

Evaluation of Chitosan-Nisin Coating on Quality Characteristic of Fresh Chicken Fillet under Refrigerated Conditions

B. Sotoudeh¹, M. H. Azizi^{2*}, A. Mirmajidi Hashtjin³, R. Pourahmad⁴, and H. Tavakolipour⁵

ABSTRACT

Poultry should be stored in appropriate conditions to prevent its fast spoilage. Using antimicrobial coatings is considered as one of the methods to preserve this product. In this study, Chitosan and Chitosan-Nisin coatings were examined under refrigerated conditions at a temperature of 4°C. The samples were packed in uncoated as the control, Chitosan coating, and Chitosan-Nisin coating groups. The chicken fillets were tested for microbial (total bacterial count, *Salmonella* count, and *staphylococcus aureus coagulase*) and physicochemical (pH, color, and texture) features on the first, third, fifth, and seventh days of storage. Based on the results, the Chitosan coating increased the shelf life of fresh chicken under refrigerated conditions by three-days, which demonstrated an inhibitory effect on the overall bacterial growth until the third day. Finally, Chitosan coating demonstrated an antibacterial effect on the *Salmonella* and positive *staphylococcus aureus coagulase* until the fifth day. The samples with Chitosan-Nisin were found to be more effective than the Chitosan coated samples. In addition, the Chitosan-Nisin coated samples prevented the growth of total bacteria including *Salmonella*, and positive *staphylococcus aureus coagulase*. Further, it increased the shelf life of fresh chicken under refrigerated conditions at the temperature of 4 °C for seven days.

Keywords: Biopolymer, Edible coating, Peptides, Refrigerated, Shelf life, *Salmonella*, *Taphylococcus aureus*.

INTRODUCTION

Poultry meat is regarded as an important source of protein for human nutrition and a type of white meat. In most countries, chicken meat is the most consumed poultry meat, which is preferred due to several features such as good protein quality, lower fat, less time of

cooking, and easy digestion, compared to other kinds of meat (Dave and Ghaly, 2011). Chicken meat contains 17.5–20% protein, 56–60% water, 1–2% salts, and 18–24% fat. In addition, chicken meat is considered as one of the most popular food ingredients for preparing various dishes, the consumption of which has rapidly increased in many countries

¹ Department of Food Science and Technology, Islamic Azad University North Tehran Branch, Tehran, Islamic Republic of Iran.

² Department of food science and Technology, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

*Corresponding author; e-mail : azizit_m@modares.ac.ir

³ Agricultural Engineering Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Islamic Republic of Iran.

⁴ Department of Food Science and Technology, Islamic Azad University Varamin-Pishva Branch, Varamin, Islamic Republic of Iran.

⁵ Department of Food Engineering, Islamic Azad University, Sabzevar Branch, Sabzevar, Islamic Republic of Iran.



during the recent decades (Warriss, 2010). Further, poultry meat is one of the most affordable and fastest sources of meat production in the world. The global meat production exceeded 250 million tons in 2014, in which poultry meat constituted 87 million tons and ranked in the second place preceded only by pork meat which had an annual production of 108.9 million tons. Furthermore, chicken meat is a highly perishable food item. The contaminants related to chicken meat include intestines, skin, air, water, and the equipment used during their production, which are considered as the sources of poultry carcass contamination (Cervený *et al.*, 2009). The need for production with minimal cost has increased, along with providing customer satisfaction, due to a considerable increase in the tendency of acceptance and practice of the science of food hygienity and safety in fresh processed meat. The food packaging industry has expanded and developed rapidly, especially in meat and its products. However, the major advancement in this area is related to packaging materials and its systems, although the principles related to this science have remained the same (Kerry *et al.*, 2006). Substitution of the chemical and synthetic ingredients in food and packaging materials with the natural ingredients has attracted a lot of consumers' attention. Most of the synthetic ingredients have been replaced with their natural counterparts. For example, chemical antioxidants such as BHA, BHT, and TBHQ have been replaced with a combination of tocopherol and ascorbic acid in food products, which should be continued to be adopted at the level of food packaging design. In other words, designing and producing environment-friendly packaging such as the relative replacement of synthetic and composite materials with the biodegradable or edible materials can result in decreasing the use of the total quantity of the material and increasing the amount of recyclable or reusable materials. In addition, based on the advancement of studies in packaging technology and food industry with respect to its engineering, as well as those related to the consumers of the food

