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Preparation and antibacterial activity of chitosan-silver nanoparticles for application in preservation of minced meat

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Abstract

Background: In recent years, the development of efficient and green route to synthesis metal nanoparticles has gained considerable attention in various areas of nanotechnology. Among metal nanoparticles, silver nanoparticles (AgNPs) have attracted much interest because of their potential antimicrobial agents and they are widely applied in many biological and medical fields. With respect to Ag-based nanocomposites, chitosan-silver nanoparticles (Ch-AgNPs) represent an emerging group of bio-nanostructured hybrid materials due to their biocompatibility and biodegradability. Ch is considered a non-toxic biopolymer, as well as its antimicrobial and antifungal activities against a wide range of microorganisms.

Methods: Colloidal AgNPs were prepared by chemical reduction of silver ions in the presence of Ch giving Ch-AgNPs. Physiochemical characterizations were determined by scanning electron microscopy (SEM), X-ray diffraction (XRD), and zeta potential. In vitro antibacterial activity was evaluated against *Escherichia coli* and *Salmonella typhimurium* using nutrient agar dilution method. The in vivo antibacterial activity was also tested against *E. coli* in minced meat under aerobic conditions at 4 °C for 10 days. In addition, different biochemical parameters were determined in minced meat samples.

Results: The colloidal AgNPs formed in situ by chemical reduction of Ag ions in the presence of Ch and showed a good stability. SEM and XRD confirmed the formation of nanoparticles. Zeta potential value was decreased by increase the ratio of Ag and was found to be − 0.225, − 0.193, and − 0.0695 mV for Ag ratio 0.1, 0.2, and 0.35, respectively. The in vitro antibacterial activity of Ch and Ch-AgNPs against *E. coli* and *S. typhimurium* reveal that the *E. coli* was more susceptible to these products than *S. typhimurium* and Ch-AgNPs have more influence with increasing of the silver concentrations. The in vivo antibacterial activity against *E. coli* in minced meat samples showed that the effect of Ch-AgNPs was a concentration dependent and greater compared with either controls or Ch alone.

Conclusions: The results suggest that Ch-AgNPs could be used in food preservation as antimicrobial agents and for shelf-life extension. However, further toxicological studies on mammals are needed.

Keywords: Chitosan, Silver nanoparticles, Synthesis, Meat preservative

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Background

Nanotechnology is one of the alternatives for improving food shelf life that enables the construction of active food packaging combining properties of external barrier and antimicrobial agents. Such approach is capable of inhibiting or retarding microbial growth and, as a consequence, can reduce food deterioration providing extension of shelf-life (de Azeredo 2013). Among nanomaterials, metal nanoparticles, such as silver and gold, are very important because of their potential chemical and biological properties (Daniel and Astruc 2004). Metallic silver and silver salts have been used as powerful antibacterial agents for many years (Gupta 2000). The silver-based substance has a biocide effect for many bacterial species that are promising its application for packaging (Bosetti et al. 2002). Chitosan (deacetylated chitin) is a biopolymer prepared from shellfish and has advantages such as non-toxic, biodegradable, and biocompatible. It exhibits antimicrobial activity and has therefore received attention as a potential food preservative of natural origin. Moreover, chitosan displayed an excellent antibacterial activity but it was ineffective in preventing oxidative rancidity (Badawy and Rabea 2017; Marei et al. 2018; Rabea et al. 2009).

Numerous methods have been reported for the synthesis of silver nanoparticles (AgNPs), such as chemical reduction of silver ions in aqueous solution with or without a protecting agent (Chou 2004; Nasiriboroumand et al. 2018; Park et al. 2011). Stable Ch silver nanoparticles were prepared in the range of 10–15 nm using reducing and stabilizing agents without any toxic chemicals with increased stability that enhance the antibacterial activity (Radhika et al. 2012; Shrivastava et al. 2007). The activity of AgNPs was more against gram-negative than gram-positive bacteria (Yoksan and Chirachanchai 2009). AgNPs were also prepared based on electrochemical oxidation/complexation process followed by UV irradiation reduction (Reicha et al. 2012; Venkatesham et al. 2014). The nanoparticles were characterized using transmission electron microscopy, X-ray diffraction, energy dispersive X-ray, and UV-visible spectrophotometry. The obtained nanoparticles were uniform and spherical. The antimicrobial activity of silver nanoparticles was tested against *Bacillus thuringiensis*, *Pseudomonas aeruginosa*, *E. coli*, and *Micrococcus luteus.* The nanoparticles demonstrated a relatively high antibacterial activity.

