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EFFECTIVENESS OF CHITOSAN AND AG-NANOPARTICLE FILMS ON THE QUALITY OF CHICKEN MEAT

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ABSTRACT

This study aims to evaluate the effectiveness of the composite films of chitosan (Ch) and silver nanoparticles (AgNPs) biosynthesized by the *Aspergillus niger* on the chemical qualities and microbial content of chicken breasts meat during storage at 4°C for 28 days. Over storage time pH, moisture content, chicken breast meat peroxide number were analyzed. The results indicated that the films of Ch alone or Ch-AgNPs had increased the values of all parameters during the preservation periods. Regarding the microbial content, the total bacterial count decreased in both Ch and Ag-NPs over time compared to the control. The lowest level was found in Ch-AgNPs samples. These findings demonstrated that the meat samples coated with either Ch or Ch-AgNPs performed better than those without coating during storage time. The Ch-AgNPs film became thicker after AgNPs were added. The chicken breast sample coated with Ch-AgNPs preserved the moisture content, pH values, peroxide number, percentage of F.F.A and bacterial counts better compared to the Ch sample and compared after 28 days of preservation, the values were %75.91, 6.9, 0.42 (mgO2/100g), %0.95, 5.08 (log10 cfu/g) respectively, which caused prolongation of the shelf life of the Ch-AgNPs sample.

INTRODUCTION

Nanoscience is the science that studies materials on a scale between 1 and 100 nanometers, which exhibit physical and chemical properties different from materials whose actual dimensions exceed 100 nanometers. There is a change in surface area, melting point, freezing point, and some other properties at the nano level compared to the assembly of molecules at a higher level (Filipponi and Sutherland, 2013). The nanomaterials on which most research is conducted are usually powders composed of nanoparticles that exhibit properties different from powders of the same chemical composition but with much larger partials. Its potential in the food nanotechnology sector, including food packaging, foods and dietary supplements, is being researched due to its unique functionality and applications of nanomaterials (Chaudhry *et al.*, 2008).

Ag has been known since ancient times as an effective antimicrobial agent for treating diseases, preserving food and keeping water safe (Maillard and Hartemann, 2013). Particular attention is paid to AgNPs due to their antimicrobial (Al-Mahmood, 2022). Antioxidant activity, and potential use as drug delivery agents and other applications (Gudikandula *et al.,* 2017). AgNPs are metallic particles within the nanosize range and exhibit physical properties different from those of the base metal ions (Velusamy *et al*., 2016).

Ch is a dietary fibre with chains of linear polysaccharides derived from chitin, the second most common natural polysaccharide after cellulose (Muthu *et al.,* 2021). This compound's first discovery and research dates back to the nineteenth century (Khoushab and Yamabhai, 2010; Iber *et al.*, 2022). Ch currently refers to a group of biopolymer materials obtained in chitin's chemical or enzymatic deacetylation to varying degrees. Ch is a linear polymer consisting of D-glucosamine and N-acetyl-D-glucosamine linked together by β-(1 → 4) glycosidic bonds (Hidangmayum *et al.*, 2019).

Using antibacterial and anti-fungal coatings in packaging food products reduces the microbial load. Studies have demonstrated their capacity to prevent the development of particular microbes, ensuring the food product's safety and quality. Natural antimicrobial compounds where the most interest of researchers to improve the food safety (Ibrahim *et al.,* 2023). Research has proven using coatings in packaging food that they can inhibit the growth of specific types of microorganisms, ensuring the food product's safety and quality (Ahmed and Varshney, 2011). Coatings made of Ch were considered excellent and ideal because they have intermolecular bonds along the chain, which give them gas and moisture-impermeable properties (Florez *et al.,* 2022).

Coating foods with Ch reduced the numbers of lactic acid bacteria, aerobic mesophilic bacteria, and the amount of total volatile essential nitrogen. It also inhibited *Staphylococcus aureus* bacteria in meat stored for 15 days ،Ch can be used as a preservative in the meat and meat products industry due to its anti-oxidative rancidity properties of fats and microorganisms (Duran and Kahve, 2020). The research aimed to investigate the reliability of nanofilms made of chitosan alone or incorporated with AgNPs to preserve chicken breast meat under refrigeration temperature.

MATERIALS 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.

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 (Vásconez *et al.,* 2009) 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 (Sun *et al.,* 2021).