industry, consumers are constantly demanding new materials with new functionalities. These new approaches toward food-packaging systems are related to new development in the areas of processing technology, life cycle changes, political decision-making, and scientific research (Testin and Verrgano, 1990). Edible layers and coatings increase the quality of food products by their protection against physical, chemical, and biological degradation. Edible layers and coatings are used to facilitate an easy method for modifying the physical resistance of food products so that they can reduce the clustering of particles, and increase the visual and tangible features of the food products. Further, they can protect the products against oxidation, absorption, dryness, microbial growth, and other chemical reactions. Creating a barrier and preventing food products against oils, gases, and vapors are considered as the most common function of the edible layers and coatings. Furthermore, these products may contain active substances such as antioxidants, antimicrobials, colors, and flavors, which further enhance the food items. Therefore, edible layers and coatings increase the quality of food products, which result in creating longer shelf-life including their "safety and health" (Guilbert and Gontrad, 1995). Nowadays, there is a great desire for enhancing the knowledge related to the use of natural and antimicrobial coatings for preserving the quality of meat and increasing its shelf life, due to the consumers' awareness and interest in consuming natural and healthy foods (Embusead and Huber, 2007). In addition, edible coatings are used in the food product. Hence, the coating is considered as a part of the product. In general, coatings are applied by several methods such as waxing/spraying and dipping (Ghanbarzadeh and Orommiehie, 2008). Chitosan is widely used in the food industry as an antimicrobial coating, which is a multi-sugary form of glucosamine and N-acetyl glucosamine. Further, Chitosan is obtained from the deacetylation of chitin, which is considered as one of the most abundant natural polymers

(Coma *et al.*, 2002). An antimicrobial agent can display a specific inhibitory activity or a mechanism against any microorganism. Therefore, selection of the appropriate antimicrobial agent depends on its effectiveness against the target microorganisms. In this regard, bacteriocin is regarded as one of the effective antimicrobial factors that is widely used in the food industry. The term "Bacteriocin" was coined in 1953 to define the colicin produced by *Escherichia coli* (Settanni and Corsetti, 2007). The bacteriocins are synthesized in ribosomal terms, which are found intracellularly in peptides, and release low-volume molecular proteins that play a bacteriostatic impact (inhibitory impact on the bacterial growth or slowing their growth) on the other bacteria related to one species (narrow spectrum) or several species (broad spectrum). However, the bacteriocins are not considered as antibiotics in order to differentiate them from therapeutic antibiotics that can detect allergic reactions in humans although they involve antibiotic properties (Settanni and Corsetti, 2007). Various bacteriocins such as Nisin, Pediocin, Lacticin, and so on can be combined with food or they can be introduced into the food packaging system in order to prevent food from degradation by pathogenic microorganisms (De Vuyst, and Leroy, 2007). The combination of bacteriocin-Nisin was used as the antibacterial compound. Nisin is produced by certain breeds of *Lactococcus lactis* (Naidu, 2000).

The present study aimed to evaluate preservative and antimicrobial effect of Chitosan-Nisin on chicken fillets under refrigerated conditions for prolonging shelf life and quality of chicken fillets.

MATERIALS AND METHODS

In this study, Cellophane was obtained from Iranian packaging industry, culture media, reagents and other chemicals from Merck, and Nisin and Chitosan from Sigma. Then, pH meter (Met Rohm, Switzerland), Texture

analyzer (Hounsfield, UK), Colorimeter (Hunter lab, USA), Digital scale (Sartorius, Germany), Microbial Laminar Hood (Jall Tajhiz, Iran), and Colony counter (Gerber, Swiss) were used as the instruments in the present study. The samples were chicken fillets weighting 5 kg, which were provided immediately after slaughter from one of the slaughterhouses around Tehran, Iran. Then, the fillets were packed immediately after delivery. In addition, the Chitosan coating was prepared by dissolving the chitin crust of a crab (deacetylation degree of 80 percent) in an acetic acid solution of 1% and obtaining 2% concentration. Around 5–6 hours were required to dissolve Chitosan into the acetic acid solution at 40°C. A magnetic stirrer was used to dissolve Chitosan. Then, the resulting Chitosan solution was filtered using Whatman filter paper No. 3 under vacuum. In the present study, coating with Chitosan and coating with Chitosan-Nisin (an antibacterial material) were used. The chicken was coated with Chitosan by preparing a certain amount of suspension in which 200 g fresh chicken parts were placed and kept at the room temperature of 25°C during 3 hours until they were completely dried. Then, chicken breasts were sent to laboratory immediately after being slaughtered in the slaughterhouse of Varamin. There was no possibility of cross contamination by applying immediate packaging under microbial hood equipped with UV. Next, the Nisin coating was prepared by adding to a suspension of Chitosan solution in the proportion of 2.5 mcg per mL, and mixed with the solution with a stirrer at 7,000 rpm for 90 seconds. The fresh chicken parts were coated by placing them in a certain volume of the suspension and coated by keeping at room temperature until drying. Finally, the freshly coated pieces of the meat were packaged with common cellophane. Regarding the control samples, they were packaged with cellophane at the room temperature of 25°C under the microbial hood equipped with UV, without leaving any possibility of cross contamination.

Later, 200 g samples of chicken breasts were floated in the active coverage. Then, they were



completely drowned and hanged under microbial hood at 25°C for 3 hours to dry. The control samples consisted of fresh chicken fillets without any active coating and packaged with common cellophane and were stored at the refrigerated temperature (4°C). Finally, the experiments were performed on the first, third, fifth, and seventh days, with three replications.

Microbiological Tests

First, one gram of the sample was weighted in the laboratory under microbial hood and diluted in 10 mL of a ringer solution. Then, a series of 6 tubes containing sterile distilled water was prepared using the CFU method. In addition, the diluted sample (1 mL) was added to the dilution tube 1 and cultured in the Nutrient Agar culture medium by using the Pour plate method. Later, the cultures were incubated at 37°C for three days for calculating the total number of microorganisms (Benson, 1984).