The antimicrobial effect of nanosilver packaging was evaluated in minced beef during storage at refrigerator temperature, and the data showed that an inhibitory effect on microbial growth was reported after 7 days of application (Carbone et al. 2016). Bacterial growth was slowed down, allowing an increase of the shelf-life of minced meat that usually spoiled after 2 days of storage in common food packaging. Chitosan, lysozyme, and nanocolloidal silver (NAg) showed antimicrobial activity against *Bacillus cereus*, *Micrococcus flavus, Escherichia coli*, and *Pseudomonas fluorescens*. Addition of lysozyme to sol composition significantly increased the antioxidant activity (Zimoch-Korzycka and Jarmoluk 2015). In addition, the mixture of enzyme and chito-oligomers exhibits a strong inhibiting effect against *Staphylococcus aureus* in minced meat stored in the refrigerator (Biswas et al. 2012; Kanatt et al. 2013).

Therefore, the present study aimed to prepare and characterize three Ch-AgNPs and study their antimicrobial activity in vitro and in vivo. The chemical analysis of the treated minced meat with Ch-AgNPs was also considered during shelf life periods.

Materials and methods

Chemicals and tested bacterial strains

Low molecular weight Ch (89% degree of deacetylation), Tween 80, dimethyl sulfoxide (DMSO), sodium tripolyphosphate (STPP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid (vitamin C), silver nitrate, sodium phosphate dibasic (Na_2HPO_4), sodium phosphate monobasic (NaH_2PO_4), sodium-potassium tartrate, and potassium iodide were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Nutrient broth (NB) and nutrient agar (NA) media were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK). All other commercially available chemicals were purchased from El-Gomhouria Company for trading chemicals and medical appliances (Alexandria, Egypt) and used without further purification.

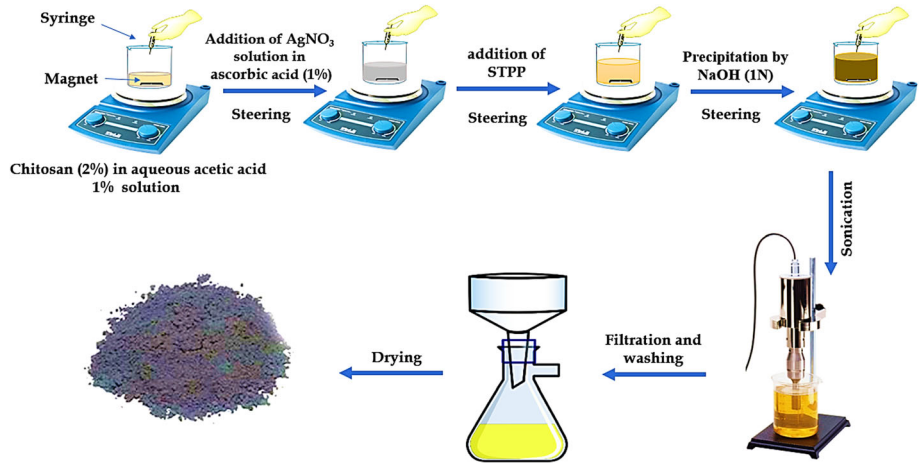
Two food pathogenic bacteria *Escherichia coli* (ATCC 8739) and *Salmonella typhimurium* (ATCC 1402) were obtained from Microbiology Laboratory, Department of Dairy Science, Faculty of Agriculture, Alexandria University, Egypt. The selected bacterial cultures were maintained on NA medium at 37 °C and stored in refrigerator at 4 °C.

Minced meat

Fresh meat from thigh was purchased from local meat packer at Smouha, Alexandria, Egypt. Samples were obtained within 72 h of slaughtering. Samples were sterile by immersion in water solution of sodium hypochlorite (10 mg/L) for 1 h, then rinsed twice with sterilized distilled water, then minced using ground meat.

