 20 days was investigated. The results of X-Ray diffraction confirmed the dispersion of ZnO-NPs on the chia seed mucilage matrix. Also, the number of total aerobic mesophilic and psychrophilic bacteria, coliforms and lactic acid bacteria, and the pH, total volatile nitrogen, peroxide and free fatty acids indexes in the control fillets were significantly increased compared to the fillets coated with chia seed mucilage during storage. While, in the samples coated with chia mucilage containing ZnO-NPs the number of the above-mentioned bacteria decreased in the first stage and then significantly increased during storing. Based on our findings, the shelf life of fillets could be increased at least 20 days by coating them with the chia seed mucilage containing ZnO-NPs.

Introduction

Chicken is known as one of the most consumed food in the world due to its properties such as low price, low fat and cholesterol, easy to cook and digest compared to other meats. However, meat is an ideal medium for growing many microorganisms due to its high moisture content, optimal pH value, richness in nitrogenous components, minerals, and growth factors.[1,2] Since spoilage of fresh chicken is an economic burden to the producers as well as a threat to consumer health, the preservation of the quality and increasing the shelf life of chicken is one of the goals of producers. Packaging as well as edible coatings and film*s* have been recently considered to substitute chemical coating for enhancing nutrients and shelf life.[1]

In this respect, chia seed mucilage can be a new source for preparing of edible coatings and films.[3] Chia (*Salvia hispanica* L.*)*, is an annual herbaceous crop of the *Lamiaceae* family. Chia seed produces a highly clear and viscous solution when is immersed in water leading to adhere the crust of seeds. Chia seed mucilage mainly contains xylose, glucose and methyl glucuronic acid and a branched polysaccharide with high molecular weight. The chia seed contains approximately 5.00 - 6.00% of mucilage that can absorb 27 times more water than their weight. This mucilage is widely used in the food industry as a foam stabilizer, suspending agent, emulsifier, binder or adhesive.[3] However, their application is limited due to the fragility and poor barrier to gas exchange of biodegradable films. These limitations can be eliminated by using nanocomposite containing zinc oxide nanoparticles (ZnO-NPs). Since the ZnO-NPs are cheaper and more biocompatible and safer than other nanoparticles, they are widely used to improve the properties of the used polymers in food packaging process.[4]

***Correspondence:**

Shahin Zomorodi. PhD
Department of Engineering Research, West Azerbaijan Agricultural and Natural Resources Research and Education Center, AREEO, Urmia, Iran
E-mail: s.zomorodi@areeo.ac.ir

Based on our knowledge, there was no study on the synthesized nanocomposite nano-biofilm from chia seed mucilage containing ZnO-NPs. Therefore, in this piece of work, the effects of coating of chia seed mucilage containing ZnO-NPs on microbial and physicochemical properties of chicken fillets were investigated during storage in retailers.

Materials and Methods

The chia seed mucilage extraction. The chia seeds (Kian Food Company, Tehran, Iran) were mixed with deionized water in a ratio of 1: 40 (seed: water) at 30.00 ˚C and the pH was adjusted to 8.00 using 0.20 M NaOH (Merck, Darmstadt, Germany). The solution was then stirred using a magnetic stirrer (Pole Ideal Pars Co., Tehran, Iran) for 3 hr to separate the mucilage. The mixture of chia seed and mucilage was dried at 50.00 ˚C for overnight. The seed mucilage was separated by sieving on a 40-mesh screen.[5]

Film and coating solutions preparation. A 1.50 g of chia seed mucilage was added to 50.00 mL distilled water and homogenized using a magnetic stirrer for 3 hr at 25.00 ˚C. The pH of solution was adjusted to 8.00 using 0.10 N NaOH. The solution was then stirred at 120 rpm within a water bath at 80.00 ˚C for 30 min. The ZnO-NPs (10 - 30 nm; > 99.00% pure, Inc., Houston, USA) at two levels of 0.25% and 0.50% were separately dissolved in 50.00 mL of 1.00% acetic acid (Merck, Darmstadt, Germany). In order to have a better distribution of nanoparticles, the solution was subjected to ultrasound using a sonicator ultrasound bath (Sonica, Catania, Italy) at 40.00 kHz frequency for 30 min. The nanoparticles suspension was then added to the solution containing coating and was sonicated again in the ultrasound water bath at 40.00 kHz frequency for 15 min. Glycerol (Merck) 40.00% (w/w) of chia seed mucilage was subsequently added to the mixture as a plasticizer and gently mixed for 60 sec.[6] To produce film, 250 g of the film formation solution was poured on a clean vessels polytetrafluoroethylene with 25.00 cm in diameter and dried at 50.00% relative humidity and 50.00 ˚C. A film was produced similarly without adding of NPs as the control. The dried film was then removed from surface of plate and stored at 25.00 ˚C and 50.00% relative humidity.[6]

Coating of chicken fillet. The chicken fillets were obtained from Urum Chakavak Company (Urmia, Iran). The samples were transferred to the laboratory in hygienic conditions. They were then sliced in 100 g portions, washed, and divided into eight equal groups which containing of 10 slices. The treatments included: C: control (without using any coating), Ch: the treatments coated with chia mucilage solution, Zn 0.25 and Zn 0.50 the treatments coated with chia mucilage solution containing 0.25% and 0.50% ZnO-NPs respectively. The fillets were immersed in the prepared solutions for 20 min. The coated samples were then dried at 24.00 ˚C and 50.00% relative humidity for 5 hr. The fillets were placed in the sterile zipper bags and stored at 4.00 ˚C for 20 days. The sampling was carried out at days 1, 8, 12, 16, and 20 for analysis of microbial and physic-chemical experiments.