Detecting and Counting Salmonella

Detection of *Salmonella* was conducted as follows. First, the enrichment was initialized, in which 25 g of the sample was mixed with 225 mL of the lactose culture medium and incubated at 37°C for 24 hours. Then, the secondary enrichment was obtained by using the Tetrathionate Broth culture medium, which was incubated for 24 hours at 37°C. Next, the isolation of the *Salmonella* and *Shigella* was done in the culture media of Mac Conkey Agar (MCA) and Brilliant Green Agar (BGA) in the CFU by diluting a series of six tubes containing sterile distilled water prepared by adding 1 mL to the tube number 1, which was cultured in the above-mentioned culture mediums by the linear culturing method and incubated for 24-48 hours. Subsequently, the tubes of Lysine, Iron, and Agar (LIA) were incubated in the sloping culture at the

temperature of 37°C for 48 hours (Andrews et al., 1995).

Identifying and Counting

Staphylococcus

First, 10 g of the sample was weighted under the microbial hood, placed in 90 mL of sterile serum, and incubated at 37°C for 24 hours. Then, a series of 6 tubes containing sterile distilled water was prepared using the CFU method and 1 mL of the sample was added to the dilution tube with serial number one. Next, it was cultured in the Baird-Parker Agar culture medium by using the Pour plate method and incubated at 37°C for 48 hours. Finally, the coagulase test was performed on the incubated product (Benet and Lancette, 1998).

Color

The color changes of the samples were assessed by using the Hunter Lab device from the Agricultural Research Center. This is a type of a spectrophotometer device used for analyzing the color of the products. The L^* indicates the brightness (white-black), a^* shows (red-green), and b^* represents yellow-blue. The greater value of L^* means higher severity of the brightness. Similarly, the tendencies to red and yellow are determined by the values of a^* and b^* , respectively. The device was calibrated by white and black tiles before the test, and controlled by red tiles. In this way, the color indices of L^* , b^* and a^* were determined (Salva, 2009).

Evaluation of the Texture Analyzing Properties

The texture analysis device Hounsfield UK, from the Agricultural Research Center, was used to assess the texture properties of the samples. A piece from the central part of

each sample with dimensions 4×6 cm was cut and the texture resistance against the shear bond strength of stainless steel was measured. The force equivalent of the load cell was 500 N, the end-point of the test was 25 mm, and the velocity of the probe was 100 mm per minute. Each test was performed with three replications. Finally, the maximum force required for shearing was reported (Polidori, 2007).

pH Measurement

The pH-meter device (Met Rohm Switzerland) was calibrated to 4 and 7 tampon solutions. Then, 50 g of the uniform sample was put into a 100 mL beaker and its pH at 25°C was recorded (Sotoudeh *et al.*, 2013).

Statistical Analysis

In order to analyze the data, Pearson correlation coefficient was used to evaluate the presence or absence of significant differences between the values of each index on the days 1, 3, 5, and 7.

RESULTS AND DISCUSSION

Counting of the Total Number of Microorganism

Based on the results of the mean comparison, a significant difference was observed between the control sample with the samples B and C on the first and third

days ($P < 0.05$). In addition, a significant difference was observed between the samples A, B, and C on the fifth day ($P < 0.05$). However, as shown in Table 1, no significant difference was observed between the samples A and B on the seventh day while they were significantly different from the sample C ($P < 0.05$).

Identifying and Counting Salmonella

Salmonella was uncountable on the first day and a significant difference was observed between the treatments B and C on the third and fifth days ($P < 0.05$). The *Salmonella* treatment increased on the seventh day, which was uncountable and indicated a significant difference with the treatment C ($P < 0.05$). As shown in Table 2, no growth of *Salmonella* was observed in the treatment C.

Identifying and Counting Staphylococcus

Regarding the treatment A, *Staphylococcus* was uncountable on the first day. In addition, a significant difference was observed between treatments A, B, and C on the third and fifth days ($P < 0.05$). *Staphylococcus* increased in the treatment B, which was uncountable and indicated a significant difference with the treatment C ($P < 0.05$). In the treatment C, no growth of *Staphylococcus* was reported. Regarding the total bacterial count, in the samples with Chitosan coating, no bacteria were observed until the end of the day 3. Additionally, in

Table 1. Total count of microorganisms during storage (log CFU g⁻¹).^a

Treatment	Storage time (day)			
	1	3	5	7
A	433/33b	10 ⁹ < u	10 ⁹ < u	10 ⁹ < u
B	0a	0a	2.5×10 ⁸ c	10 ⁹ < u
C	0a	0a	0a	0a

^a The averages displayed in a column with different letters are significantly different with each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.



the samples with Chitosan-Nisin coating, no bacteria were seen until the end of the day 7. (Table 3). However, the presence of bacteria was observed from the very first day in the control sample.

Product Colorimetry

As indicated in Table 4, the mean comparison in the color for the first, third, and seventh days indicated a significant difference between the samples A, B, and C ($P < 0.05$). Sample B had the highest mean on the days 1, 3, and 7. The highest mean was related to the sample B. On the day 5, no significant difference was observed between the samples B and C. However, a significant difference was observed based on the mean comparison between the control and the other samples ($P < 0.05$).

