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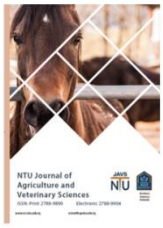

The Effect Interacting of Chitosan as a Biofilm with Nisin on the microbial content Chicken Breast Meat Preserved in Cold Storage

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A B S T R A C T

This study aims to evaluate the effectiveness of the chitosan (ch) and nisin (Ni) composite films on chicken breast meat's microbial content during storage at four °C for 28 days. The results indicated that adding nisin to chitosan increased the inhibitory diameter of Gram-negative and positive bacteria compared to chitosan and nisin alone. Microbial tests of chicken breasts included estimating the total number of all aerobic bacteria, proteolytic Bacterial Count and lipolytic Bacterial Count, the composite films of chitosan -nisin performed better than those of chitosan alone and on the control samples because they revealed a drop in all bacteria during the preservation periods. The addition of nisin to the chitosan casings increased the water vapour (vapor) permeability.

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Introduction

Chitosan, a deacetylated form of the second most abundant natural polysaccharide chitin, is a resource-abundant biopolymer that is recovered mainly from marine crustacean waste. Owing to its intrinsic antimicrobial, metal chelating, film-forming, and ease of chemical modification, it has been widely studied for application in environmental remediation, food preservation, and drug delivery [1].

The biofilm containing Ch in food product packaging has suitable properties that reduce the microbial load. It has been proven that they can inhibit the growth of contamination microbes, ensuring the food product's safety and quality [2]. Chitosan films are considered excellent and ideal because they have intermolecular bonds along the chain that give them gas- and moisture-proof properties [3].

Food packaged with Chitosan coating reduces the counts of all species of microbes that contaminate meat and meat products due to its anti-oxidative rancid properties of lipids and anti-microorganisms [4].

Nisin (34 amino acid residues) produced by a *Lactococcus lactis* subspecies strain is a natural bioactive antimicrobial peptide. Nisin has high antibacterial activity, especially for Gram-positive bacteria, and it can form holes by interacting with bacterial cell membranes [5]. These pores lead to the release of substances in the cell and finally result in the death of bacteria [6]. Besides, nisin with excellent bacteriostatic activity has good edible safety. It has been widely used in the food industry and recognized as a safe food bacteriostat by the World Health Organization (WHO) in 1969, it was approved by the U.S. FDA in 1980 [7].

The study aims to increase the shelf life of chicken breasts by preservation quality of chicken breast flesh wrapped for four weeks at refrigeration temperature by filme chitosan or a mixture of chitosan-nisin filmes. After four weeks of preservation, the meat samples were tested for their ability to withstand microbiological contamination.

MATERIAL AND METHODS

Chicken meat

Freshly slaughtered chicken meat was purchased from different locations in the local markets of Mosul, Iraq. After 48 hours of slaughter, the meat samples were washed with distilled water, and the chicken breasts were isolated and cut into cubes of 3 x 3 x 3 cm^3 [8].

Chitosan

Ch powder was obtained from the company Biorigins (U.K.) Membrane solutions were prepared by the weighting of 10g of dry Ch and dissolved in 1000 mL of distilled water [9]. and all components were mixed using a hot plate magnetic stirrer at a temperature of 55°C for 60 minutes and adding 8-10 mL of glacial acetic acid plus 10 ml of glycerol, then the mixture was mixed well. The pH of the mixture by using ph meter was adjusted to 7, then, it was stored in the refrigerator until use (Figure 1) [10].

Nisin solution preparation

The Nisin was obtained from Smart Kimya (Turkey). It was prepared according to [11]. by dissolving 0.5 g of Nisin powder in 500 ml (0.02 M) of HCl (pH 2). the mixture was filtered through an injection filter fitted with a 0.22 µm hole size using a Syringe filter for sterilizing and purification. The solution was then stored in the refrigerator until it was needed.

Figure 1. films of chitosan.

