



## *Preservative Effect of Chitosan Coating on Shelf Life and Sensory Properties of Chicken Fillets during Chilled Storage*

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### ABSTRACT

**Background:** Chicken fillets contain essential amino acids besides many minerals and vitamins, which are necessary for maintaining life and promoting growth. Moreover, it is low in calories and cholesterol; therefore, it can be used for feeding infants, young children, and some patients. **Methods:** Chicken fillets were initially coated by dipping in different concentrations of chitosan (1.0%, 1.5%, and 2.0%), and then the shelf life of coated samples was investigated under refrigeration storage ( $4 \pm 1$  °C) for 15 days. The control (uncoated) and coated samples were analyzed periodically for bacteriological, pH value, and sensory characteristics. **Results:** The sensory evaluation results correlated with the microbial analyses. Chitosan-coated samples achieved a shelf-life extension of 12 days at chilled storage temperature ( $4 \pm 1$  °C) whereas the non-coated samples had a shelf life of 3 days at the same storage temperature. There were no significant organoleptic changes within the chitosan-coated samples ( $P > 0.05$ ). The pH values of all coated samples were significantly lower than the control group ( $P < 0.05$ ). However, the obtained data revealed that chicken fillets samples coated with chitosan (1.0%, 1.5%, and 2.0%) led to a significant reduction ( $P < 0.05$ ) of the total aerobic bacterial count (TBC), total Enterobacteriaceae, and total Staphylococcus counts along the storage period. **Conclusion:** The present study established that application of chitosan coating on chicken fillets could have a potential for preserving the microbiological quality and enhancing sensory attributes during chilled storage.

**Keywords:** *Chicken fillets; Chilled storage; Chitosan coating; Microbiological quality; Shelf life*

### Introduction

Consumers all over the world favor chicken meat because of its low fat, low cholesterol content and high nutritional value including essential amino acids, proteins, a good source of vitamins, minerals and other growth factors. However, chicken meat is a highly perishable food

commodity that provides an almost perfect medium for microbial growth, including both spoilage and pathogenic microorganisms (Vasilatos and Savvaiddis, 2013). The spoilage of fresh poultry products is an economic burden to the producer; consequently, developing methods to

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extend the shelf life and overall quality represents a major task of the poultry processing industries. By increasing the consumer demand for minimum processing, preservative-free, more stable and safe foods, the development of natural preservative with high antibacterial activities for improving the quality and extending the shelf life of food products is desirable. In this regard, the application of natural antimicrobial and/or antioxidant edible coatings have become a novel way to maintain the freshness and quality of foods (Lopez-Caballero *et al.*, 2005, Ojagh *et al.*, 2010, Wan *et al.*, 2007).

Chitosan and its derivatives, which are natural, biodegradable, bio-renewable, and nontoxic, represent the most promising agents for effective preservation of food (Rosca *et al.*, 2005). It is usually prepared from chitin (2 acetamido-2-deoxy b-1, 4-D-glucan) and it has been found in a wide range of natural sources (crustaceans, fungi, insects, annelids, molluscs etc.). However, chitosan, commercially produced from processing the waste of crustaceans, is an important biopolymer that possesses antimicrobial and antioxidant activity as well as enormous economic value. The inhibitory effect of chitosan depends on its concentration, molecular weight and type of bacteria (Zheng and Zhu, 2003). This polymer is given the generally recognized as safe (GRAS) status, which is a safety guarantee in use as a natural food component (Shepherd *et al.*, 1997, Terbojevich and Muzzarelli, 2000).

Chitosan have drawn much attention and have been considered for applications in the food industry due to its particular physico-chemical properties, short time biodegradability, biocompatibility with human tissues, film-forming and barrier properties against pathogenic microbes, antimicrobial and antifungal activities, and non-toxicity (Hassanzadeh *et al.*, 2017, Özdemir and Gökmen, 2017, Yu *et al.*, 2017). Chitosan coatings have been investigated as a microbial hurdle in some meat products (Georgantelis *et al.*, 2007, Giatrakou *et al.*, 2010, Kanatt *et al.*, 2013, Roller *et al.*, 2002, Yingyuad *et al.*, 2006). Other potential applications of chitosan as biopreservative have also been studied in fresh or frozen seafood

(Chaiyakosa *et al.*, 2007, Duan *et al.*, 2010, Jeon *et al.*, 2002). This is mainly due to a fact that chitosan exhibits a good antimicrobial activity against many pathogenic and spoilage microorganisms, including gram positive and gram-negative bacteria, molds and yeasts (Jeon *et al.*, 2001, Kong *et al.*, 2010, Lee *et al.*, 2003, Prashanth and Tharanathan, 2007, Tsai *et al.*, 2002). The antimicrobial activity of chitosan is largely dependent on deacetylation degree, molecular weight, pH value, and type of microorganism (Dutta *et al.*, 2009, Lim and Hudson, 2003). Moreover, it exhibits antioxidative activity when is used as a food additive because of its ability to chelate metal ions involved in the catalysis of oxidative reactions (Agulló *et al.*, 2003).