Based on the mean comparison in Table 5, all samples had a significant difference with each other ($P < 0.05$). Finally, a significant difference was observed between the means of all samples ($P < 0.05$) (Table 6).

Evaluating Properties of the Texture Analysis

Comparison of the means on the first day indicated a significant difference among the samples and the highest difference was observed for the sample C ($P < 0.05$) (Figure 1). In addition, mean comparison for day 3 indicated no significant difference between the samples A and B, while a significant difference was reported for the samples A and C ($P < 0.05$). Further, a significant difference was observed among the samples on the fifth day and the highest difference was observed for the sample A ($P < 0.05$). Finally, the

Table 2. *Salmonella* bacteria count during storage (log CFU g⁻¹).^a

Treatment	Storage time (day)			
	1	3	5	7
A	10 ⁹ < u	10 ⁹ < u	10 ⁹ < u	10 ⁹ < u
B	0a	0a	0a	10 ⁹ < u
C	0a	0a	0a	0a

^a The averages displayed in a column with different letters are significantly different with each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.

Table 3. *Staphylococcus aureus* coagulase (+) bacteria count during storage (log CFU g⁻¹).^a

Treatment	Storage time (day)			
	1	3	5	7
A	10 ⁹ < u	10 ⁹ < u	10 ⁹ < u	10 ⁹ < u
B	0a	0a	0a	1/710 ⁸ b
C	0a	0a	0a	0a

^a The averages displayed in a column with different letters are significantly different with each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.

Table 4. Intensity of Light change of chicken fillet during storage.^a

Treatment	Storage time (day)			
	1	3	5	7
A	58.72a	59.09a	59.48a	56.02a
B	96.91c	69.32c	64.48b	66.36c
C	63.83b	64.42b	64.93b	65.91d

^a The averages displayed in a column with different letters are significantly different with each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.

Table 5. Redness change of chicken fillet during storage.^a

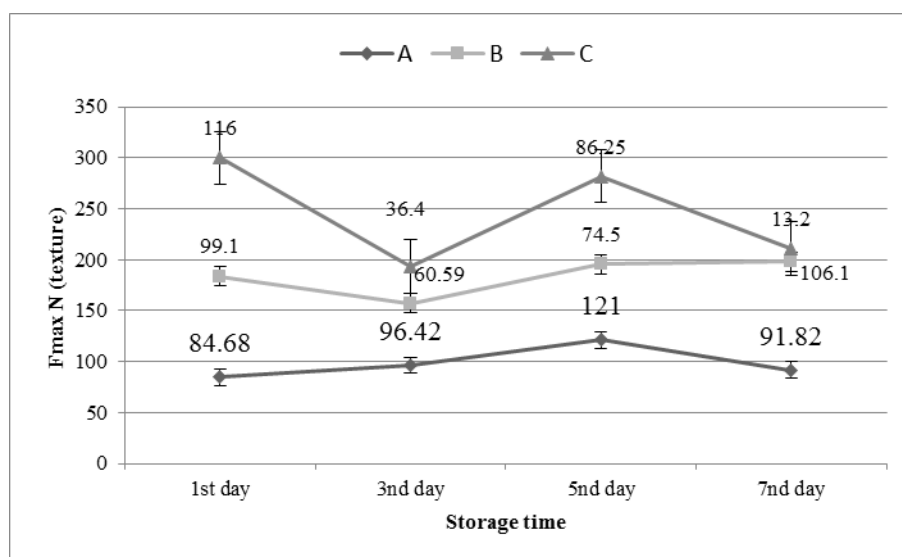
Treatment	Storage time (day)			
	1	3	5	7
A	4.88b	2.47b	1.65b	2.63c
B	0.12a	0.94b	0.51a	0.16a
C	9.27c	3.39c	2.97c	1.71b

^a The averages displayed in a column with different letters are significantly different with each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.

Table 6. Yellowness change of chicken fillet during storage.^a

Treatment	Storage time (day)			
	1	3	5	7
A	7.81a	5.81a	7.62a	4.9b
B	11.2c	10.17c	8.76b	10.29a
C	9.74b	14.4d	10.5c	10.13c

^a The averages displayed in a column with different letters are significantly meaningful with respect to each other ($P < 0.05$). Legend: A, Control; B, Chitosan; C, Chitosan-Nisin.

**Figure 1.** Texture Fmax (N) change of chicken fillet during storage: (A) Control; (B) Chitosan, (C) Chitosan-Nisin.

comparison of the means on the seventh day indicated a significant difference among the samples and the highest difference was observed for the sample B ($P < 0.05$).

pH Measurement

As illustrated in Figure 2, the pH of each treatment during storage (seven days)

indicated a significant difference compared to the means of the different samples on the first, third, and fifth days ($P < 0.05$). Further, no significant difference was reported between the samples B and C on the seventh day, while they were significantly different with respect to the sample A ($P < 0.05$).