X-ray diffraction (XRD) analysis. The structure of the ZnO-NP was studied by X-ray diffraction (XRD-6000, Shimadzu, Tokyo, Japan) with Cu K_α radiation (λ = 0.1541 nm) at 40.00 kV and an electricity current of 30.00 mA. The samples were scanned at a diffraction angle of 0.05° (2θ) with a speed of 10 sec at room temperature.[7]

Microbiological analysis of coated chicken fillet samples. To prepare the dilution, 10.00 g of each homogenized sample was transferred into sterile stomacher bags under aseptic conditions and 90.00 mL of 0.10% sterile peptone water (Sigma-Aldrich, St. Louis, USA) was added. It was then homogenized for 2 min by Stomacher (Seward Stomacher, West Sussex, UK). A series of dilutions was prepared by adding 1.00 mL of each concentration to 9.00 mL of 0.10% peptone water. Lactic acid bacteria (LAB), were studied using pour plate method in MRS agar (de Man, Rogosa, Sharpe; Merck) and incubated for 48 hr at 37.00 ˚C under anaerobic conditions. Total aerobic mesophilic bacteria (TAMB) and total aerobic psychotropic bacteria (TAPB) were studied using pour plate method in plate count agar (PCA) (Merck) and incubated under aerobic conditions at 30.00 ˚C for 48 hr and at 7.00 ˚C for 10 days respectively. Total coliform bacteria (TCB) were studied bilayer using violet red bile agar (Merck) by pour plate method under aerobic conditions at 37.00 ˚C for 48 hr. The results were calculated as a logarithm of colony forming units per g (log CFU g^{-1}) of sample.[8]

Physicochemical analysis of coated chicken fillet samples. To determine the pH, 10.00 g of minced and homogenized chicken fillet sample was mixed with 90.00 mL of distilled water and stirred for 30 min and then filtered. The pH was measured at room temperature using a digital pH meter (model 691; Metrohm, Zurich, Swiss).[9] Total volatile basic nitrogen (TVB-N) value was also determined using a Kjeldahl apparatus (Kimia Tajhiz Gharb Co., Tehran, Iran). 5.00 g of the chicken fillet sample mixed with 1.00 g of MgO (Sigma-Aldrich), 250 mL of distilled water were transferred into the distillation flask. The distillate was absorbed by 40.00 mL of 2.00% boric acid solution containing methyl red (Merck) indicator for 20 min and then was tittered with 0.10 N H_2SO_4 (Merck) solution. The TVB-N value was then calculated.[9] The oil in the chicken fillet sample was first extracted. For this purpose, the fillet pieces were minced twice to obtain uniform dough. Then the chloroform-(Neutron Pharma-chemical Co., Tehran, Iran) methanol (Sigma-Aldrich) solvent and water (2:1:1

ratio) were added to the chicken fillet paste and homogenized with homogenizer (IKA, Staufen, Germany) for 1 min and the chloroform layer was separated by a glass decanter. The oil in the solvent was evaporated by vacuum rotary evaporator (Strike300, Wiggens GmbH, Baden-Württemberg, Germany) under at 40.00 ˚C. To measure the peroxide value (PV), about 1.00 g of fillet oil extracted was weighed in the Erlenmeyer flask (Pole Ideal Pars Co., Tehran, Iran), and 20.00 mL of a solution mix of acetic acid (Neutron pharmachemical Co.) and chloroform (1: 1) was added to it, and thoroughly mixed to dissolve the fat in the solvent. Then 0.50 mL of saturated potassium iodide was added and mixed. After one min, 30.00 mL of distilled water was added to Erlenmeyer and titrated with 0.10 N sodium hyposulfite (Merck) solutions in the presence of starch reagent. Then the PV value was calculated in mEq 100 g^{-1} of fat. To measure the free fatty acid (FFA), to 25.00 mL of an equal mixture of diethyl ether (Merck) and ethanol (Hamon Teb Co., Tehran, Iran) neutralized was added 1.00 g of fillet oil extracted and thoroughly mixed to dissolve the fat in the solvent and titrated with 0.10 N NaOH in the presence of a phenolphthalein (Merck) reagent. Then the FFA was calculated.

Statistical analysis. The results were analyzed using a factorial statistical design with two factors in three replications. The data were subjected to analysis of variance (ANOVA) using the MSTAT-C and Microsoft Excel 2016 (Redmond, Washington, USA) software. To compare means Duncan's multiple range test was used at 95.00% confidence interval level. The data were represented as the mean ± standard deviation (SD).

Results

X-Ray diffraction. The XRD patterns of Ch films and nanocomposite films containing ZnO-NPs are shown in Figure 1. As shown in Figure 1A, in the diffraction of pure chia seed mucilage film, a crystalline peak was observed at the angle θ = 14.77. As can be seen from Figures 1 (B and C), the important peaks of these shapes in nanofilms are in the areas of 31.52, 34.97, 36.47, 37.78, 47.46 and 57.77 degrees.

Microbial count in the chicken fillet. The changes in the number of bacteria are presented in Figure 2. According to Figure 2, the numbers of TAMB, TAPB, total TCB and LAB were 4.65, 4.20, 2.69, and 2.42 log CFU g^{-1} respectively. During refrigerated storage, the number of bacteria in the control and chia mucilage coated significantly increased ($p < 0.05$). However, in the chia mucilage coated containing ZnO-NPs, the number of these bacteria firstly decreased or remained constant and then increased during storage ($p < 0.05$). At the end of storage period the lowest count of microorganisms was seen in the treatments containing 5.00% ZnO-NPs. According to Figure 2A, the average count of TAMB in the treatment containing 0.25% and 50.00% ZnO-NPs reached to 5.67 and 4.53 log CFU g^{-1}, respectively, at the end of storage period that was less than 6.00 log CFU g^{-1} indicating freshness of meat. The average of TAPB was also less than acceptable amount in these groups (Fig. 2B).