Although chitosan has been shortly reviewed in particular for antimicrobial and antioxidant properties, which are useful in the food industry to enhance food quality and shelf life (Aider, 2010, Hamed *et al.*, 2016, No *et al.*, 2007), no much data exist on the application of antimicrobial edible coatings in meat systems. Recently, research endeavors have focused on the application of these natural antimicrobials to meats as a novel option to preserve them against spoilage and pathogenic microbes. Thus, the objectives of this study were to evaluate the effects of chitosan on the sensory, pH value, and bacteriological characteristics of chicken fillets as well as the shelf life of chitosan-coated chicken fillets under refrigerated condition ( $4 \pm 1$  °C).

## Materials and Methods

*Preparation of the chitosan solutions:* Chitosan of low molecular weight (MW = 340) in powder from crab shells was obtained from Marine Hydrocolloids Company (Meron, India). The moisture content was less than 10%, and chitosan had a deacetylation degree of 75-85%. Briefly, 1 g of chitosan was dissolved in 100 mL of 1% (w/v) glacial acetic acid for preparation of chitosan 1% (1.5 g and 2 g of chitosan were used to prepare chitosan 1.5% and 2%, respectively), and then stirred with a magnetic stirrer for 3 h at 55 °C (Fernandes *et al.*, 2012).

*Preparation of the chicken fillets:* Fresh chicken breasts meat (skinless and boneless fillet, each slice weight 200 g) were purchased from a local market and immediately transported to the laboratory. The chicken fillets were divided into four groups, including uncoated group (Control), and three coated groups (Group I, II and III). The Control group consists of chicken fillet dipped in sterilized distilled water for 1.5 min. For the three coated groups, samples were individually dipped in different concentrations of chitosan (Group I, 1%), (Group II, 1.5%), and (Group III, 2%) for 1.5 min. The excess solution was drained off immediately after dipping. Finally, all samples were stored in refrigeration condition ( $4 \pm 1$  °C), and bacteriological, chemical and sensorial tests were performed on zero, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, and 15<sup>th</sup> day of storage.

*Sensory evaluation:* It was performed according to Petrou *et al* (Petrou *et al.*, 2012). Seven panelists were asked to evaluate the acceptability (total sensory evaluation score) as a composite of odor, taste and appearance using a nine-point hedonic scale. The scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (first off-odor, off-taste development) < 6; a score of 6 was taken as the lower limit of acceptability. The sample was defined as unacceptable after development of first off-odor or off-taste.

*Measurement of pH value:* The determination of the pH values of different chicken samples were done according to the method described by (Basiri *et al.*, 2014). The pH value was measured in duplicate by homogenizing 10 g of whole ground chicken fillet with 90 mL of deionized water for 1 min and was kept at room temperature for 10 min. The pH values of the supernatant solution of homogenate was recorded by using a pH meter (Schott pH meter, mode CG824, Germany) at each sampling interval over the storage period.

*Bacteriological analyses:* Duplicate samples (10 g) from the coated and uncoated samples were homogenized with 0.1 % sterile peptone water (90 mL) in a Stomacher (Seward, BA6021, UK) for 1

min. One mL of the original homogenate was transferred into a sterile test tube containing 9 mL of 0.1 % sterile peptone water solution then appropriate serial dilutions were carried out. For the total aerobic plate count (International Organization for Standardization (ISO), 2013) one mL of each previously prepared serial dilution was carefully transferred into separate, duplicate, appropriately marked Petri dishes, and thoroughly mixed with about 15 mL of previously melted and adjusted ( $45 \pm 1$  °C) plate count agar. After solidification, the inoculated plates as well as the control one were inverted and incubated promptly for  $48 \pm 2$  h at 37 °C. A volume of 0.1 mL from each prepared dilution was evenly spread into duplicated plates of violet red bile glucose agar (VRBGA) incubated at 37 °C (for 24 h) for Enterobacteriaceae counts (International Organization for Standardization (ISO), 2004), and Baird-Parker agar medium (37 °C for 24 h) for enumeration of Staphylococcus spp. (International Organization for Standardization (ISO), 1999). All media for the bacteriological analyses were purchased from HiMedia Laboratories, Mumbai, India. The results were expressed as the logarithm of the colony forming units per gram (log CFU/g).