Evaluation of the Antimicrobial ability of Chitosan and Nisin

The inhibition ability of Chitosan and Nisin against food poisoning-causing isolates of *S. aureus* and *E. coli* that were identified from chicken flesh samples used in the experiment [12]:

The bacterial suspension accounts for each isolate were adjusted at 1.5×10^8 cells/ml using 0.5 McFarland's standard tube solution. Subsequently, 0.1 ml of every suspended solution was taken out and applied to the Muller Hinton Agar culture

medium, and it was left for 15 minutes. Then 100 µL of each treatment was transferred at a concentration of 1% Chitosan and 0.1% Nisin, and a mixture of the two treatments by adding 10% Nisin to the Chitosan was prepared and were transferred to holes with a diameter of 6 mm made on the surface of the culture medium using a sterile Cork-borer. The plates were then incubated at 37 °C for 24 hours. Then, the inhibition ability of each treatment against each bacterial species was determined by measuring the diameter inhibition zone in millimeters [12].

Determination of Film

Using a digital micrometer, Film thickness was measured at eight random positions [13].

Water Vapor Transmission Rate (W.V.T.R.)

The water vapor permeability of chitosan-based edible films was measured using a W.V.T.R. tester (Qualitest Perme-W3/030). The measuring range of the equipment ranged from 0.04 to 1000 g/m^2/24 h. Each film specimen was conditioned for 24 h in a desiccator at 25 °C and 55 % relative humidity before analysis. The test films were cut into round shapes (31 cm^2). The relative humidity and temperature used in the test were 90% and 38 °C, respectively. The water vapor permeability of the edible films was calculated using the following formula; $WVTR = \Delta W/(A.t)$ ΔW = change in sample weight (gm), A = sample area (m^2), T = sample placement time (Sec) [14].

Tensile strength and elongation testing

Measuring the tensile strength and elongation of samples using the universal testing machine (H10KT/USA), at the headquarters of the Industrial Research and Development Authority/National Center for Packaging and Packaging/Bagdad, which depends mainly on preparing the sample with a width of 10 mm x a length of 100 mm, where the sample is placed vertically inside the device, which has a holder on both sides. It begins to pull in two different directions until the sample is crushed [15].

Packaging of chicken breast samples

Chicken breast samples were prepared by cutting them into pieces weighing 30-35 g per sample to ensure that the envelopes completely contained the samples, and they were covered with wrappers as follows: First: chicken breasts without packaging, second: The chicken breasts are coated with the previously prepared chitosan wrap alone, third: Chicken breasts coated with Chitosan and 10% Nisin. The samples were packaged individually for each group and put inside cork boxes. The relevant information for each type was put on the outside of the box to separate the samples. The boxes were then stored in the refrigerator at 4°C. Various estimates were made on uncoated and coated chicken breasts using the treatments used in the research, at a rate of every seven days of storage in the refrigerator at 4°C for 28 days and with three replicates, as follows.

Figure 2. Chicken breast coated with chitosan.

Bacteria total counts

The total number of aerobic Bacteria was calculated by the method [16].

Total Proteolytic Bacterial Count

The total number was calculated by the method [17].

Total Lipolytic bacteria Count

The total number was calculated by the method [17].

Statistical Analysis

The experiment was carried out using the SAS program (2002) and the averages were statistically analyzed using Duncan Duncan's multiple range test according to [18]

RESULTS AND DISCUSSION

The antibacterial activity against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S.aurous*) was evaluated for the treatments used in the experiment. Figure (4) showed that Chitosan at a rate of 1% gave good effectiveness as an antibacterial against gram-negative bacteria with an inhibitory diameter of 7mm and those that were gram-positive with a diameter of 9mm. These results agreed with [19]. According to [20].the results also demonstrated that Nisin was unsuccessful against *E. coli*, while it was efficient against *S. aureus* bacteria, exhibiting a 13 mm inhibitory diameter. The results also confirmed the efficacy of the Chitosan and Nisin combination, demonstrating the sensitivity of *E. Coli* and *S. aureus* to the combination with inhibitory diameters of 10 mm and 15 mm, respectively Figure (3). The synergistic effect of Chitosan and Nisin increased the antibacterial efficacy [21].