Effect of coating on pH of chicken fillet. As shown in Table 1, the pH value in the control and coated samples increased during storage, so that the highest level of pH belonged to the samples coated with mucilage. However, the pH decreased in mucilage-coated sample containing 0.25% and 0.50% ZnO-NPs up to day 12 and 16, respectively, and then increased. On days 8 and 16 of storage, a significant difference was observed amongst the samples containing ZnO-NPs in terms of pH value ($p < 0.05$). However, there was no significant difference amongst treated groups in terms of pH at the end of storage time.

Effect of coating on peroxide value (PV) of chicken fillet. As Table 1 shows, the PV of chicken fillet was not detectable at the first day of storage indicating freshness chicken fillet used in the present study. During storage period PV was significantly increased in the control and coated with mucilage alone. While, in the samples coated with ZnO-NPs the PV of chicken fillet was not detectable at days 4 and 8 of storage.

Effect of coating on TVB-N in chicken fillet. As shown in Figure 3A, TVB-N level within the chicken fillet in the control and coated with chia seed mucilage increased during storage. This was slower at the beginning but increased more at the end of the storage period that is due

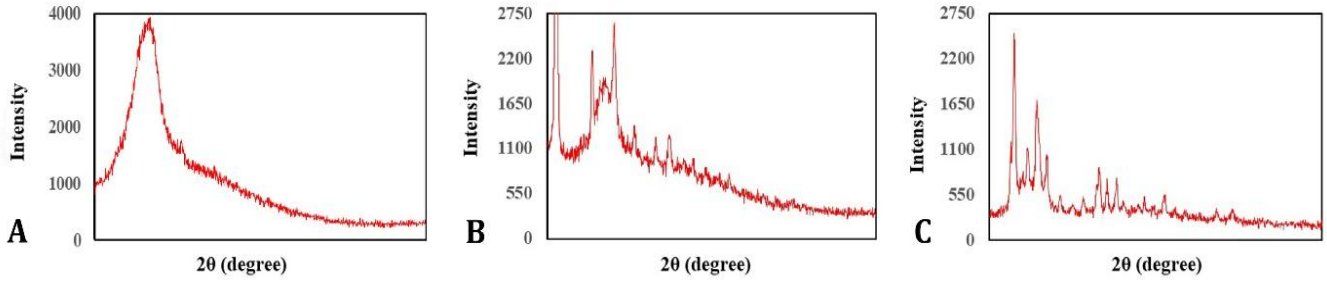

Fig. 1. Profile X-Ray diffraction (XRD) of films. **A)** Chia seed mucilage film (Ch), **B)** Chia seed mucilage film with 0.25% ZnO-NPs (ZnO 0.25) and **C)** Chia seed mucilage film with 0.50% ZnO-NPs (ZnO 0.50).

to TVB-N-producing bacteria. In the early days of storage, the bacterial population was in the basal phase and increased slowly and then increased rapidly. According to Figure 3A, this index decreased in the samples containing ZnO-NPs up to 4 day and then increased. However, this process was slower than those of other treated groups.

Effect of coating on FFA in chicken fillet. The initial value of FFA index in chicken fillet samples was about 0.05% (Fig. 3B), which indicates the freshness of the fillets. During storage, FFA content increased in the control and coated by chia seed mucilage treatments, which the rate of this increment was higher in the control samples.