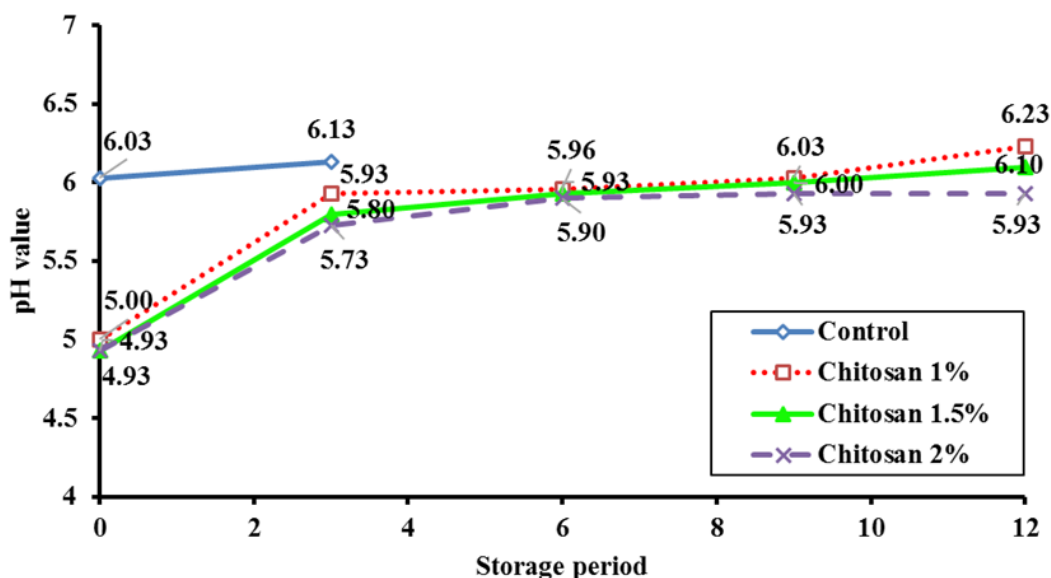
*Data analysis:* Analyses were run in triplicate ( $n = 3$ ) on different occasions with different chicken meat samples. Results were reported as mean values  $\pm$  standard errors (SEs). Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to investigate the statistically significant differences between means ( $P < 0.05$ ). The bacterial counts were converted to log CFU/g and were subjected to ANOVA using the SPSS software package, version 20 (SPSS Inc., Chicago, Ill).

## Results

The sensory evaluation results of chicken fillets immersed in different concentrations of chitosan (1.0%, 1.5%, and 2.0%), and control samples during zero, 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup>, and 15<sup>th</sup> day of refrigerated storage are represented in **Table 1**. The sensory scores (appearance, color, odor, texture, and overall acceptability) of uncoated

samples were given acceptable scores by the third day but not measured on 6<sup>th</sup> day due to the presence of spoilage signs (slimy appearance and off-odor). However, chitosan-coated samples had an acceptable sensory score till 12<sup>th</sup> day of storage. **Figure 1** illustrates the changes in pH value of control and chitosan-coated samples during storage at the temperature of ( $4 \pm 1$  °C). The lowest amounts of pH at zero time for 1%, 1.5% and 2% chitosan-coated samples were 5.00, 4.93, and 4.93, respectively; while the highest amounts were 5.93, 6.10, and 6.23, respectively on the 12<sup>th</sup> day. **Table 2** shows the effect of chitosan coating on the microbiological quality of control and chitosan-coated chicken fillets during storage at  $4 \pm 1$  °C. The initial total aerobic bacterial count (TBC) in uncoated samples was 6.29 log CFU/g, which reduced to 5.54, 5.19, and 4.95 log CFU/g in 1%, 1.5%, and 2% chitosan-coated samples, respectively. Moreover, the mean value of TBC increased to 6.87 log CFU/g in uncoated samples after 3 days of storage; whereas, coated samples had counted in the range of 4.81-5.42 log CFU/g. After 12 days of storage, the TBC mean was