Synthesis of Nanoparticles

In potato dextrose broth (PDB), *A. niger* was cultivated aerobically to prepare biomass for biosynthetic experiments. Following *A. niger* inoculation, the flasks were shaken at 120 rpm and kept at 25 °C in an orbital shaker. After growing for 72 hours, the biomass was extracted by passing it through the Whatman filter paper (No.1). The harvested biomass was washed three to four times with distilled water to remove any medium component from the biomass. In Erlenmeyer flasks, the biomass (about 20 g, fresh weight) was mixed with 150 mL of distilled water for 24 hours at 25 °C while agitated at 120 rpm. Following incubation, Whatman filter paper (No. 1) separated the fungal filtrate from the biomass. For the synthesis of nanoparticles, 50 mL of $AgNO_3$ (1 mM) was mixed with 50 ml of fungal filtrate in 250 ml Erlenmeyer flasks. The mixture was shaken in the dark at 25 °C and 120 rpm. The control group, which contained only filtrate and no Ag ions, were stored with the experimental flasks (Gursoy, 2020).

Detection of Nanoparticles

Following a two-hour incubation period, the absorbance of the fungal filtrate containing nanoparticles was determined using a UV-visible spectrophotometer (Perkin Elmer, Labda-25). The experiments were performed in triplicate. According to (Bhainsa and D'souza 2006), the medium changes colour from yellow to dark brown when AgNPs are produced.

Scanning Electron microscope (S.E.M.)

For S.E.M., a drop of an aqueous solution containing the AgNPs was placed on the carbon-coated copper grids and dried under an Infrared lamp. Samples were measured by an S.E.M. device (Inspect F50 Fei company- Holland) (Vigneshwaran *et al.,* 2007).

Synthesis of edible films

900 ml of prepared Ch was mixed with 100 ml of previously scheduled AgNPs (v/v). The mixture was mixed using a magnetic stirrer at room temperature for two hours to ensure the distribution of AgNPs within the Ch solution. After pouring this solution into plastic plates with an inner diameter of 13 cm and volume of 18 mL, the plates were allowed to dry for 48–72 hours at room temperature (25–30 °C). Subsequently, the films were taken off the plates to wrap the chicken breast meat, which was refrigerated for four weeks at 4°C.

Determination of Film

Using a digital micrometer, Film thickness was measured at eight random positions (Han Lyn and Nur Hanani, 2020).

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) (Souza *et al.,* 2019).

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 (Briassoulis and Giannoulis, 2018).

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 Ch with 10% AgNPs. The samples were packaged individually for each group and put inside cork boxes using the previously described methods. 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 four °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 four °C for 28 days and with three replicates, as follows:

Moisture content

It was estimated according to method decribed by (Sebo *et al.,* 2019).

pH value

The Senso Direct ph200 meter was used to determine the pH value. A 5:1 ratio of distilled water was applied as the sample solvent. After that, the electrode of the pH meter was dipped into the sample solution, and the pH value was noted (Nardoia *et al.,* 2018).

Peroxide value (P.V.)

P.V. was measured according to (Calvo *et al.,*2017).

Bacteria total number

Calculate the total count of bacteria of contaminated meat. Take out 5 g of chicken breast sample was taken and the sample was mixed with 20 ml of distilled water in a volumetric flask, then the mixture was shaken well to obtain a homogeneous mixture and the first dilution was considered 10:1, then the appropriate dilution was carried out to obtain the ideal numbers in the dishes, the spreading method was used to estimate the total number of bacteria in chicken breast samples by drawing 0.1 ml of the appropriate dilution and spreading it on the nutrient medium Agar, then the dishes were incubated at a temperature of 37 °C for 24 hours(Da Silva *et al.,* 2018).

RESULTS AND DISCUSSION

The yellow color of the *A. niger* mycelial cell filtrate is evident before it is immersed in $AgNO_3$. The color changed from the initial stage to yellowish-brown after being incubated for 24 hours (Figure 1). The brown color indicated the medium's formation of AgNPs. Similar color findings were reported in some other studies.