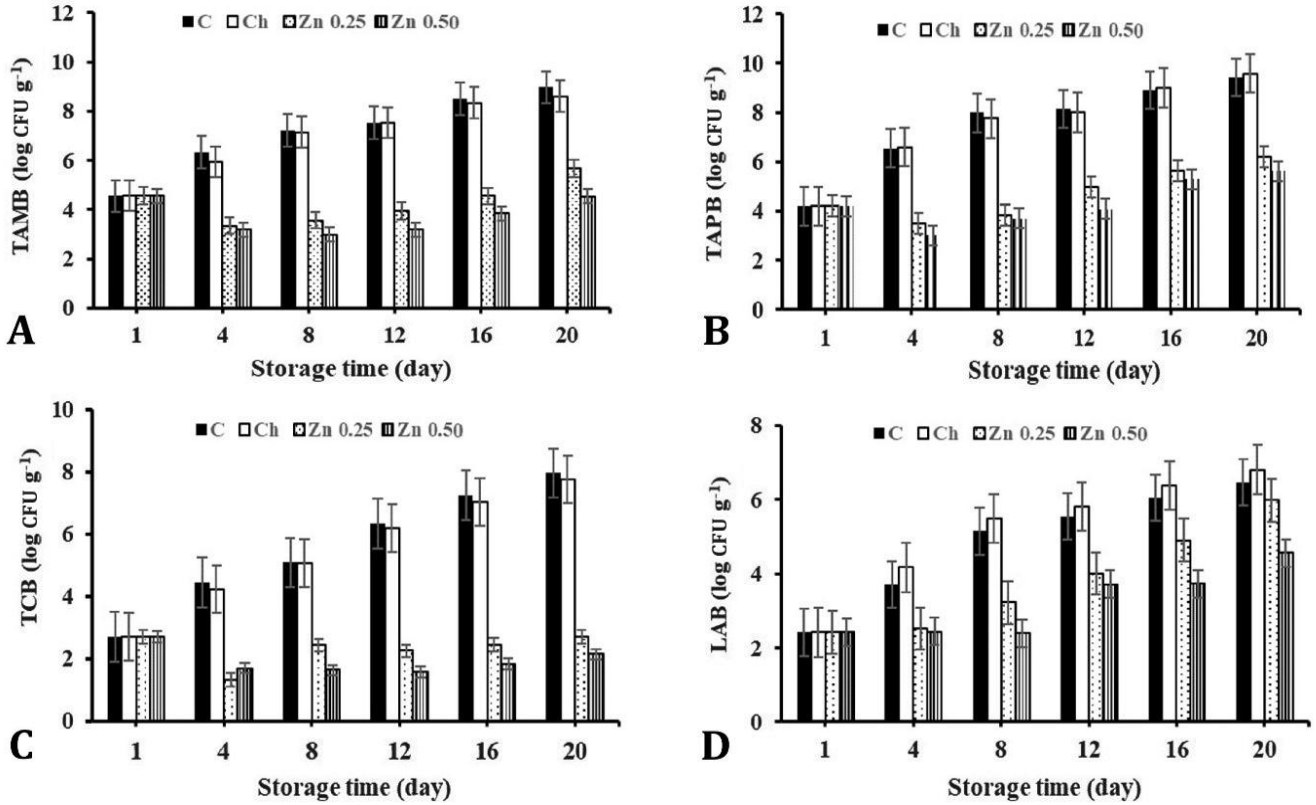

Fig. 2. The effect of treatments on the numbers of bacteria. **A)** Total aerobic mesophilic bacteria (TAMB), **B)** Total aerobic psychrophilic bacteria (TAPB), **C)** Total Coliform bacteria (TCB) and **D)** Lactic acid bacteria (LAB). Treatments: Control (C), Chia seed mucilage (Ch), Chia seed mucilage with 0.25% ZnO-NPs (Zn 0.25) and Chia seed mucilage with 0.50% ZnO-NPs (Zn 0.50).

Table 1. The effect of treatments on the pH and peroxide value (PV) of chicken fillet. Data are presented as means of replicates ± SD.

Test	Treatments	Storage time (day)					
		1	4	8	12	16	20
pH	C	5.58 ± 0.23^{dA}	5.92 ± 0.02^{dB}	6.25 ± 0.01^{cB}	6.64 ± 0.04^{bA}	7.07 ± 0.05^{aA}	7.06 ± 0.08^{aA}
	Ch	5.58 ± 0.23^{cA}	6.32 ± 0.01^{bA}	6.66 ± 0.02^{aA}	6.50 ± 0.01^{aA}	6.67 ± 0.01^{aB}	6.73 ± 0.07^{aB}
	Zn 0.25	5.58 ± 0.23^{bA}	5.19 ± 0.21^{cC}	5.11 ± 0.01^{cC}	5.26 ± 0.05^{cC}	6.00 ± 0.02^{aC}	6.02 ± 0.05^{aD}
	Zn 0.50	5.58 ± 0.23^{bA}	5.18 ± 0.14^{cC}	4.95 ± 0.04^{dD}	5.20 ± 0.01^{cC}	5.10 ± 0.02^{cD}	5.98 ± 0.04^{aD}
PV (mEq kg^{-1})	C	ND	4.89 ± 0.96^{eA}	7.76 ± 1.34^{dA}	10.19 ± 1.08^{cA}	11.93 ± 0.98^{bA}	12.67 ± 1.23^{aA}
	Ch	ND	3.21 ± 1.21^{eA}	6.62 ± 0.96^{dA}	9.06 ± 1.12^{cA}	10.59 ± 1.02^{bA}	11.78 ± 1.44^{aA}
	Zn 0.25	ND	ND	ND	0.95 ± 0.87^{cC}	1.83 ± 0.67^{bC}	2.23 ± 0.81^{aC}
	Zn 0.50	ND	ND	ND	0.45 ± 0.75^{cC}	1.03 ± 0.79^{bC}	1.13 ± 0.75^{aC}

Treatments: Control (C), chia seed mucilage (Ch), chia seed mucilage with 0.25% ZnO-NPs (Zn 0.25), and chia seed mucilage with 0.50% ZnO-NPs (Zn 0.50). ND: Not detected.

^{abcd} Different lowercase letters in the rows for each treatment ($p < 0.05$); ^{ABCD} Different uppercase letters in the columns for each time indicate statistically significant differences ($p < 0.05$).