Figure 3. Antibacterial activity of the chitosan+ nisin complex on *E.coli* and *S. aureus*

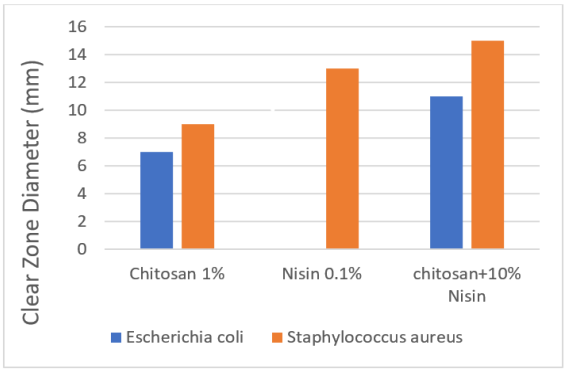

Figure 4. antibacterial activity of chitosan, nisin, and chitosan-nisin against *S. aureus* and *E. coli*

Thickness is an important variable affecting films' properties, such as tensile strength, elongation, and water vapor permeability [22]. The thickness of the film material directly depends on its preparation methods, such as drying, solvent evaporation time, relative humidity, and dish surface [23].Table (1) shows the average thickness of the Chitosan film used in the experiment, 0.081 mm. The results agreed with [24]. The findings demonstrated that the thickness of the membrane, which reached 0.070 mm, was not significantly altered by adding nisin to Chitosan. However, the membrane's tensile strength decreased due to the addition of Nisin. These results may be related to the interactions between the chains of antibacterial agents, which easily penetrated the membrane matrix, so there was greater mobility between the chains, resulting in the production of casings with a small tensile strength, according to [25].

The values of water vapor permeability (WVP) are displayed in Table (1), where it was discovered that chitosan produced a permeability of 55.27 g/m^2. The water vapor permeability of Nisin and Chitosan was 55.282 g/m^2 for 24 hours. The results proved that the addition of Nisin to the Chitosan gave a higher water vapor permeability compared to the rest of the coefficients, where the result agreed with [26].

Table 1. Thickness, water vapor permeability (WVP) and Tensile strength (TS) of Chitosan's and Chitosan-Nisin's films.

Treatment	Chitosan	Chitosan+ Nisin
Thickness (mm)	0.080 ± 0.03 a	0.070 ± 0.03 a
TS (MPa)	21 ±0.03 a	9.7 ±0.79 b
WVP (g/ m^2.24h)	55.27± 0.07 b	55.28± 0.09 a

The different letters in the column indicate that there is a significant difference at (P <0.05)

which showed that increasing the values of water vapor permeability with increasing the addition of antimicrobial agents.

Testing the total count of aerobic bacteria contaminated of chicken's breast samples is essential to know the duration of the efficiency of the packaging and the packaging process. The results (Figure 5) showed that the total number of aerobic bacteria isolated from uncoated samples was 4.86 log10 cfu/g. A gradual increase in these numbers occurred during the preservation storage at 4°C, while a decrease in the total number of bacteria in Chitosan-coated samples occurred during the 7,14, and 21-day preservation periods compared to the control. These outcomes agreed with the findings of [27].

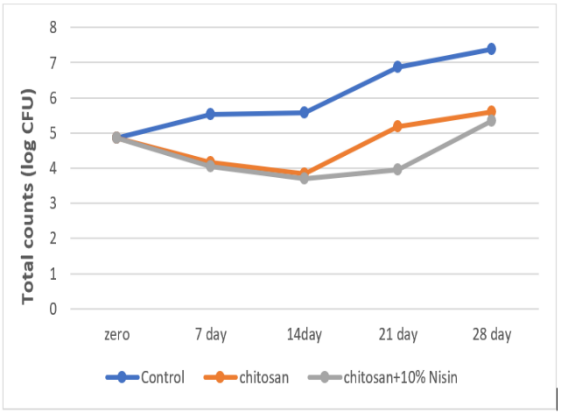

Figure 5. Bacteria total count (log_{10} CFU/g) of Chicken Breast samples treated with chitosan and chitosan-Nisin after storage for 28 days at four °C

Due to the synergistic activity, the data also demonstrated a decrease in the bacterial counts for the Chitosan-Nisin coated samples compared to other samples throughout four preservation periods.

