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Effect of Chitosan Powder and Nanoparticles as Natural Preservatives for Beef Minced Meat

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EA conduct the experiment and wrote the first draft of the manuscript. NM and GK presented the idea of research, did formal analysis of data and supervision.ME perfromed data analysis and interpretation, writing and revising the manuscript. GK revised the manuscript.

Key words
Minced meat, Refrigerated storage, Chitosan, Antioxidant, Microbiological quality

ABSTRACT

The need for natural products to be used as healthy food preservative alternatives for synthetic preservatives is increasing rapidly. This study aimed to compare the effect of chitosan powder (CP) and nanoparticles (CNPs) on the microbiological quality and shelf life of beef minced meat during its refrigerated storage for 9 days. The size of CNPs ranged from 150 to 350 nm as determined by transmission electron microscopy. Comparing the effect of the two chitosan products, CNPs exhibited higher antibacterial properties against *S. aureus* and *E. coli*, as revealed by lower minimal inhibition concentrations than the CP. Among these bacteria, *E. coli* was the most sensitive to CP and CNPs. Minced meat samples treated with CNPs showed significantly lower *E. coli* and total mesophilic counts than samples treated with CP. The pH values and antioxidant properties (as measured by the DPPH radical scavenging assay) of CNPs-treated samples were significantly higher than CP-treated samples. There were no significant differences in organoleptic characteristics between the two treatments. With these antimicrobial and antioxidant properties, CP and CNPs, with a better effect for CNPs, could be used as preservatives for the minced meat to extend its shelf life.

INTRODUCTION

Meat plays a pivotal role in safeguarding human health by giving all essential nutrients such as protein, vitamins, and minerals. However, meat can also act as a vehicle for many microorganisms, which may lead to food spoilage and poisoning, and subsequent severe economic losses. Microbial contamination of meat usually occurs during improper processing practices (Lonergan *et al*., 2019). Microbial spoilage and oxidation of meat are two main obstacles influencing meat product quality and shelf-life. Therefore, evolving techniques to expand meat shelf life could be a big target of the meat processing industry. Many consumers prefer low processed, preservative free, more steady, and unharmed foods. Thus, the evolution of safe natural food preservatives with high antimicrobial and antioxidant properties becomes an urgent demand (de Farias *et al*., 2019; Khan *et al*., 2016; Tayel, 2016).

Chitosan, a derivative of abundantly available chitin, is a biodegradable, bio-renewable, non-toxic natural polymer that is usually prepared from crustacean shell wastes (Kou *et al*., 2021). It has antibacterial, antifungal, and anticancer effects (Elkeiy *et al*., 2018; Khamis *et al*., 2019). In food technology, chitosan acts as the most favorable agent for the effective preservation of food due to not only its safety, health-promoting potential, and low cost but also for its physicochemical properties such as water-binding capacity, bioactivity, and toughness (Khan *et al*., 2016; Kumar *et al*., 2020; Tayel, 2016). Chitosan at a concentration range of 0.5%, 1.5%, 2% has potent antimicrobial properities against a large variety of food spoilage and pathogenic microorganisms (Dutta *et al*., 2009; No *et al*., 2002). Moreover, it has antioxidant effects since it can interfere with free radicals released during oxidative stress (Abd El-Hack *et al*., 2020). As most consumers do not prefer using synthetic antioxidants as food additives, chitosan was successfully used as an alternative antioxidant in food processing and packaging (Sabaghi *et*

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al., 2015). To increase its efficiency, bioavailability, and specificity, chitosan was transformed into its nanoparticle form. Chitosan powder (CP) and nanoparticles (CNPs) were commonly utilized as a vehicle for drug delivery (Huang *et al*., 2004). Moreover, CNPs are reported to possess more potent *in vitro* antimicrobial effects than the parent chitosan (Divya *et al*., 2017). Apart from this previous study, little data are available in the literature regarding the comparison between the preservative effects of chitosan and CNPs on meat products. The present study aimed, therefore, to compare the effect of CP and CNPs on the microbiological quality, antioxidant properties, and shelf life of beef minced meat during its refrigerated storage.

MATERIALS AND METHODS

Materials

Low molecular weight edible chitosan (MW:340 KDa, >10% moisture and 95% degree of deacetylation) prepared from carb shells was purchased from the Marine Hydrocolloids Company (Meron, India) in a powder form. Bacterial media (Nutrient agar, Baird Parker agar, and Eosin Methylene Blue agar) were obtained from Oxoid (Basingstoke, Hampshire, UK). Sodium tripolyphosphate (TPP) and 2, 2-diphenyl-picrylhydarzy1 (DPPH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Food pathogenic Gram-positive bacteria *S. aureus* (ATCC 8095) and Gram-negative bacteria *E. coli* (ATCC 25922) were provided by the Laboratory of Bacteriology, Department of Food Hygiene, Animal Health Research Institute, Dokki, Egypt, and were stored at 4°C. Fresh beef meat was purchased from a local butcher shop in Kafer EL-Sheikh city, Egypt within 3 days of slaughter. Before mincing, meat samples were sterilized by soaking in 10 mg/L sodium hypochlorite for 60 min, followed by three times sterilized distilled water washes.