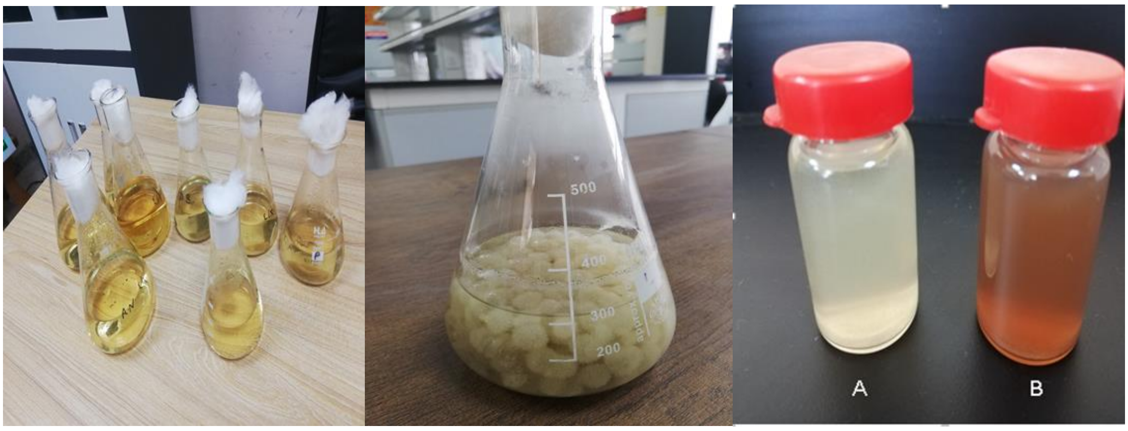

Figure (1): Cell-free filtrate of A. niger: (a) without (b) without and with AgNO3 solution (1mM) after 24 hours of reaction.

It could be attributed to the nitrate reductase and anthraquinone of the fungi, which allows for the form of Ag ions to be obtained by filtering AgNPs using the *A. niger* (Elsayed *et al.*, 2018).

UV-Vis Spectroscopy

The color intensity of AgNPs biosynthesized by fungal biosynthesis was confirmed by measuring absorbance at different wavelengths in the 300–600 nm range to identify the most incredible surface plasmon resonance. The most significant peak of AgNPs was seen at 360 nm (Figure 2), confirming that AgNPs production has occurred, the results agreed with (Iqtedar *et al.*, 2019).

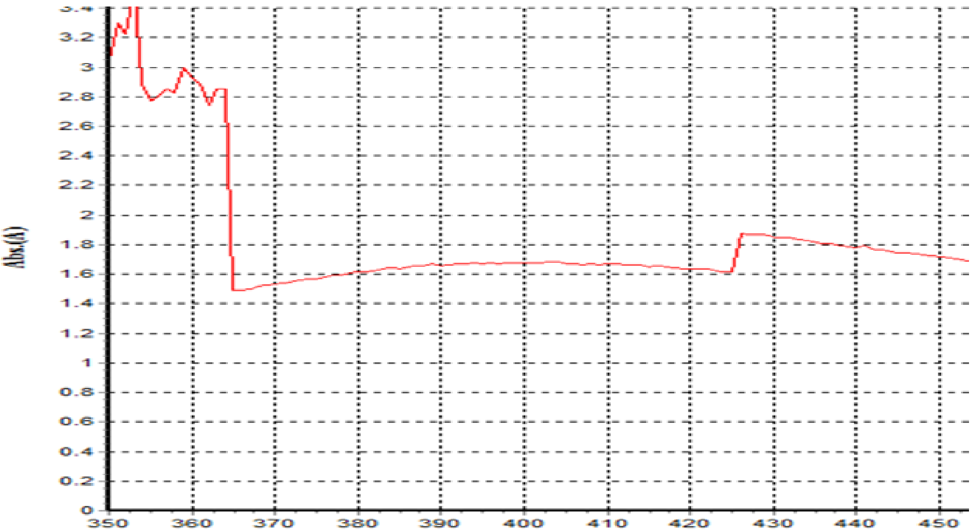

Figure (2): UV-Vis spectrum of AgNPs biosynthesized by A. niger

Scanning electron microscopy (S.E.M.)

The morphological examination of AgNPs was investigated using a scanning electron microscope. Images from the S.E.M. micrograph of these AgNPs showed a rough surface appearance with a diameter range of particles from (30-60) nm (Figure 3) (Nehal and Singh, 2022).