ranged 5.99-6.97 log CFU/g in chitosan-coated samples. Regarding Enterobacteriaceae counts, the initial analysis of uncoated samples showed that the mean value of total Enterobacteriaceae count was 5.73 log CFU/g; whereas, the values were in the range of (4.14-4.60 log CFU/g) in the chitosan-coated samples. Moreover, the mean value of Enterobacteriaceae count increased to 6.31 log CFU/g in uncoated samples after 3 days of storage; whereas, the mean value was ranged (4.86-4.93 log CFU/g) in the coated samples. After 12 days of storage, the mean count of Enterobacteriaceae was ranged 5.99-6.97 log CFU/g in chitosan-coated samples. On the other hand, the initial Staphylococcal count was 5.65 log CFU/g; whereas, the mean values was ranged (3.20-3.48 log CFU/g) in the chitosan-coated samples. Furthermore, the mean value of Staphylococcal count increased to 6.14 log CFU/g in uncoated samples after 3 days of storage; whereas, the mean value was ranged (4.01-4.23 log CFU/g) in the coated samples. The mean values Staphylococcal count was in the range of (5.61-6.26 log CFU/g) in chitosan-coated samples after 12 days of storage.



**Figure 1.** Changes in pH value of control and chitosan-coated samples (1%, 1.5 % and 2%) during storage at the temperature of ( $4 \pm 1$  °C).

**Table 1.** Sensory characteristics of control and chitosan coated chicken fillet samples during chilled storage at ( $4 \pm 1$  °C).

Storage period	Uncoated samples		Chitosan-coated chicken fillets		
	Control	Chitosan 1%	Chitosan 1.5%	Chitosan 2%	
Zero day	9.67 ± 0.33 <sup>A</sup>	9.67 ± 0.33 <sup>A</sup>	9.67 ± 0.33 <sup>A</sup>	9.67 ± 0.33 <sup>A</sup>	
3 <sup>rd</sup> day	8.00 ± 0.58 <sup>B</sup>	7.83 ± 0.17 <sup>B</sup>	7.67 ± 0.17 <sup>B</sup>	7.67 ± 0.17 <sup>B</sup>	
6 <sup>th</sup> day	NA	7.67 ± 0.17 <sup>B</sup>	7.67 ± 0.17 <sup>B</sup>	7.17 ± 0.17 <sup>B</sup>	
9 <sup>th</sup> day	NA	7.33 ± 0.33 <sup>B</sup>	7.17 ± 0.17 <sup>B</sup>	7.17 ± 0.17 <sup>B</sup>	
12 <sup>th</sup> day	NA	6.67 ± 0.67 <sup>B</sup>	6.67 ± 0.67 <sup>B</sup>	7.00 ± 0.58 <sup>B</sup>	
15 <sup>th</sup> day	NA	NA	NA	NA	

Means carrying different superscript letters on the same column are significantly different ( $P < 0.05$ ). NA= sample not analyzed as it had spoiled. Samples were considered spoiled if total bacterial counts were above 7 log CFU/g or had sensory score less than 6.

**Table 2.** Effect of chitosan coating on the microbiological quality of control and chitosan-coated chicken fillets during chilled storage at  $4 \pm 1$  °C.