Preparation of silver nanoparticles

Ch-AgNPs were prepared by direct reduction of $AgNO_3$ with vitamin C as shown in Scheme 1 and Table 1 (Cao et al. 2010) with some modifications. Three concentrations (0.1, 0.2, and 0.3%, *w*/*v*) of silver nitrate were prepared in distilled water then 5 mL of each solution was added dropwise under magnetic stirring onto 50 mL of Ch solution (2%, *w*/*v*) to obtain a homogeneous solution. After magnetic stirring for 30 min, 5 mL of vitamin C



Scheme 1 Preparation scheme of Ch-AgNPs

solution (1%) was added dropwise onto the mixture and the pH value was adjusted to 6. The reaction continued for another 60 min while stirring under 50 °C temperature. Fifteen milliliters of STPP solution (1%) was added dropwise to the previous mixture. The pH was adjusted to 10 using NaOH (1 N) then the mixture was sonicated for 15 min at a sonication power of 10 kHz and 9 cycles/s which controlled by the software of the device (Ultrasonic Homogenizers HD 2070 with HF generator (G 2070), ultrasonic converter UW2070, booster horn (SH 213 G), and probe microtip MS 73, Ø 3 mm). Finally, the solution was stored in water bath at 60 °C for 3 h and the precipitate was filtered, washed by distilled water, and dried in an oven at 70 °C for 3 h.

Characterization of Ch-AgNPs

Scanning electron microscope (SEM)

Ch-AgNP samples were inspected with a JEOL scanning electron microscope (SEM Inc., Japan) with a magnification of × 20,000 and acceleration voltage 19 kV. Dry particles were suspended in ethyl alcohol by sonication to dismantling the assembled particles. After that, the particles were mounted on metal stubs with double-sided tape, sputtered with gold, and viewed in a SEM. SEM has also measured the particle size of the products (Badawy et al. 2018).

Zeta potential

Surface charge of the prepared Ch-AgNPs was examined by using a Malvern Zeta nano-sizer instrument (Enigma Business Park, Grovewood Road, and Malvern WR14 1XZ, UK). Fixed weight (0.1 g) of prepared particles was suspended in glycerol (50%) in isopropanol (*v*/*v*) then sonicated for 30 min. The suspension was transferred to a zeta-potential cell (Honary and Zahir 2013a).

X-ray diffraction (XRD)

X-ray diffractograms on powder samples were obtained using a Bruker's X-ray Diffraction (USA) with Cu tube radiation ($k = 1.54184$ Å), a graphite monochromator and Lynxeye detector at 30 kV, and a current of 10 mA. The diffractometer was controlled and operated by a PC with the DIFFRAC.SUITE™ Software package. Measurements were taken over an angular range of $0.99° \le 2\theta \le 89.99°$ with a scanning step of 0.05 and a fixed counting time of 10 s. Divergence, scattered, and receiving radiation slits were 1°, 1°, and 0.2 mm, respectively (Badawy et al. 2018).

Microbiological studies

In vitro antibacterial activity of Ch-AgNPs

The in vitro antibacterial activity of Ch-AgNPs was assayed using NA dilution method according to the European

Table 1 Preparation conditions and characterizations of silver and chitosan nanoparticles

Code	$AgNO_3$ conc. (%)	Chitosan 2% (mL)	Vitamin C 1% (mL)	STPP 1% (mL)	Particle size (nm) ± SE
Ch-AgNP1	0.1	50	5.0	15	76.86 ± 8.65
Ch-AgNP2	0.2	50	5.0	15	63.03 ± 6.98
Ch-AgNP3	0.3	50	5.0	15	61.57 ± 6.68

Ch chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), *Ch-AgNP3* (0.3% Ag), *STPP* sodium tripolyphosphate

Committee on Antimicrobial Susceptibility Testing (EUCAST) (Kahlmeter et al. 2006) against *E. coli* (ATCC 8739) and *S. typhimurium* (ATCC1402). Preliminary screening tests were performed at concentrations ranging from 100 to 2000 mg/L of each product. For determination of minimum inhibitory concentration (MIC), different concentrations of the compounds were added to NA medium immediately before it was poured into the Petri dishes at a temperature of 40–45 °C. Parallel controls were maintained with distilled water mixed with NA medium. One loopful of bacteria in NB medium (≈ 5 μL) was placed on the surface of NA medium (ten per plate) then incubated at 37 °C for 24 h. Each concentration was tested in triplicate. The MIC was recorded in each case as the minimum concentration of compound that inhibited the growth of tested bacteria after incubation for 24 h at 37 °C. From the observed MIC, intermediate concentrations between MIC values were prepared by suitable dilutions of stock solution and the accurate MIC values were determined.