Preparation of CNPs

CNPs were chemically prepared using sodium TPP as previously described (Du *et al*., 2009). In brief, 100 mL chitosan (2%, w/v) dissolved in acetic acid solution (1%, v/v) were mixed with 4 ml sodium TPP (2%, w/v) dissolved in distilled water. The mixture was first stirred for 1 h, then, was sonicated at 1.5 kW for 10 min using Ultrasonic Homogenizers HD 2070. The resultant CNPs were refined by centrifugation at 10000g for 1 h followed by twice rinsing of the precipitate (CNPs) with distilled water and then freeze-dried.

Characterizations of CNPs

The size and shape of the CNPs were determined using a JEOL transmission electron microscopy (TEM, JEM-2100) at 100 kV. After a brief sonication in ethanol to separate the aggregated dry CNPs, 200 µl of CNPs were mounted on a carbon-coated copper grid covered with nitrocellulose and then examined directly by TEM. Size distribution of CNPs were quantified by dynamic light scattering (DLS) using a Nano ZS zetasizer system (Malvern Instruments) with 633 nm laser wavelength and 173° scattering angle. CNPs size was determined by the average of three measurements and expressed as mean diameter (nm).

Determination of minimum inhibitory concentration

Minimum inhibitory concentrations (MICs) for CP and CNPs on *S. aureus* (ATCC 8095) and *E. coli* (ATCC 25922) were determined by agar dilution method, as recommended by the European Committee for antimicrobial susceptibility testing (EUCAST) (Testing, 2000) and as previously described (Badawy *et al*., 2020). CP and CNPs were tested at concentrations of 4 to 2048 µg/ml and 1.44 to 737.28 µg/ml nutrient agar media in liquid form (before being poured into the Petri dishes), respectively. All dilutions were used in three replicates. Control plates contained nutrient agar free from CP and CNPs. One loopful inoculum of each pathogenic organism (~1×10^4 CFU/g) in nutrient broth medium was spotted (10 spots/plate) on the surface of nutrient agar medium then incubated for 48 h at 37 °C. The minimum concentration of CP and CNPs that inhibited the growth of *S. aureus* and *E. coli*, was considered the MIC.

In vivo *antimicrobial activity*

The minced meat samples (100 g each) were divided into 4 groups as follow: group 1 (G1, –ve control group), samples were left without bacterial inoculation; G2 (+ve control group), samples were inoculated by *E. coli* ($1x10^4$ CFU/g); G3 (CP group), samples were inoculated by bacteria and treated with CP at a concentration of 1g/kg minced meat; and G4 (CNPs group), samples were inoculated by bacteria and treated with CNPs at a concentration of 300 mg/kg minced meat. Each group contained 7 samples and the obtained results were presented as means from three independent experiments. The concentrations of CP and CNPs were chosen based on the results obtained from the *in vitro* antimicrobial assay which showed better inhibition at these two concentrations. The contaminated minced meat samples were thoroughly mixed in polyethylene bags and then homogenized for 3 min at 37°C to confirm the appropriate distribution of the bacteria. A second round of homogenization was also performed following the addition of CP and CNPs. The polyethylene bags containing samples were stored under

aerobic conditions at 4 °C until further use.

Total mesophilic count

Total mesophilic count was carried out according to the technique recommended by ISO. Samples were treated with either CP at a concentration of 1g/kg minced meat or CNPs at a concentration of 300 mg/kg minced meat.

Measurement of pH

The pH of minced meat samples was calculated by the method described by Jansen (2001). A homogenization of 10 g minced meat with 90 mL deionized water was done at room temperature. Following filtration, the pH was measured by a digital pH meter (Schott pH meter, CG824 mode).

DPPH- radical scavenging assay

DPPH (2, 2-diphenyl-1-picryl-hydrazil) radical scavenging assay was performed as previously described (Azaam *et al*., 2018). Minced meat samples (5 g/ sample) were homogenized in methyl alcohol (10 ml). Following centrifugation at 7000 rpm for 7 min, 150 µl of the resultant supernatant was added to 1 ml of 0.1% DPPH. After 1 h incubation in dark, the yellow color was formed, and the absorbance was measured at 515 nm. Ascorbic acid was used as a positive control. The experiment was repeated three times. The DPPH scavenging activity was calculated from this equation: DPPH scavenging (%)= [(control absorbance - sample absorbance) / control absorbance] × 100.

Sensory evaluation

Sensory evaluation was performed as previously described (Petrou *et al*., 2012). A composite of odor, flavor, and appearance on a 9-point scale was requested from seven panelists to determine acceptability (total sensory evaluation). The scale points were excellent (9), very good (8), good (7), bad (6), first off-odor, and off-taste development (< 6). After first off-smelling or off-tasting, the sample was deemed inappropriate.