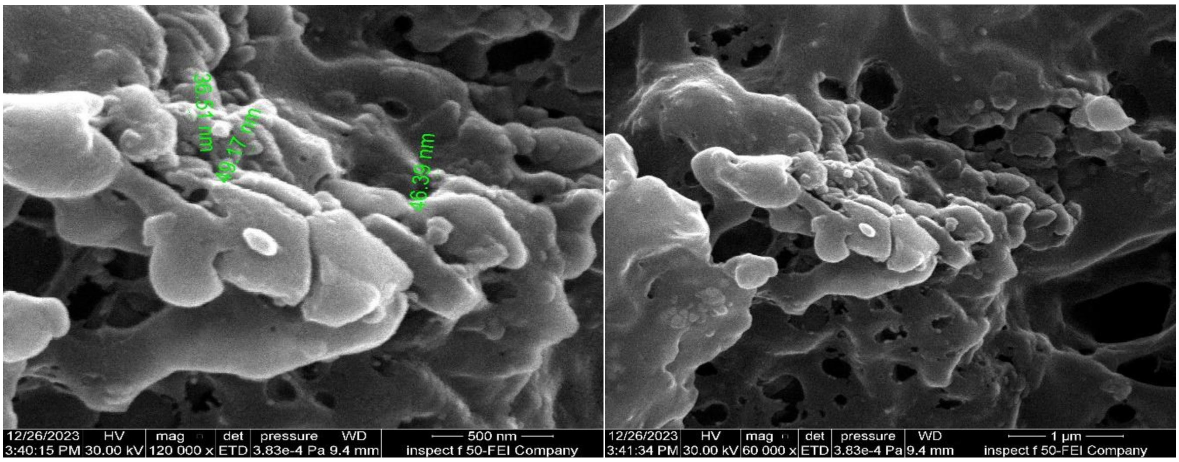

Figure (3): S.E.M. images of AgNPs.

Barrier properties of membranes

Thickness is an important variable that affects the properties of films, such as tensile strength, elongation, and water vapour permeability (Siracusa *et al.,* 2018). Table (1) shows the average thickness of the Ch film used in the experiment, which was 0.081 mm, which was agreed with (Zheng *et al.* 2019). The addition of AgNPs increased the thickness of the Ch-AgNPs film to 0.120 µm, which may be due to the increase in total solid content. At the same time, sample Ch produced a W.V.P.R. of 55.27 g/m^2/24 hours. In contrast, a slight decrease was found for the Ch-AgNPs sample, which was 55.196 /m^2/24 hours. AgNPs aggregated together to create a more tortuous channel for water vapour diffusion, mainly responsible for decreased W.V.P. in the Ch-AgNPs film. These results agreed with previous studies (Qiao *et al.,* 2019). Tensile strength was increased in the Ch films in the presence of AgNPs, given that the tensile strength of Ch by itself was 21 MPa, but the addition of AgNPs increased it to 29 MPa. The outcomes concurred with (Shankar *et al.* 2021).

Table (1): Thickness, water vapour permeability and Tensile strength of Chitosan-based composite films.

Sample	(MPa) T.S.	WVP (g/m^2/24h)	Thickness (mm)
Ch	21± 0.03 b	55.270± 0.07 a	0.080± 0.03 a
Ch-AgNPs	29± 0.42 a	55.196± 0.04 b	0.120± 0.03 a

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

pH value

Since there is no significant difference (p<0.05) between the Ch sample and the control sample during the first week of preservation, results (Figure 4) indicate that the pH value of the chicken breast samples coated with Ch and CH-AgNPs increases gradually over the preservation period. A significant difference (P<0.05) was indicated in the pH values of the chicken breast samples coated with CH-AgNPs compared to the control sample for all preservation periods. However, there were significant differences (P<0.05) during the final three weeks of preservation. The unwrapped samples from the first, second, third, and fourth weeks had pH values of 6.1, 6.42, 6.8, and 7.4, respectively, which agreed with the findings (Febrianta *et al.,* 2021). The samples coated with Ch had pH values of 6.1, 6.18, 6.73, and 7, whereas those covered with Ch-AgNPs had pH values of 6, 6.1, 6.25, and 6.9 throughout four weeks. The high pH values are due to the increased microbial content during the preservation period (Sharma *et al.*, 2017).