In vivo antimicrobial activity of prepared Ch-silver nanoparticles

Three replicates of minced meat samples each 100 g were used for the in vivo antibacterial activity. Three treatments of Ch and Ch-AgNPs at two different concentrations (1000 and 2500 μg/g) showed good inhibition in the in vitro experiments; two controls, inoculated and none inoculated with *E. coli*, were tested in vivo for their antibacterial activity. Sterilized minced beef meat samples were placed in the polyethylene bags and contaminated with 25 μL of *E. coli* (ca10^4 cfu/g). In order to ensure proper distribution of the pathogen, the inoculated samples were homogenized for 2 min at room temperature. Following homogenization, different concentrations of treatments were added, and to ensure uniform distribution of added compounds, treated meat samples were further homogenized as previously described. The polyethylene bags with samples from all treatments were wrapped and stored under aerobic conditions at 4 °C for 10 days intervals (3, 7, and 10 days) until analysis (Emiroğlu et al. 2010; Shavisi et al. 2017).

Chemical analysis of prepared minced meat

Moisture content and pH

The moisture content was determined by drying 5 g of minced beef meat treatments in an air drying oven at 105 °C to a constant weight (AOAC 2003). The pH was determined by blending 1 g of minced beef meat sample in 10 mL of deionized distilled water. The mixture was filtrated and measured using a digital pH meter.

Soluble and total proteins assay

One gram of minced beef meat was homogenized in 4 mL phosphate buffer pH 7.4, and the soluble protein content was determined according to the Lowery et al. method (Lowery et al., 1951). A protein standard curve generated using BSA and the measurements were done in three triplicates. Three samples of protein content were determined comparing to the standard curve of BSA. In addition, total protein content was determined by the micro Kjeldahl's method (AOAC 2003). The total protein content was calculated by multiplying the total nitrogen.

Fat content

Fat content of prepared minced beef meat samples (10 g each) was determined according to the AOAC method (AOAC 2003). The extraction was done by a mixture of chloroform and methanol (2:1). Fat content was expressed as percentage (g fat/100 g of meat samples).

Antioxidant activity assay

DPPH radical scavenging activity of the test samples was estimated (Shimada et al. 1992). Five grams of minced beef meat samples were homogenized in 10 mL of methanol and centrifuged at 5000 rpm for 10 min. Fifty microliters of the resultant soluble fraction was transferred to 1 mL of 0.1 M DPPH then vortexed and absorbance measured at 517 nm after 20 min incubation in the dark. Percent of DPPH scavenging activity was calculated as follows:

Inhibition (%) = [(absorbance of the control – absorbance of the sample)/absorbance of the control)] × 100

Determination of peroxide value (PV)

Extracted fat (0. 5 g) was dissolved in 10 mL of a mixture of glacial acetic acid and chloroform solution (3:2 *v*/*v*) then shaked for 3 min to dissolve the fat (AOAC 1989). Potassium iodide (2 g) was dissolved in 3 mL of deionized distilled water to form a saturated solution of KI. Then, 1 mL of KI was transferred to fat sample and shaked for 1 min. Twenty milliliters of distilled water was added to the solution and 1 mL of starch indicator (1%) was added, and the reaction mixture was titrated by sodium thiosulphate solution (0.01 N) with vigorous shaking. Titration continued until the blue color disappeared. PV was calculated and expressed in milliequivalents of active oxygen per kilogram (meq. O_2/kg meat).

Determination of ash content

Minced beef meat samples (10 g each) were ignited in a muffle furnace at 550 °C to constant weights and ash content was determined (AOAC 2003).

Determination of minced meat color

Minced meat color was determined using a HUNTERLAB Colorimeter (Hunter Associates Laboratory Inc., Reston, USA), calibrated to black and white standards.

Tristimulus L*a*b* measurement mode was used (Hunter and Harold 1987). L*(lightness), a*(redness), and b*(yellowness) values were measured and recorded as the mean of these measurements. From the measured values, chroma $[(a^{*2} + b^{*2})^{1/2}]$ and hue (b*/a*) were also calculated.

Statistical analysis

Statistical analysis was performed using the Cohort software (Costat 1986). Means and standard error (SE) were obtained from three independent replicates performed for each treatment. Two-way analysis of variance (ANOVA) (Kotz et al. 2006) was conducted, and mean property values were separated with Duncan's multiple range test to determine significant differences among the mean values at a probability level of ≤ 0.05.

Results

Characterization of Ch-AgNPs

Scanning electron microscopy (SEM)

The SEM was used to examine the surface morphology and particle size of Ch-AgNPs nanoparticles as shown in Fig. 1. The shape of Ch-AgNP1-3 was almost spherical in shape. The mean of particle size was ranged between 61.57 and 76.89 nm (Table 1).