Statistical analysis

The analytics of statistics were conducted using SAS (Statistical Analysis System, 2004) using one-way ANOVA followed by the Duncan test as a *post hoc* test to determine the difference between groups. Data were presented as mean ± standard error of mean (SEM) and the significant values were set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Identification and characterization of the prepared CNPs

As examined under TEM, CNPs appeared as mono-dispersible spheres with different sizes ranging from 150 to 350 nm (Fig. 1). Size distribution as measured by dynamic light scattering (DLS) showed CNPs sizes ranging from 120 to 450 nm with a mean diameter of 297.6 ± 17.32 nm. Consistent with our findings, other studies also prepared CNPs with similar size ranges (Elkeiy *et al*., 2018; Feyzioglu and Tornuk, 2016; Loutfy *et al*., 2016). However, Badawy *et al*. (2020) prepared smaller CNPs than prepared in this study.

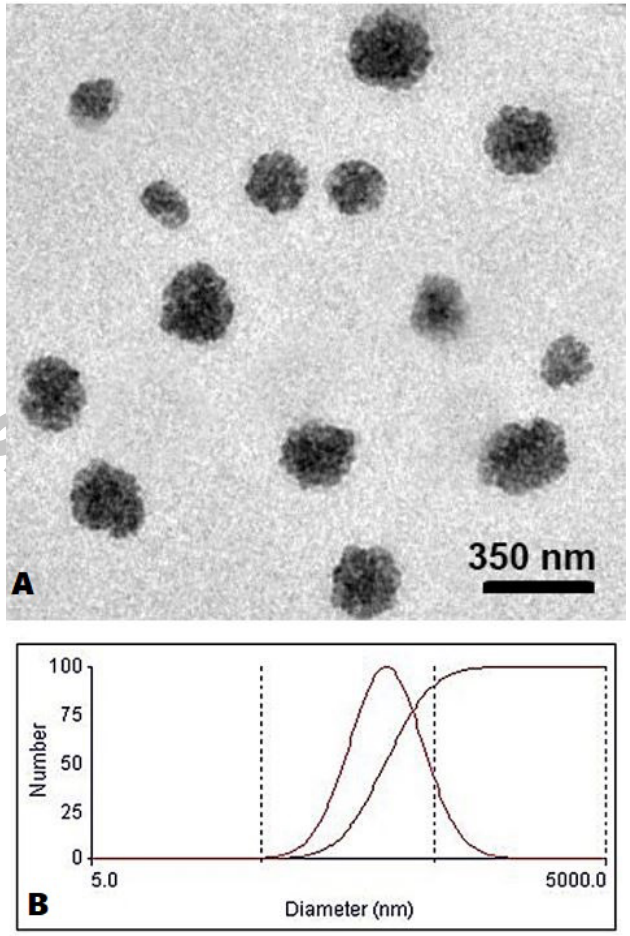

Fig. 1. (A) Transmission electron microscope and (B) size distribution by dynamic light scattering (DLS) show the prepared chitosan nanoparticles with different sizes ranging from 150 to 350 nm and 120 to 450 nm, respectively.

In vitro *inhibitory effect of CP and CNPs on bacteria*

Comparing the effect of the two chitosan products, CNPs exhibited a higher antibacterial effect, as revealed by lower MICs (170 mg/L for *E. coli* and 260 mg/L for *S. aureus*), than the CP (360 mg/L for *E. coli* and 550 mg/L for *S. aureus*). This indicates that *E. coli* is more sensitive to CP and CNPs than *S. aureus* and consequently, we used

E. coli in the further investigation. Nearly close MICs of chitosan against *E. coli* (165 mg/L) were obtained by Badawy *et al*. (2020).

In vivo *inhibitory effect of CP and CNPs on* E. coli

Table I shows counts of *E. coli* in control -, control +, CP, and CNPs groups in the minced meat samples stored at 4 °C for 9 days. The *E. coli* counts at 3, 6 and 9 days of storage were 125.31±1.98, 267.69±2.54, and 296.17±2.86 x 10^4 cfu/g in the control-group, respectively. On the other hand, the control + group exhibited significantly higher counts of 317.54±3.28, 690.33±5.72, and 739.28±6.81 x 10^4 cfu/g than all other groups. In contrast, groups treated with either CP or CNPs showed significant lowered bacterial counts compared to control + and control – groups. In treated groups, minced meat treated with CNPs showed significantly lower counts (35.28±0.63, 39.28±0.75, and 45.06±0.81 x 10^4 cfu/g) compared to the CP group (84.11±1.03, 129.33±1.64, and 156.19±1.75) at 3, 6, and 9 days of storage, respectively. Subsequently, these results infer that treatment of minced meat with either CP or CNPs showed a lower *E. coli* number with best effect for CNPs. Therefore, it would be possible that if fresh minced meat was contaminated accidentally with *E. coli*, the addition of CP or CNPs would decrease the bacterial load.