Chitosan coating with Nisin had higher antimicrobial effect than Chitosan without nisin. Furthermore, the rate of bacterial

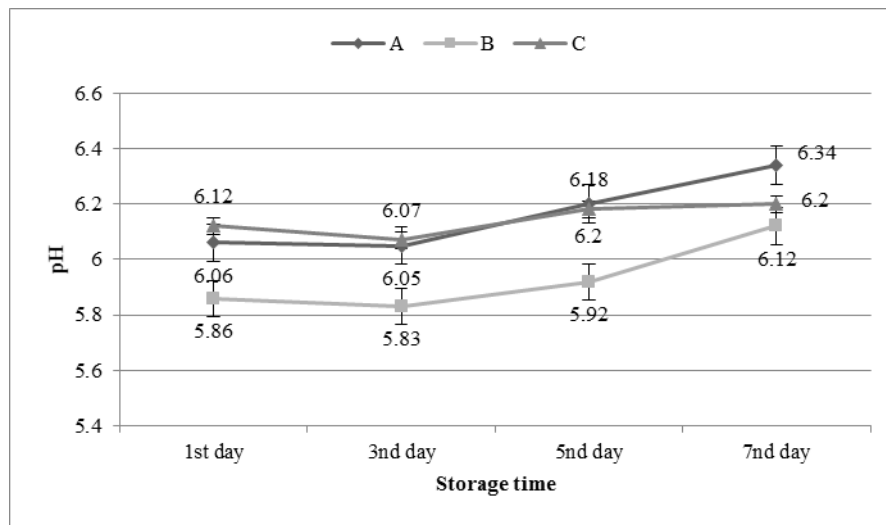


Figure 2. Change in pH of chicken fillet during storage. A: control B: Chitosan C: Chitosan-Nisin.

growth was lower than that of the other samples in the Chitosan coatings containing Nisin due to the presence of Chitosan, which is consistent with the results of Chun *et al.* (2009), which reported Chitosan coatings in fish resulted in reducing the rate of bacterial counting significantly during storage in cold conditions. Although the mechanism of antimicrobial activity of Chitosan was not indicated clearly, there were some suggestions in this regard. First, the presence of positive-charge amino groups that bond with large coarse molecules with a negative charge at the microbial level resulted in breaking the membrane, leaking the cellular material, and ultimately creating cellular death (Wang *et al.*, 2003). In another research conducted on Chitosan coating and chickpea coliform in glacial conditions, the researchers found that the number of bacteria decreased to about 1-2 logarithmic cycles compared to the amount in the control samples regarding the percentage of Chitosan (Fan *et al.* 2008). Tao *et al.* (2005) reported the minimum inhibitory concentration of Chitosan for *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella Bacillus cereus* as 0.038, 0.75, and 0.3%, respectively. The bacterial action may be bactericidal or bacteriostatic, which may slow down the bacterial activity and

lead to death or prolonged latency, respectively (Settanni and Corsetti, 2007). Jeevararatnam *et al.* (2004) reported that Nisin can reduce the growth of the bacteria in mesophilic and aerobic pyrotrophic bacteria and lactic acid bacteria, as well. Nisin as bacteriocin on *Salmonella typhimurium* was first evaluated in this study. In addition, the growth of *Salmonella typhimurium* was evaluated in commercial soup medium at 8 and 30°C for 21 days. Further, Nisin at 8°C prevented the growth of this bacterium. In the growing process of *Staphylococcus aureus*, the Chitosan coating with Nisin had better reaction than Chitosan coating alone. *Staphylococcus aureus* bacteria can normally cause food poisoning, as well as digestive diseases in humans. Furthermore, the samples containing Chitosan with bacteriocin (Nisin) have been more successful in reducing growth rate of *Staphylococcus aureus* bacteria. This is probably related to the high antimicrobial activity of Nisin against these microorganisms. Based on the results, the rate of bacterial reduction increased by enhancing Chitosan concentration. The results are in line with those of Darmadji *et al.* (1996), which indicated that 1% Chitosan solution decreases microbial counts by an average of 1-2 log CFU g⁻¹ in minced beef

patties stored at 4°C for 10 days. Additionally, a large number of studies reported the ability of Chitosan coating to reduce microbial load in different meat products. Sagoo *et al.* (2002) demonstrated that the total viable counts of yeasts, and molds are reduced by approximately 1-3 log CFU g⁻¹ on skinless and standard sausages dipped in 1% Chitosan solution before being stored at 7°C for 18 days. Furthermore, the addition of Chitosan at 1% in fresh pork sausages reduced the amounts by 0.5-1.5 log CFU g⁻¹ according to Soutos *et al.* (2008). Elsaid *et al.* (2018) reported that Chitosan coating (1, 1.5 and 2%) improves the microbial quality and sensory characteristics of chicken fillets under chilled storage (4 ± 1°C). In addition, the uncoated samples spoiled and had a slimy appearance and off-odor up to 3 days of storage due to rapid microbial growth. Based on the results, Chitosan-coated samples were acceptable. Chitosan has the potential to bind to many different food components such as proteins, fats and other anionic substances available in complex food matrices such as meat due to its polycationic nature. Thus, it may influence the antimicrobial action of Chitosan (Devlieghere *et al.*, 2004; Kubota and Kikuchi, 1999).