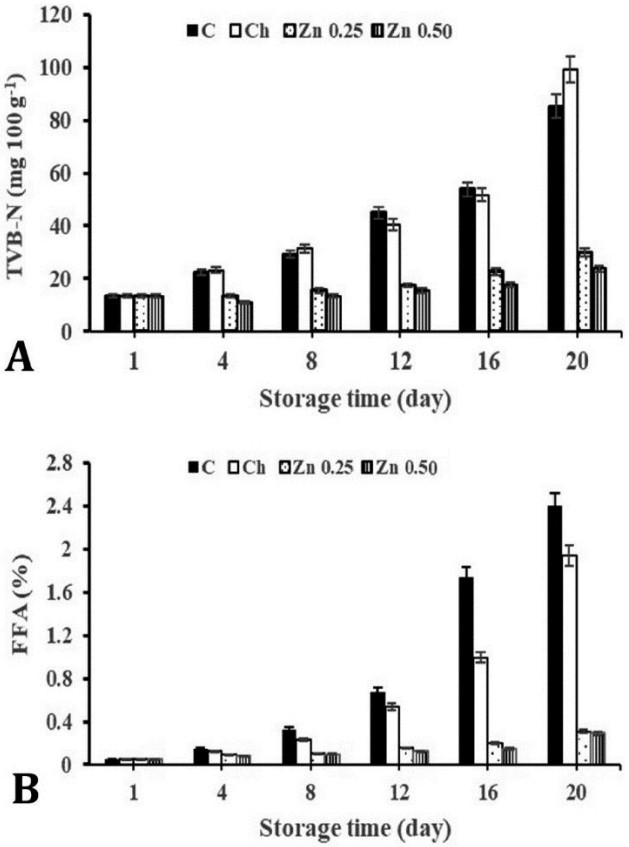

Fig. 3. The effect of treatments on **A)** Total volatile basic nitrogen (TVB-N), and **B)** Free fatty acid (FFA). Treatments: control (C), chia seed mucilage (Ch), chia seed mucilage with 0.25% ZnO-NPs (Zn 0.25), and chia seed mucilage with 0.50% ZnO-NPs (Zn 0.50).

Discussion

The main crystalline peak observed (θ = 14.77) in pure chia seed mucilage film may be strong due to the presence of intermolecular and intramolecular hydrogen bonds.[10] The crystal structure depends on the molecular weight of the polymer, process parameters and the type of solvent .[11] Fernandes *et al.*[12] also reported a strong crystalline peak in the region of about 15.00° for chia seed mucilage. Pure ZnO-NP had a wurtzite or hexagonal structure.[13]

The important peaks of nanofilms are in agreement with the peaks reported by Ghamsari *et al.*[13] and corresponds to the data of the Joint Committee on Powder Diffraction Standards (JCPDS),[14] which indicates the formation of zinc oxide in composite films. These diffraction angles were in consistent with the standard properties for ZnO hexagonal crystals. [14]

Similar results have been reported by other researchers.[7,15] These researchers observed peaks at different angles in nanocomposite films containing zinc oxide that belonged to hexagonal ZnO atomic plates with the indices of (100), (002), (101), (103) and (112). These results confirm the dispersion of ZnO-NPs on the chia seed mucilage matrix. Comparison peaks of chia seed mucilage film containing different amounts of ZnO-NPs showed that the crystal structure of ZnO was not change by the presence of chia seed mucilage. However, with increasing amount of ZnO-NPs, the intensity and area under the peak at 2θ=14.77 decreased. These results showed that the decrease in the crystallization degree, increase the amorphous region, which in turn increases the conductivity of nanocomposite films. This behavior indicates that alignment between ZnO and Ch polymer matrix occurs in amorphous regions.[15] Chu *et al.*[7] also showed that by addition of ZnO-NP to the poly lactic acid (PLA) film, the observed diffraction peaks decreased or disappeared.

Also, with increasing the concentration of ZnO in the chia seed mucilage film, the height of some of the available peaks increased, which is in agreement with the results obtained by Chandrakala *et al.*[16] Strong and narrow diffraction peaks indicate a good crystalline structure of the particles.

Fernández-Pan *et al.*[8] reported that in the chicken meat, the initial numbers of TCB, TAMB, TAPB and LAB were found to be 3.34, 3.43, 2.17, and 3.37 log CFU g^{-1} that increased to 6.99, 7.03, 7.55, and 8.96 log CFU g^{-1} after 13 days of refrigerated storage. The average count of TAPB in chicken fillets were found to be 4.00 log CFU g^{-1} in the reported study by Smolander *et al.*[17] While Alvarez-Astorga *et al.*[18] reported that the mean count of TAMB and TCB are 5.79 and 3.56 log CFU g^{-1} respectively. This discrepancy can be due to increasing of meat bacterial load during slaughtering, peeling and slicing.[19] The initial mean count of evaluated bacteria in our study was in the range all previous studies. According to Smolander *el al.*[17] findings the number of LAB in chicken was 4.00 log CFU g^{-1} at initial and then increased up to 7.00 log CFU g^{-1} during seven days of post-storage.

Initial microbial contamination of poultry carcasses is originated from two main sources, namely the slaughter-house environment, poultry carcasses contamination and the lack of maintaining hygienic standards in poultry slaughterhouses can have a significant impact on the development and spread of carcass contamination.[20] The chicken can provide a suitable medium for bacterial growth due to its nutrients and optimal pH. Therefore, it is contaminated by microbes when it is not stored properly. During refrigerated storage, the number of bacteria in the control and chia mucilage coated significantly increased ($p < 0.05$). However, in the chia mucilage coated containing ZnO-NPs the number of the bacteria firstly decreased or remained constant and then increased during storage ($p < 0.05$). At the end of storage period the lowest count of microorganisms was seen in the treatments containing 5.00% ZnO-NPs. The permissible limit for number of TAMB less than 10^6, between 10^6 - 10^7, and more than 10^7 CFU g^{-1} on the chicken meat, indicate

freshness, acceptable, and non-consumable, respectively.[21] The acceptable count of 7.00 log CFU g^{-1} has been reported for TAPB.[21] Therefore, the shelf life of chicken fillet for the control and coated with chia gum was 4 days.

Although the use of chia seed mucilage coating alone decreased the microbial load compared to the control sample, it could not increase the shelf life of chicken fillets. The reason for this can be attributed to the lack of antimicrobial properties of chia seed mucilage. Similar findings using alginate-based edible coating for chicken fillet,[22] and coating of whey isolates proteins for chicken have been previously reported.[8]

Therefore, the results of the present study demonstrated that chia seed mucilage containing various concentrations of ZnO-NPs can enhance the shelf life of chicken fillet up to 20 days.