E. coli count in the control– group was so high and exceeded the acceptable *E. coli* count (6.0 x 10^4 cfu/g) as indicated by other studies (Cao *et al*., 2013; Hu *et al*., 2015). However, Badawy *et al*. (2020) detected a nearly similar higher count in the untreated (control -) minced meat samples. The antibacterial effect of chitosan, when added to meat samples, was previously reported by several studies (Abd El-Hack *et al*., 2020; Badawy *et al*., 2020; Cao *et al*., 2013; Dutta *et al*., 2009; Hu *et al*., 2015) and was attributed to chitosan ability to bind firmly with the negatively charged molecules on bacterial cell membrane causing their damage and leakage of their cytoplasmic contents (Ojagh *et al*., 2010). Chitosan particles also can form a coat surrounding the bacteria and preventing the entrance of oxygen and subsequently death of bacteria (Devlieghere *et al*., 2004). Moreover, the antimicrobial activity of chitosan is largely dependent on its molecular weight and size, as well as the type of microorganism. Indeed, low molecular weight, smaller diameter chitosan as in CNPs showed higher antibacterial activity (Badawy *et al*., 2020; No *et al*., 2002). In support, we also found that CNPs had a higher antibacterial activity which could be attributed to their smaller size and higher penetration ability to bacteria. Gram-negative bacteria, such as *E. coli*, have thin peptidoglycan layer in the cell membrane which could be the cause for higher sensitivity to chitosan (Goy *et al*., 2016). The antimicrobial activity of chitosan could also be due to its high degree of deacetylation properties which can kill many food spoilage and pathogenic microorganisms (No *et al*., 2002).

Effect of CP and CNPs on total mesophilic count

The CNPs group showed significantly lowest total mesophilic counts of 1.15±0.04, 2.59±0.09, and 7.28±0.19 x 10^3 cfu/g at 3, 6 and 9 days of storage, respectively compared to all other groups (Table I). The CP group showed significantly lower total mesophilic counts of 1.86±0.05, 4.67±0.15, and 11.35±0.23 x 10^3 cfu/g than the control– group (1540.23±12.60, 18450.38±25.60, 60210.17±29.53 x 10^3 cfu/g) at 3, 6 and 9 days of storage, respectively. These findings imply that treatment of minced meat with either CP or CNPs showed a lower total mesophilic count with better antibacterial potential for CNPs. Meat samples were considered spoiled if total bacterial counts were above 1 x 10^7 cfu/g. Reducing total mesophilic count is also associated with longer shelf life of meat samples. Indeed, the shelf life increased 3 days more than normal meat after the addition of CP or CNPs.

Table I. Effect of chitosan on *E. coli* and total mesophilic count in minced meat samples stored at 4 °C for 9 days.

Treatment	Day 3	Day 6	Day 9	Total
***E. coli* count (10^4 cfu/g)**				
Control (-)	125.31±1.98 [b]	267.69±2.54 [b]	296.17±2.86 [b]	229.37±2.16 [b]
Control (+)	317.54±3.28 [a]	690.33±5.72 [a]	739.28±6.81 [a]	582.23±4.59 [a]
CP	84.11±1.03 [c]	129.33±1.64 [c]	156.19±1.75 [c]	123.19±1.28 [c]
CNPs	35.28±0.63 [d]	39.28±0.75 [d]	45.06±0.81 [d]	39.62±0.67 [d]
Total mesophilic count (10^3 cfu/g)				
Control (-)	1540.23 ± 12.60 [a]	18450.38 ± 25.60 [a]	60210.17 ± 29.53 [a]	26733.33±23.54 [a]
CP	1.86 ± 0.05 [b]	4.67 ± 0.15 [b]	11.35 ± 0.23 [b]	5.96±0.17 [b]
CNPs	1.15 ± 0.04 [c]	2.59 ± 0.09 [c]	7.28 ± 0.19 [c]	3.67±0.12 [c]

Data were presented as mean ± SEM (n = 3). Columns carrying different letters are significantly different at $p < 0.05$. Before treatment: 130 × 10^4 cfu/g for *E. coli* and 20.03 × 10^3 cfu/g for total mesophilic count. Control (−), minced meat without *E. coli*; control (+), minced meat treated with *E. coli*, CP, chitosan powder; CNPs, chitosan nanoparticles.

Table II. Effect of chitosan on pH and antioxidant activity of minced meat samples stored at 4 °C for 9 days.