Zeta potential

The zeta potential values for the three Ch-AgNPs were ranged between − 0.0695 and − 0.225 mV (Fig. 2), indicating the dispersal of particles in suspension at pH 7 and 25 °C. The negative charge of zeta potential values obtained denotes the surface charge of the particles.

X-ray diffraction (XRD)

The crystallographic structure of Ch-AgNPs was determined by XRD and is shown in Fig. 3. Figure 3a shows the characteristic peaks at $2\theta = 9.89°$ and $2\theta = 19.93°$. However, these two peaks are very weak in the spectra of Ch-AgNPs (Fig. 3b–d), which suggest a low crystallinity and an amorphous nature of the products. In addition, these two characteristic peaks were significantly decreased with the increase in the ratio of silver (0.1, 0.2, and 0.3%) in the products. The weak peaks reflect great disarray in chain alignment of chitosan with the production of new peaks that identify the presence of silver. Figure 3b displays the X-ray diffraction patterns of Ch-AgNPs1. A number of Bragg reflections are observed with 2θ of 37.73°, 43.92°, 64.09°,76.99°, 81.11°, and 97.93°, which correspond to the characteristic face-centered Ch-AgNPs1 with count indexes (737), (309), (207), (195), (95), and (62), respectively. The peak corresponding to the (737) plane is more intense than the other planes. The broadening of these peaks is mostly due to the effect of nano-sized particles. The diffraction angles observed at 10.98° and 20.53° corresponding to count indexes (142) and (191), respectively, refer to the chitosan shell. The X-ray diffraction patterns of Ch-AgNPs2 (Fig. 3c) demonstrated diffraction angles of 37.59°, 43.75°, 63.94°,76.91°, 81.12°, and 97.44°, which correspond to the count indexes (1124), (488), (323), (325), (127), and (57), respectively. The X-ray diffraction patterns of Ch-AgNPs3 (Fig. 3d) demonstrated diffraction angles of 37.93°, 44.09°, 64.26°,77.20°, 81.37°, and 97.99°, which result to the characteristic face-centered Ch-AgNPs3 with count indexes (947), (364), (282), (298), (118), and (55), respectively.

Microbiological studies

In vitro antibacterial activity

Data in Table 2 shows the estimated MIC values of Ch and Ch-AgNPs against *E. coli* and *S. typhimurium.* The MIC values reflected that *E. coli* was more susceptible to the compounds than *S. typhimurium*. The antibacterial effect was increased with increasing the concentration of silver content in the products, and Ch-AgNP3 was the most active product (MIC = 150 and 200 mg/L against *E. coli* and *S. typhimurium*, respectively). At the lowest ratio of silver (0.3%), the MIC values were 200 and 250 mg/L against *E. coli* and *S. typhimurium*, respectively.

In vivo antibacterial activity on minced meat

The antibacterial activity against *E. coli* in minced beef meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C is shown in Table 3. It can be seen that the activity of the three treatments of Ch-AgNPs were 8.17 and 3.67×10^4 cfu/g, 11.50 and 5.83×10^4

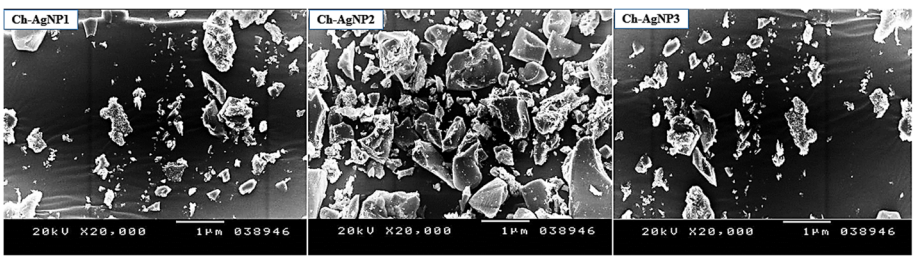


Fig. 1 SEM of the Ch-AgNPs. The right images show the particles in agitation form while the left images show the spherical particles with scale bar 1 μm and magnification of × 20,000 at 20 Kv. Ch-AgNPs1 (0.1% Ag), Ch-AgNPs2 (0.2% Ag), and Ch-AgNPs3 (0.3% Ag)