In this study, most of pH changes associated with Chitosan coating was related to the effect of Chitosan on its antibacterial activity. The increase in pH with time can be attributed to production of volatile compounds such as ammonia and tetra methylamine, derived from bacteria causing corrosion and increasing the pH values gradually with increasing the storage period due to endogenous enzymes, bacterial metabolites, and volatile organic compounds such as amines (Gill, 1986). The obtained results were consistent with those of Sharafati Chaleshtori *et al.* (2016) and Hassanzadeh *et al.* (2017) who reported that Chitosan-coated samples had lower pH values than those in the uncoated samples. Furthermore, the use of Chitosan coating in chicken meat samples can stabilize the pH value during storage (Hassanzadeh *et al.*, 2017; Sharafati Chaleshtori *et al.*, 2016).

An increase in the pH is not desirable since it can lead to microbial corruption and the subsequent production of lactic acid, formic acid, propionic acid, and other compounds. In addition, an increase in pH creates a sort of rancidness in the chicken meat. Therefore, a pH level of 5.4 is highly preferred since it can minimize the favorable conditions for growing microorganisms with its acidic environment, and it is not too acidic to affect the capability of the meat to retain its juiciness and texture (Hyttia *et al.*, 1999). The color of chicken meat, which is regarded as one of the most crucial criteria for selecting meat by consumers, is related to the concentration of pigments, especially myoglobin and the chemical status of myoglobin in the meat level. In our study, brightness (L*) in the Chitosan-Nisin coating had a roughly constant trend, while it had the highest brightness intensity in the Chitosan coating on the first day, which gradually decreased from the third day to the seventh, along with a steady decrease in the light intensity. In general, depending on the type of coating that affects myoglobin absorption or light penetration (Salva, 2009), redness (a*) is considered as the most direct source of pigment in chicken meat by involving myoglobin. Besides, there is a direct increase in redness with the amount of oxymyoglobin on meat, depending on the type of coating, which produces reddish color, indicating that the highest redness is related to Chitosan-Nisin coating on the first day, and decreases in the following days. Further, in the case of Chitosan coating, a reduction in redness was observed that could be attributed to the low permeability to oxygen, as in the control sample. Also, the reason for the lack of coverage and formation of red oxymyoglobin was observed. Furthermore, the highest yellowness (b*) was observed in the Chitosan coating on the first day, depending on the type of packaging and the various interactions that occur in packaging the chicken fillet. Chicken fillet is the most important qualities of chicken meat. The results of this study indicated that the greatest shear force for chicken meat belonged to the first day of Chitosan-Nisin



coating, although this force decreased after some time, due to the effect of the type of packaging, coating, and the related chemical reactions and enzymes (Salva, 2009). In addition, the results of the experiments conducted on color, odor, taste, texture, and overall acceptance reported that the control samples treated with Chitosan had high values of all sensory parameters without any significant difference (Jonaidi Jafari *et al* 2017). Regarding the natural enzymes and microorganisms in meat, several changes occurred in meat texture. The effect of proteolytic enzymes led to the breakdown of the structure in meat protein and an increase in the amount of free ammonia in meat (Polidori, 2007).

CONCLUSIONS

Based on the results, *Salmonella* and *staphylococcus aureus* were uncountable on the first day in the control sample, while they did not grow until the end of the fifth day in the Chitosan coated samples and until the end of the seventh day in the samples with the combined coating of Chitosan-Nisin. In this study, the highest pH changes were related to the Chitosan coated samples. This can be related to the impact of Chitosan on the texture of the food, and its chemical and antimicrobial activities. Based on the types of coating considered in this study, the observations indicated that the highest redness was in the Chitosan-Nisin coating for the first day and the redness decreased in the following days. Further, more redness was observed in the control sample, due to the lack of coating and the subsequent formation of oxy-myoglobin. The results of this study indicated that the maximum shear force was in the Chitosan-Nisin coated samples, while this force decreased over time, which can be related to the effects of the type of coating used in the packaging. Furthermore, in the Chitosan coated and control samples, the capability of the meat to retain its juiciness diminished over time and, consequently, more force was

required for cutting the meat. In general, the results indicated that the combined coating of Chitosan-Nisin is significantly effective in increasing the shelf life of the chicken fillet.

ACKNOWLEDGEMENTS

This work was supported by Islamic Azad University North Tehran, and Agricultural Research, Education and Extension Organization (AREEO).