Treatment	Day 3	Day 6	Day 9	Total
pH values				
Control (-)	6.37±0.15 ^{aA}	5.92±0.13 ^{abAB}	5.61±0.11 ^{aB}	5.93±0.12 ^{b}
Control (+)	6.03±0.12 ^{bA}	5.57±0.14 ^{cB}	5.55±0.10 ^{bB}	5.71±0.13 ^{c}
CP	6.30±0.19 ^{abA}	6.01±0.16 ^{bAB}	5.84±0.17 ^{aB}	6.05±0.17 ^{b}
CNPs	6.86±0.15 ^{aA}	6.42±0.14 ^{aAB}	6.14±0.12 ^{aB}	6.47±0.13 ^{a}
Antioxidant activities (%)				
Control (-)	42.34± 0.24 ^{bA}	26.16 ± 0.16 ^{cB}	15.82± 0.17 ^{cC}	28.11 ± 0.19 ^{c}
Control (+)	38.47 ± 0.25 ^{dA}	19.22 ± 0.18 ^{dB}	13.58 ± 0.16 ^{dC}	23.76 ± 0.21 ^{d}
CP	40.09 ± 0.22 ^{cA}	30.11 ± 0.20 ^{bB}	24.28 ± 0.21 ^{bC}	31.47 ± 0.20 ^{b}
CNPs	47.33 ± 0.24 ^{aA}	39.71 ± 0.21 ^{aB}	33.67± 0.19 ^{aC}	40.24 ± 0.25 ^{a}

Data were presented as mean ± SEM (n = 3). Columns carrying different lower-case letters are significantly different at $p < 0.05$. Rows of the three time points carrying different upper-case letters are significantly different at $p < 0.05$. Before treatment, pH = 6.52 and the antioxidant activity = 27.46%. Control (−), minced meat without *E. coli;* control (+), minced meat treated with *E. coli*, CP, chitosan powder; CNPs, chitosan nanoparticles.

Effect of CP and CNPs on pH

The pH values of the minced meat samples at 3, 6, and 9 days of storage in the control + group were significantly lower than the control – group (Table II). However, samples treated with either CP or CNPs showed significantly higher pH values compared to the control +. In treated groups, minced meat treated with CNPs showed significantly higher pH values than the CP group. No significant difference was noticed between the control and CP groups. These findings indicate that treatment of minced meat with CNPs showed the highest pH value compared to all other groups. Regarding the three time points, the pH values in all groups were significantly reduced by increasing the storage time. Thus, pH values at day 9 were the lowest.

Minced meat is not appropriate for consumption with a pH below 5.6 or above 7.0 (Karabagias *et al*., 2011). In the present study, the overall mean of the pH values ranged from 5.85 to 6.86 which is compatible with the normal limit of meat pH (5.6–7.0). Similar pH values in beef meat were also recorded by other studies (Badawy *et al*., 2020; Niyonzima *et al*., 2013). Bacterial growth stopped at acidic pH with a minimum limit varied from 4.6 to 5 based on types of bacteria (Claus and Fritze, 1989). The minimal inhibitory pH for *E. coli* is 5 (Cowan and Steel, 1965). Lactic acid produced from glycogen during the storage time of the meat is responsible for lowering pH values from 7 (before slaughtering) to 5.6 (after slaughtering) (Soriyi *et al*., 2008). Protein degradation resulted in excessive production of nitrogenous metabolites which elevate the pH of the meat (Aksu *et al*., 2005).

Effect of CP and CNPs on antioxidant activity

The antioxidant activity (%) of the minced meat samples at 3, 6, and 9 days of storage in the control + group were significantly decreased compared to all other groups (Table II). Treatment with CP or CNPs significantly increased the antioxidant activity compared to the control +. Minced meat treated with CNPs showed a significantly higher antioxidant activity followed by samples treated with CP. Moreover, the antioxidant activity in all groups was significantly reduced by increasing the storage time with lowest % on day 9 and the highest % on day 3.

Similar to our findings, previous studies have also reported higher antioxidant activity for chitosan when used in different forms with the best effect for the CNPs when added to minced meat samples (Badawy *et al*., 2020; Wan *et al*., 2013). It is well-known that potent antioxidants like chitosan can reduce lipid oxidation and so increase the shelf life of the stored meat (Karre *et al*., 2013). Therefore, chitosan as a safe, cheap, and potent antioxidant can be used as food additives (Li *et al*., 2010). CNPs decreased lipid peroxidation marker, MDA (El-Denshary *et al*., 2015; Subhapradha *et al*., 2017) and increased the activities of antioxidant enzymes (SOD, CAT, GPx) to inhibit the overproduction of reactive oxygen species (ROS) and protect tissue from oxidative damage (Elkeiy *et al*., 2018).

Effect of CP and CNPs on sensory evaluation

Table III shows the results of the effect of CP and CNPs on the sensory scores (appearance, color, odor, texture, and overall acceptability) of the beef minced meet at 3, 6, and 9 days of storage. Samples were considered spoiled if had a sensory score less than 6. Accordingly, the sensory scores of control – and + samples were only acceptable (above 6) till day 6 but on day 9 the scores dropped below 6 and spoilage signs (slimy appearance and off-odor) appeared. However, minced meat treated with either CP or CNPs had an acceptable sensory score till day 9 of storage. No significant difference in the sensory scores was noticed between the two treated groups.