Microbiological quality	Storage period	Uncoated samples		Chitosan-coated chicken fillets		
		Control	Chitosan 1%	Chitosan 1.5%	Chitosan 2%	
Total aerobic bacterial count	Zero day	6.29 ± 0.39 <sup>Aa</sup>	5.54 ± 0.19 <sup>Ab</sup>	5.19 ± 0.17 <sup>Ab</sup>	4.95 ± 0.39 <sup>Ab</sup>	
	3 <sup>rd</sup> day	6.87 ± 0.07 <sup>Aa</sup>	5.42 ± 0.62 <sup>Ab</sup>	5.13 ± 0.48 <sup>Ac</sup>	4.81 ± 0.15 <sup>Ac</sup>	
	6 <sup>th</sup> day	AD	6.24 ± 0.31 <sup>A</sup>	5.43 ± 0.63 <sup>A</sup>	5.27 ± 0.55 <sup>A</sup>	
	9 <sup>th</sup> day	AD	6.31 ± 0.71 <sup>A</sup>	6.07 ± 0.69 <sup>A</sup>	5.99 ± 0.56 <sup>A</sup>	
	12 <sup>th</sup> day	AD	6.97 ± 0.49 <sup>A</sup>	6.68 ± 0.79 <sup>A</sup>	5.99 ± 0.56 <sup>A</sup>	
	15 <sup>th</sup> day	AD	AD	AD	AD	
Total Enterobacteriaceae count	Zero day	5.73 ± 0.13 <sup>Aa</sup>	4.60 ± 0.30 <sup>Ab</sup>	4.18 ± 0.16 <sup>Ab</sup>	4.14 ± 0.09 <sup>Ab</sup>	
	3 <sup>rd</sup> day	6.31 ± 0.17 <sup>Aa</sup>	4.93 ± 0.54 <sup>Ab</sup>	4.87 ± 0.44 <sup>Ac</sup>	4.86 ± 0.54 <sup>Ac</sup>	
	6 <sup>th</sup> day	AD	5.99 ± 0.34 <sup>A</sup>	5.05 ± 0.50 <sup>A</sup>	5.03 ± 0.55 <sup>A</sup>	
	9 <sup>th</sup> day	AD	6.01 ± 0.97 <sup>A</sup>	5.41 ± 0.76 <sup>A</sup>	5.25 ± 1.15 <sup>A</sup>	
	12 <sup>th</sup> day	AD	6.52 ± 0.44 <sup>A</sup>	6.48 ± 0.72 <sup>A</sup>	6.32 ± 0.64 <sup>A</sup>	
	15 <sup>th</sup> day	AD	AD	AD	AD	
Total <i>Staphylococcus</i> count	Zero day	5.65 ± 0.29 <sup>Aa</sup>	3.48 ± 0.18 <sup>Cb</sup>	3.46 ± 0.16 <sup>Bb</sup>	3.20 ± 0.10 <sup>Bb</sup>	
	3 <sup>rd</sup> day	6.14 ± 0.38 <sup>Aa</sup>	4.23 ± 0.57 <sup>BCb</sup>	4.07 ± 0.61 <sup>ABb</sup>	4.01 ± 0.62 <sup>ABb</sup>	
	6 <sup>th</sup> day	AD	4.67 ± 0.15 <sup>ABC</sup>	4.53 ± 0.19 <sup>AB</sup>	4.27 ± 0.22 <sup>AB</sup>	
	9 <sup>th</sup> day	AD	5.42 ± 0.47 <sup>AB</sup>	5.31 ± 0.51 <sup>A</sup>	5.09 ± 0.53 <sup>A</sup>	
	12 <sup>th</sup> day	AD	6.26 ± 0.36 <sup>A</sup>	5.84 ± 0.19 <sup>A</sup>	5.61 ± 0.28 <sup>A</sup>	
	15 <sup>th</sup> day	AD	AD	AD	AD	

Means carrying different superscript letters on the same column are significantly different ( $P < 0.05$ ). AD = Apparent Decomposition. Samples were considered spoiled if total bacterial counts were above 7 log CFU/g or had sensory score less than 6. Results are (mean±SE) of three independent experiments.

## Discussion

The initial scores of sensory attributes in the samples were not affected by chitosan coating. These findings suggested that chitosan coating of samples did not lead to any off-flavor and the appearance of the products was not objectionable,

either of which could potentially lead to rejection of products by the consumer. The obtained results revealed higher sensorial scores in chitosan-coated samples, which indicate the effects of chitosan coating on preserving sensory characteristics of chicken meat. The results were in line with the

result of Hassanzadeh *et al* (Hassanzadeh *et al.*, 2017). These attributes may be explained as Furda and Knorr (Furda, 1980, Knorr, 1983) who reported that chitosan demonstrated lipid-binding and water binding capacities. Therefore, the sample containing chitosan had a better sensory appearance than the control sample. Moreover, chitosan has antioxidant properties and may maintain redness in muscle foods, due to its ability to act as a chelator on transition of metal ions, which catalyze oxidation of myoglobin (Yen *et al.*, 2008).

The pH values of all treated samples were significantly lower than control ( $P < 0.05$ ) during storage. This direct effect was related to the acidic properties of chitosan solution and prevention of microbial growth on the surface of the samples. However, the pH values increased gradually with increasing storage period due to endogenous enzymes, bacterial metabolites and volatile organic compounds as amines (Gill, 1986). The obtained results were coincided with Sharafati Chaleshtori *et al* and Hassanzadeh *et al* who reported that chitosan-coated samples had lower pH values than the uncoated samples; furthermore, the chitosan coating application in chicken meat samples could stabilize the pH value during storage (Hassanzadeh *et al.*, 2017, Sharafati Chaleshtori *et al.*, 2016).