The results Figure (6) showed the total number of proteolytic bacteria for non-coated chicken breast samples at zero time 3.32 Log10/g. after that, an

increase in these numbers was observed with the continuation of the preservation process; after 28 days of preservation, it reached 4.62 Log10/g at a temperature of 4 m°. The results converged with [28], which confirmed an increase in the total number of proteolytic bacteria with continued preservation periods While a decrease in the bacterial numbers of the chitosan-coated chicken breast sample was found by 3.25 Log10/g after 7 days of keeping the same temperature compared to the control sample, after that, the gradual increase in these numbers continued during the other periods of preservation; the highest value was estimated at 4.37 Log10/g, which was low compared to the control sample. A decrease in the values of the total number of proteolytic bacteria was observed for chicken breast samples coated with chitosan-coated fortified with Nisin compound after 7, 14, 21 days of preservation, reaching 3, 3.23, 3.45 log10 cfu/g.

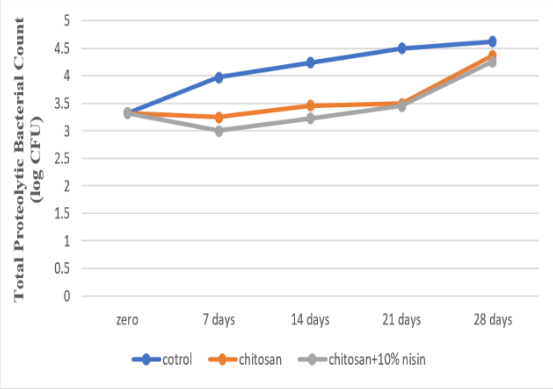

Figure 6. Total proteolytic bacteria count (log10 CFU/g) of Chicken Breast samples treated with chitosan and chitosan-Nisin after storage for 28 days at four °C

The results of Figure (7) show the total number of lipolytic bacteria isolated from unwrapped chicken breast samples coated with chitosan films with Nisin added at a temperature of 4 m° for 28 days; the total number of bacteria for unwrapped chicken breast samples (zero time) was 2.28 Log10/g. the results agreed with [28]. While a decrease in the numbers of these bacteria was observed during the first period of preservation (after 7 days) of the chicken breast sample coated with chitosan and chitosan with nisin, the numbers of which reached 2.22 and 2.04 Log10/g, respectively, compared to the control sample. After that, a gradual significant increase in the number of bacteria was observed during the periods of preservation, reaching the highest after 28 days of preservation; the values reached 3.86, 3.47 and 3.40 Log10/g for the sample of chicken breast uncoated and coated with chitosan and chitosan with Nisin, respectively.

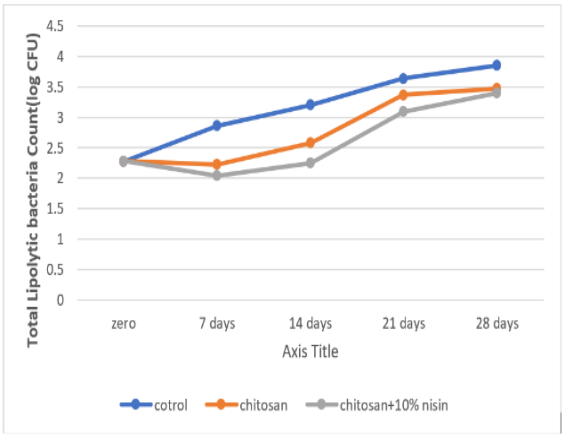

Figure 7. Total proteolytic bacteria count (log10 CFU/g) of Chicken Breast samples treated with chitosan and chitosan-Nisin after storage for 28 day at four °C

Conclusion

The research results showed an increase in the shelf life of chicken breast meats coated with Ch and Nisin, and the composite coatings of both proved their effectiveness by acting as an antimicrobial. To boost the effectiveness of the coatings in preserving food, more needs to be done to improve their tensile strength and water vapor permeability. This can be achieved by adding additional natural materials to the coating.

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Competing Interests

The author reports no conflicts of interest and is responsible for the content and writing of the paper.

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