The antibacterial properties of coating materials were depended on the concentration of ZnO-NPs, in which the samples with the higher concentration of ZnO-NPs (0.50%) showed more antibacterial properties than of coating that of the lower concentration. The antibacterial activity of ZnO-NPs is derived from reactive oxygen species (ROS) created by ZnO photocatalysis and the released zinc cations from surface of ZnO-NPs. The antimicrobial property of zinc is due to the production of ROS, especially H2O2 and OH, which is requires visible light for this purpose. [23] When ZnO-NPs contact with bacterial cells, they can be adsorbed directly by the cell surface and disrupt the cell wall, as ROS are highly active, they can easily enter in to bacterial cell membranes and disrupt cellular components such as DNA, lipids, and proteins through oxidative stress.

In dark condition, the antimicrobial activity of ZnO is mainly attributed to the binding of Zn^{2+} ions resulted from ZnO dissolution to the bacterial cell wall, which can penetrate to the bacterial cell and cause irreversible damage to the bacterial cell membrane, DNA, and mitochondria and eventually leads to cell death due to prevention of respiratory enzymes function.[24,25]

Amjadi *et al.*[26] reported that packaging of chicken fillet and cheese samples using biocomposite film containing ZnO-NPs significantly decreased the number of inoculated bacteria in the samples. Mohammadi *et al.*[27] also reported that the use of the film derived from okra mucilage containing ZnO-NPs (0.50%) increased the shelf life of chicken breast during storage at 4 ˚C. The effect of nanocomposite packaging containing ZnO-NPs on increasing the shelf life of grape[4], smoked salmon[28], sliced bread[29] and shrimp[30] have also been reported.

The pH value is related to several characteristics of meat quality including juiciness, color, water holding capacity, tenderness and microbial stability. Generally, pH values of poultry meat are between 5.20 to 7.00.[31]

The reason for pH enhancement during storage can be attributed to the released amines due to proteolysis as well as induced basic compounds by activity of meat spoilage bacteria such as ammonia, tetramethylamine and other biogenic amines.[9,31,32] The increasing of pH value can be the sign of bacterial growth, loss of quality, and finally meat spoilage.[31, 32]

The reason for the lower pH in samples treated with ZnO-NPs than that of the control sample can be attributed to the antibacterial properties of ZnO-NP. The similar results reported by Suo *et al.*[33] in pork meat and by Souza *et al.*[31] in poultry meat. These researchers demonstrated that the packaging of the coated with ZnO-NP delayed the pH increase of these meats during storage.

The increase in pH value during storage of the stored foods under aerobic conditions, such as chicken, is due to the growth of microbes. The elevated pH during storage in chicken has also been reported by other researchers.[22,31,32] The similar results reported by Amjadi *et al.*[26]

The oxidative reactions are caused the oxidative degradation of essential lipids, vitamins and pigments, and leading to changes on the food odor and flavor.[31] Lipid oxidation is one of the main challenges in meat storage. At the early stages of lipid oxidation, hydroperoxides are formed; therefore, the initial oxidation of fat is assessed by measuring the amount of peroxide.

The decreased PV in the samples coated with ZnO-NPs can be attributed to antioxidant property of zinc. Zn^{2+} can reduce lipid peroxidation by absorbing free radicals. In addition, Zn^{2+} reduces the production of malondialdehyde and superoxide dismutase (SOD).[34] The antioxidant property of ZnO-NPs has been widely demonstrated by other researchers.[35,36]

The standard PV for chicken is 10.00 mg kg^{-1}. According to Table 2 the PV was 7.89 and 6.21 mg kg^{-1} for the control and mucilage coated samples at the 4 days of storage, respectively, that was less than the standard level. But at the 8 days of storage the PV value was more than the standard level for the control and mucilage alone samples. The PV was 2.23 and 1.13 mg kg^{-1} for the sample coated with 0.25% and 0.50% ZnO-NPs, at 20 days of storage, respectively, that was less than the standard level. Therefore, ZnO-NPs could prevent the chicken fillet from lipid peroxidation for 20 days.

Regarding the physiological, chemical, and biological properties of ZnO-NPs, the application of this compound as a dietary supplement, growth stimulant, antioxidant and antimicrobial compounds, as well as a modulator of the immune system in the diet of various species of farmed animals has been increased.[35]

The TVB-N in chicken is an important physicochemical indicator in assessing the freshness and safety of meat,[37] which includes amino compounds (dimethylamine, trimethylamine, ammonia and other similar compounds) that are produced by the activities of internal and microbial enzymes.[38] Thus, an increase in TVB-N value normally represents the decomposition of the meat.[31]

In the early days of storage, the bacterial population was in the basal phase and increased slowly and then increased rapidly. The increased TVB-N is a limiting factor for meat shelf life. According to the available guideline, if TVB-N level in chicken should be maximum 20.00, ranged from 20.00 to 24.00, 25.00 to 27.00 mg 100 g^{-1}, and more than 27.00 mg 100 g^{-1} the meat consumption will be desirable, consumable, fast consuming, and inedible, respectively.[21]