Table III. Effect of chitosan on sensory characters of minced meat samples stored at 4 °C for 9 days.

Treatment	Sensory score on			Total
	Day 3	Day 6	Day 9	
Control (-)	8.46±0.29^{bA}	7.04±0.26^{bB}	6.32± 0.25^{cC}	7.27 ± 0.24^{b}
Control (+)	7.92±0.26^{bA}	6.92±0.11^{cB}	6.36±0.10^{cC}	7.07 ± 0.19^{b}
CP	9.42±0.39^{aA}	8.86±0.25^{aB}	7.17±0.26^{bC}	8.48 ± 0.26^{a}
CNPs	9.52±0.38^{aA}	8.94±0.25^{aB}	7.85±0.15^{aC}	8.77 ± 0.24^{a}

Data were presented as mean ± SEM (n = 3). Columns carrying different lower-case letters are significantly different at $p < 0.05$. Rows of the three time points carrying different upper-case letters are significantly different at $p < 0.05$. Before treatment, the mean of sensory score = 9.54±0.21. Control (−), minced meat without *E. coli;* control (+), minced meat treated with *E. coli*, CP, chitosan powder; CNPs, chitosan nanoparticles.

The obtained results revealed higher sensory scores in all samples containing chitosan, which implies the potential of CP and CNPs on preserving sensory characters of minced meat. Our results agreed with those obtained by Alam *et al*. (2017) who also found higher sensory scores for meet treated with chitosan. These improved sensory characteristics may be due to the higher binding affinity of chitosan to lipid and water (Cao *et al*., 2013; de Farias *et al*., 2019; Hu *et al*., 2015) and its antioxidant properties which could keep red color of meat through inhibition of myoglobin degradation in muscles (Sarbon *et al*., 2015). Chitosan can also form a nonpermeable barrier against the passage of oxygen into the muscle fibers and subsequently prevent fiber oxidation (Shleikin and Medvedev, 2014).

CONCLUSIONS

Chitosan powder and nanoparticles had antibacterial activity against *E. coli,* with a potent effect for the nanoparticles form. Treatment of minced meat with any of these chitosan products helps in keeping the quality of meat, increasing antioxidant properties, and improving sensory characteristics. Therefore, chitosan, particularly in the form of nanoparticles, can be successfully used as a food preservative in the food industry to maintain quality and extend the shelf life of various food products.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd El-Hack, M.E., El-Saadony, M.T., Shafi, M.E., Zabermawi, N.M., Arif, M., Batiha, G.E., Khafaga, A.F., Abd El-Hakim, Y.M., and Al-Sagheer, A.A., 2020. Antimicrobial and antioxidant properties of chitosan and its derivatives and their applications: A review. *Int. J. Biol. Macromol*., **164**: 2726-2744. https://doi.org/10.1016/j.ijbiomac.2020.08.153

Aksu, M.I., Kaya, M., and Ockerman, H.W., 2005. Effect of modified atmosphere packaging and temperature on the shelf life of sliced pastirma produced from frozen/thawed meat. *J. Muscle Fd*., **16**: 192-206. https://doi.org/10.1111/j.1745-4573.2005.08404.x

Alam, J., Murshed, H., Rahman, S., and Oh, D., 2017. Effect of chitosan on quality and shelf life of beef at refrigerated storage. *Bangladesh J. Anim. Sci*., **46**: 230-238. https://doi.org/10.3329/bjas.v46i4.36963

Azaam, M.M., Kenawy, E.-R., El-din, A.S.B., Khamis, A.A., and El-Magd, M.A., 2018. Antioxidant and anticancer activities of α-aminophosphonates containing thiadiazole moiety. *J. Saudi Chem. Soc*., **22**: 34-41. https://doi.org/10.1016/j.jscs.2017.06.002

Badawy, M.E.I., Lotfy, T.M.R., and Shawir, S.M.S., 2020. Facile synthesis and characterizations of antibacterial and antioxidant of chitosan monoterpene nanoparticles and their applications in preserving minced meat. *Int. J. Biol. Macromol*., **156**: 127-136. https://doi.org/10.1016/j.ijbiomac.2020.04.044

Cao, Y., Gu, W., Zhang, J., Chu, Y., Ye, X., Hu, Y., and Chen, J., 2013. Effects of chitosan, aqueous extract of ginger, onion and garlic on quality and shelf life of stewed-pork during refrigerated storage. *Fd. Chem*., **141**: 1655-1660. https://doi.org/10.1016/j.foodchem.2013.04.084

Claus, D., and Fritze, D., 1989. Taxonomy of bacillus. In: *Bacillus* (Springer), pp. 5-26. https://doi.org/10.1007/978-1-4899-3502-1_2

Cowan, S.T., and Steel, K.J., 1965. *Manual for the identification of medical bacteria*. Cambridge University Press.