REFERENCES

1. Andrews, W. H., June, G. A., Sherrod, P. S., Hammack, T. S. and Amaguana, R. M. 1995. Salmonella. In: "*Bacteriological Analytical Manual*". 8th Edition, FDA, ED: AOAC, Gaithersburg, MD.
2. Benet, W. and Lancette, G. A. 1998. *Staphylococcus aureus*. In: "*Bacteriological Analytical Manual*". 8th Edition, FDA, ED: AOAC, Int. Gaithersburg, MD, PP. 12: 1-5.
3. Benson, H. A. 1984. Microbiological Application: A Laboratory Manual in General Microbiology. 3rd Edition, Brown Company Publishers, Dubuque.
4. Cervený, J., Meyer, J. D. and Hall, P. A. 2009. Food Microbiology and Food Safety, "*Microbiological Spoilage Meat and Poultry Products*". *Compendium of the Microbiological Spoilage of Foods and Beverages*, Springer, PP. 69-86.
5. Chun, J. Y. Kim, A. Lee, D. Song, K. 2009. Effect of UV-C Irradiation on the Inactivation of Inoculated Pathogens and Quality of Chicken Breasts during Storage. *Food Control*. **21**:276-280.
6. Coma, V., Martial-Gros, A., Garreau, S., Copinet, A., Salin, F. and Deschamps, A. 2002. Edible Antimicrobial Films Based on Chitosan Matrix. *J. Food Sci.*, **67**(3): 1162-1169. <http://dx.doi.org/10.1111/j.1365-2621.2002.tb09470.x>
7. Darmadji, P. and Izumimoto, M. 1996. Effect of Chitosan on Meat Preservation. *Indonesian Food and Nutrition Progress*, **3**(2): 51-56.
8. Dave, D. and Ghaly, A. E. 2011. Meat Spoilage Mechanisms and Preservation Techniques: A Critical Review. *Am. J. Agric.*

- Biol. Sci.*, **6(4)**: 486-510.
<https://doi.org/10.3844/ajabssp.2011.486.510>
9. De Vuyst, L. and Leroy, F. 2007. Bacteriocins from Lactic Acid Bacteria: Production, Purification, and Food Applications. *J. Mol. Microbiol. Biotechnol.*, **13**: 194-199.
<https://doi.org/10.1159/000104752>
 10. Devlieghere, F., Vermeulen, A. and Debevere, J. 2004. Chitosan: Antimicrobial Activity, Interactions with Food Components and Applicability as a Coating on Fruit and Vegetables. *Food Microbiol.*, **21(6)**: 703-714.
 11. Elsaid, A. E., Abdallah, F. A. M. and Hadir, M. A. 2018. Preservative Effect of Chitosan Coating on Shelf Life and Sensory Properties of Chicken Fillets during Chilled Storage. *J. Nutr. Food Sec.*, **3(3)**: 139-148.
 12. Embusead, M. E. and Huber, K. C. 2009. Edible Films and Coating for Meat and Poultry. In: *“Edible Films and Coatings for Food Application”*. Springer, USA, PP. 245-268.
 13. Fan, W., Chi, Y., Zhang, S. 2008. The use of a Tea Polyphenol Dip to Extend the Shelf Life of Silver Crap (*Hydopthalmichthys molitrix*) during Storage in Ice, *Food Chemistry*, **108**: 148 – 153.
 14. Gill, C. 1986. The Control of Microbial Spoilage in Fresh Meats. *Advances in meat Res.* (USA).
 15. Hyytia, E., Hielm, S., Mokila, M., Kinnunen, A., Korkeala, H. 1999. Predicted and Observed Growth and Toxigenesis by Clostridiumtype E in Vacuum Packaged Fishery Products Challenge Tests. *Int. J. Food Microbiol.*, **47**: 161 - 169.
 16. Ghanbarzadeh, B. and Orommieh, A. R. 2008. Studies on Glass Transition Temperature of Mono and Bilayer protein Films Plasticized by Glycerol and Olive Oil. *J. Appl. Polym. Sci.*, **109(2848)**: 28-54.
<http://dx.doi.org/10.1002/app.28289>
 17. Guilbert, S. and Gontrad, N. 1995. Edible and Biodegradable Food Packaging In: *“Foods and Packaging Materials-Chemical Integrations”*. The Royal Society of Chemistry, Cambridge, UK, PP. 159-168.
 18. Hassanzadeh, P., Tajik, H., Razavi Rohani, S. M., and Moradi, M. 2017. Effect of Functional Chitosan Coating and Gamma Irradiation on the Shelf-Life of Chicken Meat during Refrigerated Storage. *Radiation Phys. Chem.*, **141**: 103-109.
 19. Jonaidi Jafari, N. J., Kargozari, M., Ranjbar, R., Rostam, H. and Hamed, H. 2017. The Effect of Chitosan Coating Incorporated with Ethanolic Extract of Propolis on the Quality of Refrigerated Chicken Fillet. *J. Food Process. Preserv.*, **42**:e13336.
<https://doi.org/10.1111/jfpp.13336>
 20. Jeevaratnam, K. Jamuna, M and Bawa, M. A. 2004. Biological Preservation of Foods-Bacteriocins of Lactic Acid Bacteria. *Indian J. Biotech.* **4**:446-454.
 21. Kerry, J. Grady, M. and Hogan, S. 2006. Current and Potential Utilization of Active and Intelligent Packaging Systems for Meat and Muscle-Based Products. *Meat Sci.*, **74**: 113-130.
<https://doi.org/10.1016/j.meatsci.2006.04.024>
 22. Kubota, N. & Kikuchi, Y. 1999. Macromolecular Complexes of Chitosan. In: *“Polysaccharides: Structural Diversity and Functional Versatility”*, (Ed.): Dumitriu, S. Dekker, New York, USA, PP. 595-628.
 23. Naidu, S. A. 2000. *Natural Food Antimicrobial System*. First Edition, CRC Press, Washington, 818 PP.
 24. Polidori, P. 2007. Tenderness Evaluation and Mineral Levels of Llama (*Lama glama*) and Alpaca (*Lama pacos*) meat. *Meat Sci.*, **77**: 599-601.
<https://doi.org/10.1016/j.meatsci.2007.05.011>
 25. Sagoo, S. Board, R. and Roller, S. 2002. Chitosan Inhibits Growth of Spoilage Microorganisms in Chilled Pork Products. *Food Microbiol.*, **19(2-3)**: 175-182.
 26. Salva, B. K. 2009. Nutrient Composition and Technological Quality of Meat from Alpacas Reared in Peru. *Meat Sci.*, **82**: 450-455.
<https://doi.org/10.1016/j.meatsci.2009.02.015>
 27. Settanni, I. and Corsetti, A. 2007. Application of Bacteriocins in Vegetable Food Biopreservation. *Int. J. Food Microbiol.*, **121**: 123-138.
<https://doi.org/10.1016/j.ijfoodmicro.2007.09.001>
 28. Sharafati Chaleshtori, F. Taghizadeh, M. Rafeian-kopaei, M. and Sharafati-Chaleshtori, R. 2016. Effect of Chitosan Incorporated with Cumin and Eucalyptus Essential Oils As Antimicrobial Agents on Fresh Chicken Meat. *J. Food Process. Preserv.*, **40(3)**: 396-404.
 29. Sotoudeh, B. Zand, N. and Ebrahimimi, M. 2013. The Usage of MAP for Shelflife