As can be seen in Figure 3, the initial amount of TVB-N in the fillets was 13.44 mg kg^{-1}. After 4 days of refrigeration, the TVB-N level was 20.00 and 24.00 mg 100 g^{-1}, for control sample and coated with chia seed mucilage, respectively making the chicken fillet edible. But the levels in the treated samples increased to more than 27.00 mg kg^{-1} indicating the product is not consumable. While in treatment containing 0.50% ZnO-NPs, the chicken fillet can be consumed up to 20 days of storage. In addition, in treatments containing 0.25% ZnO-NPs, the chicken fillet can be consumed on days 12 to 16 of storage with TVB-N contain of 7.55 and 22.67 mg 100 g^{-1} as desirable and consumable, respectively, and unconsumable on 20 days of storage with TVB-N = 29.92 mg 100 g^{-1}. Thus, the NPs could inhibit bacterial activities and eventually production of nitrogenous bases. The ZnO-NPs affect the volatile nitrogen compounds by decreasing of microbial load, and reducing the proteolysis by microorganisms. The increased TVB-N is mainly due to the bacterial degradation of meat and increased bacterial load during the period. Any attempt towards reducing of the bacterial load and their enzymatic activity leads to reduce the capacity and ability of bacteria in oxidation and deamination of non-protein nitrogen compounds and reduction of TVB-N. Suo *et al*.[33] also showed that ZnO-NP coating limited the increase of TVB-N by delaying the release of amino acids during storage of pork meat. Similar results have been reported by Souza *et al*.[31] in chicken.

The FFAs released during oxidation and hydrolysis of esterified fats, known as undesirable compounds as they can be converted into smelly volatile compounds. The low FFA content in the treatment with chia seed mucilage coating compared to the control can be attributed to the inhibition of oxygen permeation of the coating. Similar results have been reported by Dalvandi *et al*.[39] They also showed that FFA in chicken fillets with carboxymethyl cellulose (CMC) coating was reduced compared to the control. The FFA content of the fillets coated with mucilage containing nanoparticles was not significant until the 16th day of storage, and then increased significantly. The reason can be attributed to antioxidant effect and antimicrobial properties of these compounds.[38] This reduction in FFA content can also be due to the synergistic effect between chia seed mucilage coating and nanoparticles. These findings were in parallel with the results obtained for peroxide value of samples.

In conclusion, the samples of chicken fillets coated with mucilage containing ZnO-NPs had lower pH value, TVB-N, PV and FFA and count of TAMB, TAPB, LAB and TCB than those of other samples during storage time. The control and samples coated with chia seed mucilage also had all required standard for consumption until the 4 days of storage. However, the samples coated with mucilage containing ZnO-NPs had all required standard for consumption up to 20 days of storage. Therefore, the use of ZnO-NPs during storage period of fresh chicken fillets increased the shelf life by improving the physicochemical properties and decreasing microbial count.

Acknowledgments

The authors thank West Azerbaijan Agricultural and Natural Resources Research and Education Center for providing lab facilities in conducting this project.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Latou E, Mexis SF, Badeka AV, et al. Combined effect of chitosan and modified atmosphere packaging for shelf life extension of chicken breast fillets. LWT-Food Sci Technol 2014; 55(1): 263-268.
2. Sharma H, Mendiratta SK, Agrawal RK, et al. Use of various essential oils as bio preservatives and their effect on the quality of vacuum packaged fresh chicken sausages under frozen conditions. LWT-Food Sci Technol 2017; 81: 118-127.
3. Dick M, Costa TM, Gomaa A, et al. Edible film production from chia seed mucilage: Effect of glycerol concentration on its physicochemical and mechanical properties. Carbohydr Polym 2015; 130: 198-205.
4. Kumar S, Boro JC, Ray D, et al. Bionanocomposite films of agar incorporated with ZnO Nanoparticles as an active packaging material for shelf life extension of green grape. Heliyon 2019; 5(6): e01867. doi: 10. 1016/j.heliyon.2019.e01867.
5. Tavares LS, Junqueira LA, de Oliveira Guimarães ÍC, et al. Cold extraction method of chia seed mucilage (*Salvia hispanica* L.): effect on yield and rheological behavior. J Food Sci Technol 2018; 55(2): 457-466.
6. Kheirkhah Foghara S, Jafarian S, Zomorodi S, et al. Fabrication and characterization of an active bionanocomposite film based on basil seed mucilage and ZnO nanoparticles. J Food Meas Charact 2020; 14: 3542-3550.
7. Chu Z, Zhao T, Li L, et al. Characterization of antimicrobial poly (lactic acid)/nano-composite films with silver and zinc oxide nanoparticles. Materials