de Farias, B.S., Sant'Anna Cadaval Junior, T.R., and de Almeida Pinto, L.A., 2019. Chitosan-functionalized nanofibers: A comprehensive review on challenges and prospects for food applications. *Int. J. Biol. Macromol*., **123**: 210-220. https://doi.org/10.1016/j.ijbiomac.2018.11.042

Devlieghere, F., Vermeulen, A., and Debevere, J., 2004. Chitosan: Antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Fd. Microbiol*., **21**: 703-714. https://doi.org/10.1016/j.fm.2004.02.008

Divya, K., Vijayan, S., George, T.K., and Jisha, M., 2017. Antimicrobial properties of chitosan nanoparticles: Mode of action and factors affecting activity. *Fibers Polym*., **18**: 221-230. https://doi.org/10.1007/s12221-017-6690-1

Du, W.L., Niu, S.S., Xu, Y.L., Xu, Z.R., and Fan, C.L., 2009. Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydr. Polym*., **75**: 385-389. https://doi.org/10.1016/j.carbpol.2008.07.039

Dutta, P.K., Tripathi, S., Mehrotra, G.K., and Dutta, J., 2009. Perspectives for chitosan based antimicrobial films in food applications. *Fd. Chem*., **114**: 1173-1182. https://doi.org/10.1016/j.foodchem.2008.11.047

El-Denshary, E., Aljawish, A., El-Nekeety, A., Hassan, N., Saleh, R., Rihn, B., and Abdel-Wahhab, M., 2015. Possible synergistic effect and antioxidant properties of chitosan nanoparticles and quercetin against carbon tetrachloride-induce hepatotoxicity in rats. *Soft Nanosci. Lett*., **5**: 36-51. https://doi.org/10.4236/snl.2015.52005

Elkeiy, M., Khamis, A., El-Gamal, M., Abo Gazia, M., Zalat, Z., and El-Magd, M., 2018. Chitosan nanoparticles from Artemia salina inhibit progression of hepatocellular carcinoma *in vitro* and *in vivo*. *Environ. Sci. Pollut. Res. Int*., **27**: 19016-19028. https://doi.org/10.1007/s11356-018-3339-6

Feyzioglu, G.C., and Tornuk, F., 2016. Development of chitosan nanoparticles loaded with summer savory (*Satureja hortensis* L.) essential oil for antimicrobial and antioxidant delivery applications. *LWT Fd. Sci. Technol*., **70**: 104-110. https://doi.org/10.1016/j.lwt.2016.02.037

Goy, R.C., Morais, S.T.B., and Assis, O.B.G., 2016. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on E. coli and S. aureus growth. *Rev. Brasil. Farmacogn.*, **26**: 122-127. https://doi.org/10.1016/j.bjp.2015.09.010

Hu, J., Wang, X., Xiao, Z., and Bi, W., 2015. Effect of chitosan nanoparticles loaded with cinnamon essential oil on the quality of chilled pork. *LWT Fd. Sci. Technol*., **63**: 519-526. https://doi.org/10.1016/j.lwt.2015.03.049

Huang, M., Khor, E., and Lim, L.Y., 2004. Uptake and cytotoxicity of chitosan molecules and nanoparticles: Effects of molecular weight and degree of deacetylation. *Pharm. Res*., **21**: 344-353. https://doi.org/10.1023/B:PHAM.0000016249.52831.a5

Jansen, M.L., 2001. Determination of meat pH–temperature relationship using ISFET and glass electrode instruments. *Meat Sci*., **58**: 145-150. https://doi.org/10.1016/S0309-1740(00)00142-X

Karabagias, I., Badeka, A., and Kontominas, M., 2011. Shelf life extension of lamb meat using thyme or oregano essential oils and modified atmosphere packaging. *Meat Sci*., **88**: 109-116. https://doi.org/10.1016/j.meatsci.2010.12.010

Karre, L., Lopez, K., and Getty, K.J., 2013. Natural antioxidants in meat and poultry products. *Meat Sci*., **94**: 220-227. https://doi.org/10.1016/j.meatsci.2013.01.007

Khamis, A.A., Elkeiy, M.M., El-Magd, M.A., and El-Gamal, M.M., 2019. Chitosan nanoparticles from *Artemia salina* attenuate LPS-induced inflammation in MCF7 cells. *Arab. J. med. Sci*., **2**: 44-47.