Chitosan has been documented for its excellent film-forming property and broad antimicrobial activity against bacteria and fungi (Nadarajah *et al.*, 2006, Rabea *et al.*, 2003). The antimicrobial activity of chitosan is associated with its unique polycationic character, which interrupts the microbial cell membrane (Helander *et al.*, 2001). Furthermore, chitosan as a coating solution or film act as an oxygen barrier around the bacterial cell and thus prevent the growth of aerobic bacteria (Shahidi *et al.*, 1999, Zheng and Zhu, 2003). A significant difference was observed between the control and chitosan-coated samples for the microbiological quality. The obtained data revealed that, chicken fillets samples treated with different concentrations of chitosan (1%, 1.5% and 2%) led to a significant reduction ( $P < 0.05$ ) of TBC, Enterobacteriaceae and Staphylococcus

counts over the time of storage period. The observed reduction in microbial counts can be attributed to the inhibitory effect of chitosan on spoilage bacteria (Helander *et al.*, 2001, Knorr, 1991, Young *et al.*, 1982). However, samples were considered spoiled if total bacterial counts were above 7 log CFU/g or had a sensory score less than 6. In the current study, chicken samples treated with chitosan did not exceed the value of 7.0 log CFU/g for TBC, which was considered as the upper acceptability limit for fresh meat (Senter *et al.*, 2000) till the 12<sup>th</sup> day of storage; however, control samples exceeded this limit at 6<sup>th</sup> day. In addition, the obtained results clarified that chitosan-coated samples had the lowest Enterobacteriaceae and Staphylococcus counts at any time of chilled storage particularly the samples coated with chitosan 2% compared to the control samples and this nearly similar to the results of Sharafati Chaleshtori *et al* (Sharafati Chaleshtori *et al.*, 2016).

Based on the achieved results the bacterial reduction was increased by increasing of chitosan concentration. The obtained results have coincided with Darmaji *et al* (Darmadji and Izumimoto, 1996) who reported that chitosan 1% reduced microbial counts by an average of 1-2 log CFU/g in minced beef patties stored at 4 °C for 10 days. Additionally, various studies have been reported the ability of chitosan coating to reduce microbial load in different meat products. Sagoo *et al* (Sagoo *et al.*, 2002) demonstrated that total viable counts, yeasts, and molds were reduced by approximately 1-3 log CFU/g on skinless and standard sausages dipped in a 1% chitosan solution before storage at 7 °C for 18 days. Furthermore, the addition of chitosan at 1% in fresh pork sausages reduced counts by 0.5-1.5 log CFU/g according to Soultos *et al* (Soultos *et al.*, 2008).

From the obtained results and various research works in the literature, it is clear that chitosan can be successfully employed as food preservative or edible coating material because of biological activities that could be used in the food industry to preserve quality and extend the shelf life of various food products. However,

the inhibitory effects of chitosan depend upon the type of chitosan; particularly the molecular weight, the degree of deacetylation, the type of bacterium and the conditions of the medium in which it is applied (Jeon *et al.*, 2001, No *et al.*, 2002). Furthermore, chitosan has the potential to bind to many different food components such as proteins, fats and other anionic substances present 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). Therefore, preparation of chitosan coatings, in view of molecular weight and degree of deacetylation, must be further examined to describe effective use of chitosan in food applications.

### Conclusions

The results of the current study represented that chitosan coating (1%, 1.5 % and 2%) improves the microbial quality and sensory characteristics of chicken fillets under chilled storage ( $4 \pm 1$  °C). The uncoated samples spoiled and had a slimy appearance and off-odor up to 3 days of storage due to rapid microbial growth. In comparison, chitosan-coated samples had an acceptable

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sensory score and the lowest bacterial counts, particularly the samples coated with chitosan 2%, even after 12 days of chilled storage. Due to its antibacterial activity, chitosan coating might be used as a natural preservative to extend the shelf life (up to 12 days) of chicken fillets while preserving quality. Further future studies on the application of chitosan coating alone or in combination with other antibacterial agents such as essential oils, organic acid salts are necessary to control foodborne pathogens in different food products.

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### Authors' contributions

Elsaid A. Eldaly designed the study. Abdallah Fikry A. Mahmoud and Mohamed Abobaker, H collected the samples and carried out the experiments. Authors read and approved the final manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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