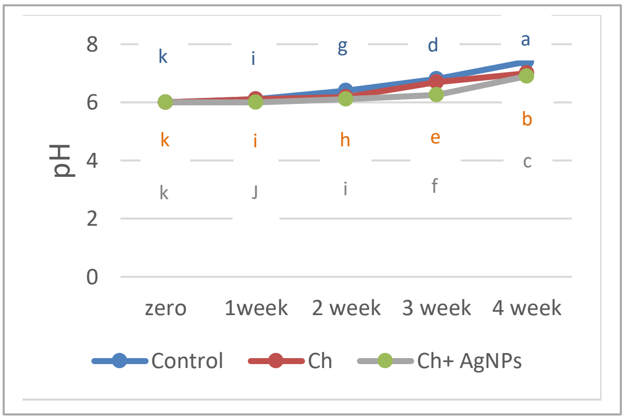

Figure (4): pH value in chicken breast samples wrapped with Ch and Ch-AgNPs after storage for 28 days at 4 °C.

Moisture content

Moisture content gradually decreased throughout the storage periods, as demonstrated by Figure (5). The results showed that the meat samples coated with Ch were reasonable compared to the unwrapped meat samples in maintaining the meat's moisture during storage periods. They were 75.98, 75.59, 75.22, and 74.53% after 1, 2, 3, 4 weeks in terms of, respectively, while the two samples of chicken breasts coated with Ch-AgNPs showed superiority over the rest of the samples as the moisture content was 76.50, 76.42, 76.23, and 75.91%, respectively, due to the combined composition of Ch-AgNPs, which forms a network that reduces moisture penetration from the samples. These findings are agreed with (Zhang *et al.*, 2019).

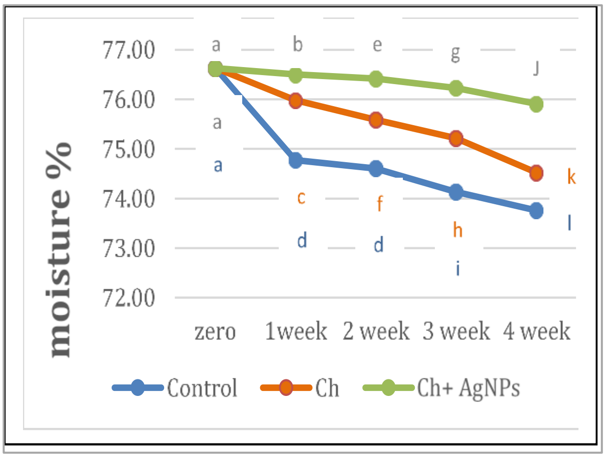

Figure (5): moisture content of chicken breast samples wrapped with Ch and Ch-AgNPs after storage for 28 days at 4 °C.

Peroxide value

Figure (6) showed the P.V. of chicken breast samples at (time Zero), which amounted to 0.12 meq O_2/kg fat. Subsequently, an increase in the value of the P.V. occurred with an increase in the duration of preservation, reaching the end of the preservation period of 1.7 meq O_2/kg, of which they agreed with Sa (Febrianta *et al.*, 2021). There was a lower increase for chicken breast samples coated with Ch, which amounted to 0.2, 0.41, 0.54 and 0.93 meq O_2/kg fat, respectively, and Ch-AgNPs, which amounted to 0.13, 0.19, 0.26 and 0.42 meq O_2/kg fat, respectively, due to the efficiency of Ch and AgNPs as an antioxidant (Sadallah and Alhaji, 2021).

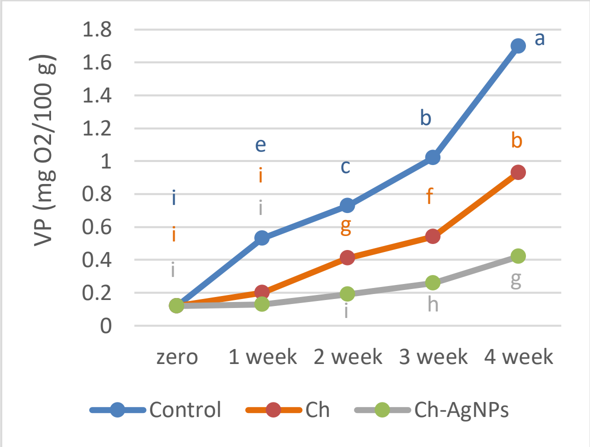

Figure (6): PV of chicken breast samples wrapped with Ch and Ch-AgNPs after storage for 28 days at 4 °C.