- Extension of Packed Spicy Chicken Meal. *Eur. J. Experiment. Bio.*, **36**: 17-623.
<http://www.imedpub.com/articles/the-usage-of-map-for-shelf-life-extension-of-packed-spicy-chicken-meal.pdf>
- Soultos, N. Tzikas, Z. Abraham, A. Georgantelis, D. and Ambrosiadis, I. 2008. Chitosan Effects on Quality Properties of Greek Style Fresh Pork Sausages. *Meat Sci.*, **80(4)**: 1150-1156.
 - TAO, W. U., Zivanovic, S., Draughon, W. A., Conway, C. S. 2005. Physicochemical Properties and Bioactivity of Fungal Chitin and Chitosan. *J. Agric. Food Chem.* **53**: 3888-3894
 - Testin, R. Verrgano, P. 1990. Food Packaging, Food Protection and the Environment. A Workshop Report. IFT Food Packing Division, The Institute of Food Technologists, Chicago, IL.
 - Verrgano, R. P. 1990. *Food Packaging, Food Protection and the Environment*. United States Department of Agriculture, USDA. <https://www.usda.gov/topics/health-and-safety>
 - Wang, Y., Zhu, J., Zhu, R., Lai, Z., Chen, Z 2003. Chitosan/Prussian Blue-based Biosensors. *Meat Sci. Technol.* **14**: 831-836.
 - Warriss, P. D. 2010. *Meat Science: An Introductory Text*. CAB International Publishers, USA.

ارزیابی پوشش کیتوزان-نیسین بر روی ویژگی‌های کیفی فیله مرغ تازه در شرایط یخچالی

ب. ستوده، م. ح. عزیزی، ع. میرمجیدی هشتجین، ر. پورااحمد، و ح. نوکلی پور

چکیده

گوشت طیور سریع فاسد می‌شود لذا لازم است در شرایط مناسب نگهداری شود. یکی از راه‌های نگهداری این محصول استفاده از پوشش‌های ضد میکروبی است. در این تحقیق از پوشش کیتوزان و کیتوزان-نیسین در شرایط یخچالی در دمای ۴ درجه سانتی‌گراد استفاده گردید. به همین منظور نمونه‌ها به ۳ گروه: بدون پوشش به‌عنوان نمونه کنترل، نمونه با پوشش کیتوزان، نمونه با پوشش کیتوزان-نیسین که سپس همه نمونه‌ها در سلفوفان بسته‌بندی شدند و مورد ارزیابی میکروبی (شمارش کلی باکتری‌ها، شمارش سالمونلا و شمارش استافیلوکوکوس اورئوس کواگولاز مثبت) و ویژگی‌های فیزیکی (شیمیایی (PH، رنگ سنجی، بافت) در طی روزهای ۷، ۱۴، ۲۱، ۲۸ گردیدند. پوشش کیتوزان-نیسین نتیجه مؤثرتری را نسبت به پوشش کیتوزان داشت. پوشش کیتوزان-نیسین تا ۷ روز از رشد کلی باکتری‌های سالمونلا و استافیلوکوکوس اورئوس کواگولاز مثبت جلوگیری کرد و باعث افزایش ماندگاری مرغ تازه در شرایط یخچال با دمای ۴ درجه سانتی‌گراد شد. همچنین پوشش کیتوزان تا ۳ روز باعث افزایش ماندگاری مرغ تازه در شرایط یخچال شد و تأثیر آن بر ممانعت از رشد کلی باکتری‌ها تا روز سوم بود و همچنین تأثیر ضد باکتری کیتوزان بر روی سالمونلا و استافیلوکوکوس اورئوس کواگولاز مثبت تا روز پنجم بود.