(Basel) 2017; 10(6): 659. doi: 10.3390/ma10060659.
8. Fernández-Pan I, Carrión-Granda X, Maté JI. Antimicrobial efficiency of edible coatings on the preservation of chicken breast fillet. Food Control 2014; 36(1): 69-75.
9. Yehia HM, Elkhadragy MF, Al-Megrin WA, et al. Citrox improves the quality and shelf life of chicken fillets packed under vacuum and protects against some foodborne pathogens. Animals 2019; 9(12): 1062; doi:10.3390/ani9121062.
10. Islam MS, Rahaman MS, Yeum JH. Electrospun novel super-absorbent based on polysaccharide–polyvinyl alcohol–montmorillonite clay nanocomposites. Carbohydr Polym 2015; 115: 69-77.
11. de Oliveira Mori CL, dos Passos NA, Oliveira JE, et al. Electrospinning of zein/tannin bio-nanofibers. Ind Crops Prod 2014; 52: 298-304.
12. Fernandes SS, Romani VP, da Silva Filipini G, et al. Chia seeds to develop new biodegradable polymers for food packaging: Properties and biodegradability. Polym Eng Sci 2020; 60(9): 2214-2223.
13. Ghamsari MS, Alamdari S, Razzaghi D, et al. ZnO nanocrystals with narrow-band blue emission. J Lumin 2019; 205: 508-518.
14. American Society for Testing and Material. Powder Diffraction Files; Joint Committee on Powder Diffraction Standards (JCPDS): Swarthmore, USA: 1999, No: 36-1451. Available at: www.astm.org. Accessed Sep 24, 2022.
15. Elashmawi IS, Hakeem NA, Marei LK, et al. Structure and performance of ZnO/PVC nanocomposites. Phys B: Condens Matter 2010; 405(19): 4163-4169.
16. Chandrakala HN, Ramaraj B, Shivakumaraiah, et al. The influence of zinc oxide- cerium oxide nanoparticles on the structural characteristics and electrical properties of polyvinyl alcohol films. J Mater Sci 2012; 47: 8076-8084.
17. Smolander M, Alakomi HL, Ritvanen T, et al. Monitoring of the quality of modified atmosphere packaged broiler chicken cuts stores in different temperature conditions A. Time-temperature indicator as quality-indicating tools. Food Control 2004; 15(3): 217-229.
18. Alvarez-Astorga M, Capita R, Alonso-Calleja C, et al. Microbiological quality of retail chicken by-products in Spain. Meat Sci 2002; 62(1): 45-50.
19. Maharjan S, Rayamajhee B, Chhetri VS, et al. Microbial quality of poultry meat in an ISO 22000:2005 certified poultry processing plant of Kathmandu valley. Int J Food Contam 2019; 8: 6. doi: 10.1186/s40550-019-0078-5.
20. Rouger A, Tresse O, Zagorec M. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms 2017; 5(3): 50. doi: 10.3390/micro-organisms5030050.
21. Iran veterinary organization (IVO). Executive directive: Hygiene processes supervision, risk assessment and risk management for poultry meat. Available at: www.ivo.ir. 2021: 1-12. Accessed 18 Sep. 2022.
22. Matiacevich S, Acevedo N, López D, Characterization of edible active coating based on alginate–thyme oil–propionic acid for the preservation of fresh chicken breast fillets. J Food Process Preserv 2015; 39(6): 2792-2801.
23. Siddiqi KS; UR Rahman A, Tajuddin, et al. Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett 2018; 13(1): 141. doi: 10.1186/s11671-018-2532-3.
24. Joe A, Park SH, Shim KD, et al. Antibacterial mechanism of ZnO nanoparticles under dark conditions. J Ind Eng Chem 2017; 45: 430-439.
25. Kim I, Viswanathan K, Kasi G, et al. ZnO Nanostructures in active antibacterial food packaging: Preparation methods, antimicrobial mechanisms, safety issues, future prospects and challenges. Food Rev Int 2020; 38: 537-565.
26. Amjadi S, Emaminia S, Nazari M, et al. Application of reinforced ZnO nanoparticle-incorporated gelatin bionanocomposite film with chitosan nanofiber for packaging of chicken fillet and cheese as food models. Food Bioprocess Technol 2019; 12: 1205-1219.
27. Mohammadi H, Kamkar A, Misaghi A, et al. Nanocomposite films with CMC okra mucilage and ZnO nanoparticles: Extending the shelf-life of chicken breast meat. Food Packag Shelf Life 2019; 21: 100330. doi: 10.1016/j.fpsl.2019.100330.
28. Baek SK, Song KB. Development of Gracilaria vermiculophylla extract films containing zinc oxide nanoparticles and their application in smoked salmon packaging. LWT-Food Sci Technol 2018; 89: 269-275.
29. Noshirvani N, Ghanbarzadeh B, Rezaei Mokarram R, et al. Novel active packaging based on carboxymethyl cellulose-chitosan-ZnO NPs nanocomposite for increasing the shelf life of bread. Food Packag Shelf Life 2017; 11: 106-114.
30. Ejaz M, Arfat YA, Mulla M, et al. Zinc oxide nanorods/clove essential oil incorporated type B gelatin composite films and its applicability for shrimp packaging. Food Packag Shelf Life 2018; 15: 113-121.
31. Souza VGL, Rodrigues C, Valente S, et al. Eco-friendly ZnO/chitosan bionanocomposites films for packaging of fresh poultry meat. Coatings 2020; 10(2):110. doi: 10.3390/coatings10020110.
32. Gram L, Huss HH. Microbiological spoilage of fish and fish products. Int J Food Microbiol 1996; 33(1): 121-137.
33. Suo B, Li H, Wang Y, et al. Effects of ZnO nanoparticle-coated packaging film on pork meat quality during cold storage. J Sci Food Agric 2016; 97(7): 2023-2029.
34. Swain PS, Rao SBN, Rajendran D, et al. Nano zinc, an alternative to conventional zinc as animal feed

supplement: A review. Animal Nutr 2016; 2(3): 134-141.
35. Bray TM, Bettger WJ. The physiological role of zinc as an antioxidant. Free Radic Biol Med 1990; 8(3): 281-291.
36. Prasad AS. Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. Front Nutr 2014; 1: 14. doi: 10.3389/fnut.2014.00014.
37. Khulal U, Zhao J, Hu W, et al. Intelligent evaluation of total volatile basic nitrogen (TVB-N) content in chicken meat by an improved multiple level data fusion model. Sens Actuators B Chem 2017; 238: 337-345.
38. Fan W, Sun J, Chen Y, et al. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chem 2009; 115(1): 66-70.
39. Dalvandi F, Almasi H, Ghanbarzadeh B, et al. Effect of vacuum packaging and edible coating containing black pepper seeds and turmeric extracts on shelf life extension of chicken breast fillets. J Food Bioproc Eng 2020; 3(1): 69-78.