Total bacteria count

Figure 7. shows the total counts of aerobic bacteria isolated from chicken breast samples. The results indicated that the bacteria count at the beginning of the storage period (zero time) was log 4.87 cfu/g. A decrease in the number of bacteria was observed for the Ch and Ch-NPs coated chicken breast samples after 1 and 2 weeks of preservation, the decrease in the Ch-NPs membrane coated sample continued after 3 weeks of preservation compared to the Ch sample and the control sample. There was an increase in the count of bacteria to reach log10 7.38 cfu/g at the end of the preservation period of the control sample at four °C compared to those coated with Ch and Ch-NPs. However, the samples coated with Ch-NPs had the lowest microbial content. These outcomes coincided with the study conducted by (Incili *et al.*, 2021).

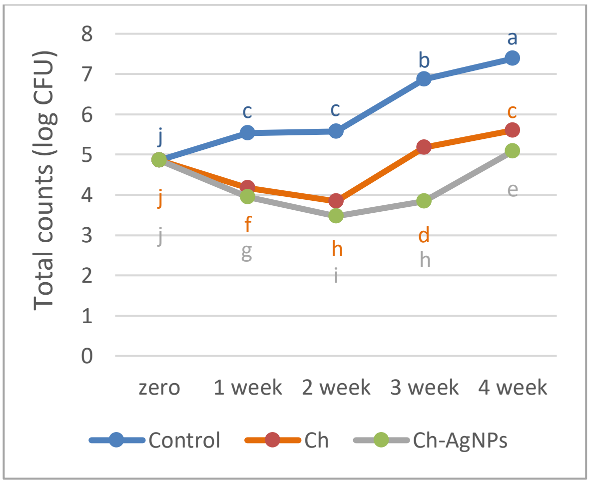

Figure (7): total bacteria count (log10 cfu/g) of chicken breast samples wrapped with Ch and Ch-AgNPs after storage for 28 days at 4 °C.

CONCLUSIONS

The results suggested that coatings containing Ch or Ch-AgNPs could preserve chicken breast meat by acting as antioxidants, antimicrobial agents, and shelf-life extenders. More research should be done on using Ch in combination with AgNPs or other naturally occurring antioxidant and antibacterial agents to preserve a range of food products.

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CONFLICT OF INTEREST

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

فاعلية أغشية الكيتوسان وجزيئات الفضة النانوية على جودة لحم الدجاج

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الخلاصة

تهدف هذه الدراسة إلى تقييم فعالية الأغشية المركبة من الكيتوسان (Ch) وجزيئات الفضة النانوية (AgNPs) المصنعة حيويا بواسطة *Aspergillus niger* على الصفات الكيميائية والمحتوى الميكروبي للحم صدور الدجاج أثناء تخزينها عند درجة حرارة 4 درجات مئوية لمدة 28 يوما. خلال فترة التخزين، تم تحليل الرقم الهيدروجيني ومحتوى الرطوبة ورقم البيروكسيد في لحم صدور الدجاج. أشارت النتائج إلى أن أغشية -Ch AgNPs وحدها أو Ch-AgNPs قد زادت من قيم جميع المعاملات خلال فترات الحفظ. فيما يتعلق بالمحتوى الميكروبي، انخفض العدد الإجمالي للبكتيريا في كل من Ch وAg-NPs مع مرور الوقت مقارنة بعينة السيطرة، حيث لوحظ أدنى مستوى في العينات المغلفة بأغشية Ch-AgNPs. وأظهرت هذه النتائج أن عينات اللحوم المغلفة إما Ch أو Ch−AgNPs كان أداؤها أفضل من تلك التي لم يتم تغليفها أثناء وقت التخزين اذ كانت النسبة المئوية. أصبح غشاء Ch-AgNPs أكثر سمكًا بعد إضافة AgNPs. حافظت عينة صدور الدجاج المغلفة بـ Ch-AgNPs على محتوى الرطوبة وقيم الأس الهيدروجيني ورقم البيروكسيد ونسبة الاحماض الدهنية الحرة وأعداد البكتيريا بشكل أفضل مقارنة بعينة Ch بعد 28 يومًا من الحفظ اذ كانت القيم 75.91% و6.9 و (mgO2/100g) 0.4 و0.95% و(log10 cfu/g) 5.08 على التوالي، مما أدى الى اطلة العمر الافتراضي للعينة المغلفة بغشاء الـ Ch-AgNPs.

الكلمات المفتاحية: جسيمات الفضة النانوي، مضاد ميكروبي، الخصائص الكيميائية، الاغشية النانوية.

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