Khan, I., Ullah, S., and Oh, D.H., 2016. Chitosan grafted monomethyl fumaric acid as a potential food preservative. *Carbohydr. Polym*., **152**: 87-96. https://doi.org/10.1016/j.carbpol.2016.06.073

Kou, S.G., Peters, L.M., and Mucalo, M.R., 2021. Chitosan: A review of sources and preparation methods. *Int. J. Biol. Macromol*., **169**: 85-94. https://doi.org/10.1016/j.ijbiomac.2020.12.005

Kumar, S., Mukherjee, A., and Dutta, J., 2020. Chitosan based nanocomposite films and coatings: Emerging antimicrobial food packaging alternatives. *Trends Fd. Sci. Technol*., **97**: 196-209. https://doi.org/10.1016/j.tifs.2020.01.002

Li, J., Zivanovic, S., Davidson, P.A., and Kit, K., 2010. Characterization and comparison of chitosan/PVP and chitosan/PEO blend films. *Carbohydr. Polym*., **79**: 786-791. https://doi.org/10.1016/j.carbpol.2009.09.028

Lonergan, S.M., Topel, D.G., and Marple, D.N., 2019. Chapter 12 meat microbiology and safety. In: *The science of animal growth and meat technology* (2nd ed.) (eds. S.M. Lonergan, D.G. Topel and D.N. Marple). Academic Press, pp. 183-204. https://doi.org/10.1016/B978-0-12-815277-5.00012-3

Loutfy, S., Alam El-Din, H., Elberry, M., Allam, N., Hasanin, M., and Abdellah, A., 2016. Synthesis, characterization and cytotoxic evaluation of chitosan nanoparticles: *In vitro* liver cancer model. *Adv. Natl. Sci. Nanosci. Nanotechnol*., **7**: 035008. https://doi.org/10.1088/2043-6262/7/3/035008

Niyonzima, E., Bora, D., and Ongol, M.P., 2013. Assessment of beef meat microbial contamination

during skinning, dressing, transportation and marketing at a commercial abattoir in Kigali city, Rwanda. *Pak. J. Fd. Sci*., **23**: 133-138.

No, H.K., Young Park, N., Ho Lee, S., and Meyers, S.P., 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. *Int. J. Fd. Microbiol*., **74**: 65-72. https://doi.org/10.1016/S0168-1605(01)00717-6

Ojagh, S.M., Rezaei, M., Razavi, S.H., and Hosseini, S.M.H., 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. *Fd. Chem*., **120**: 193-198. https://doi.org/10.1016/j.foodchem.2009.10.006

Petrou, S., Tsiraki, M., Giatrakou, V., and Savvaidis, I.N., 2012. Chitosan dipping or oregano oil treatments, singly or combined on modified atmosphere packaged chicken breast meat. *Int. J. Fd. Microbiol*., **156**: 264-271. https://doi.org/10.1016/j.ijfoodmicro.2012.04.002

Sabaghi, M., Maghsoudlou, Y., and Habibi, P., 2015. Enhancing structural properties and antioxidant activity of kefiran films by chitosan addition. *Fd. Struct*., **5**: 66-71. https://doi.org/10.1016/j.foostr.2015.06.003

Sarbon, N.M., Sandanamsamy, S., Kamaruzaman, S.F.S., and Ahmad, F., 2015. Chitosan extracted from mud crab (*Scylla olivicea*) shells: Physicochemical and antioxidant properties. *J. Fd. Sci. Technol*., **52**: 4266-4275. https://doi.org/10.1007/s13197-014-1522-4

Shleikin, A.G., and Medvedev, Y.V., 2014. Role of peroxidation and heme catalysis in coloration of raw meat. *Acta Sci. Polonorum Technol. Aliment*., **13**: 123-127. https://doi.org/10.17306/J.AFS.2014.2.1

Soriyi, I., Agbogli, H., and Dongdem, J., 2008. A pilot microbial assessment of beef sold in the Ashaiman market, a suburb of Accra, Ghana. *Afr. J. Fd. Agric. Nutr. Dev*., **8**: 91-103. https://doi.org/10.4314/ajfand.v8i1.19182

Subhapradha, N., Shanmugam, V., and Shanmugam, A., 2017. Chitosan nanoparticles from marine squid protect liver cells against N-diethylnitrosoamine-induced hepatocellular carcinoma. *Carbohydr. Polym*., **171**: 18-26. https://doi.org/10.1016/j.carbpol.2017.04.097

Tarrant, P., Pearson, A., Price, J., and Lechowich, R., 1971. Action of *Pseudomonas fragi* on the proteins of pig muscle. *Appl. Microbiol*., **22**: 224-228. https://doi.org/10.1128/am.22.2.224-228.1971

Tayel, A.A., 2016. Microbial chitosan as a biopreservative for fish sausages. *Int. J. Biol. Macromol*., **93**: 41-46. https://doi.org/10.1016/j.ijbiomac.2016.08.061

Testing, E.C.O.A.S., 2000. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. EUCAST Definitive Document E. Def 3.1. *Clin. Microbiol. Infect*., **6**: 509-515. https://doi.org/10.1046/j.1469-0691.2000.00142.x

Wan, A., Xu, Q., Sun, Y., and Li, H., 2013. Antioxidant activity of high molecular weight chitosan and N, O-quaternized chitosans. *J. Agric. Fd. Chem*., **61**: 6921-6928. https://doi.org/10.1021/jf402242e