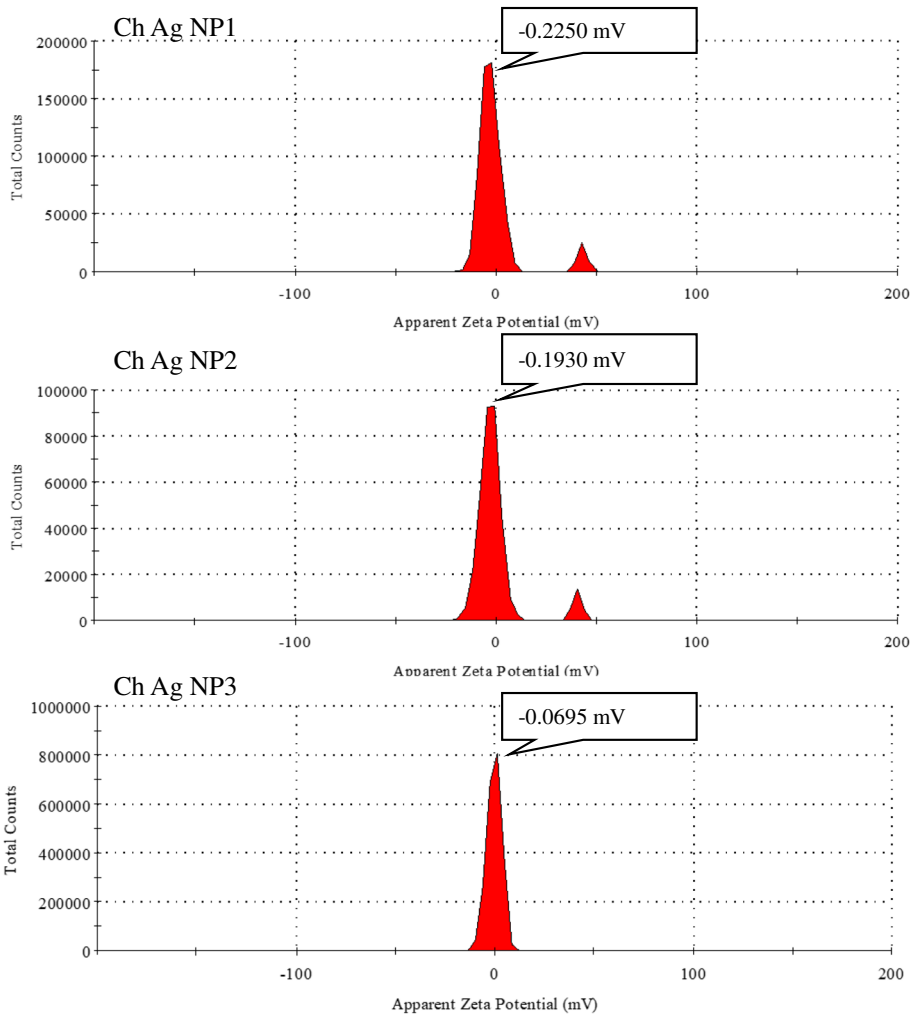


Fig. 2 Zeta potential distribution of Ch-AgNPs. Ch chitosan, NP nanoparticle, Ch-AgNP1 (0.1% Ag), Ch-AgNP2 (0.2% Ag), and Ch-AgNP3 (0.3% Ag)

cfu/g, and 14.47 and 9.00×10^4 cfu/g for Ch-AgNP3, Ch-AgNP2, and Ch-AgNP1 at the two concentrations of 1000 and 2500 mg/g, respectively, compared with > 300 and 243.67×10^4 cfu/g for control+ and control−, respectively. Moreover, the antibacterial effect was concentration dependent. Meanwhile, the antibacterial activity of most treatments was reduced with the time of storage.

Chemical analysis of treated minced meat

Moisture content and pH

Moisture content (%) in minced beef meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C is shown in Additional file 1: Table S1. The observed data showed a little variation in moisture content between all treatments of Ch-AgNPs and Ch or control treatments. However, significant differences in values of moisture content were observed between most of the tested treatments or Ch-AgNPs and control+. Moreover, no significant differences in the percentage of moisture content were observed between the different times of storage. The range of moisture contents for treatments was 71.59–73.98% and reached 76% for control +.

The pH values of minced meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C are shown in Additional file 1: Table S2. It can be noted that the pH was decreased significantly by increasing the time of storage (from 3 to 10 days). The lowest value was recorded with control+ while the pH values of the treatments with Ch-AgNPs were in the range of 6.12–6.30 compared. Moreover, no significant differences were observed between the tested products that contain different concentrations of silver.

Protein content

Soluble and total protein of minced beef meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C are shown in Additional file 1: Tables S3 and S4,

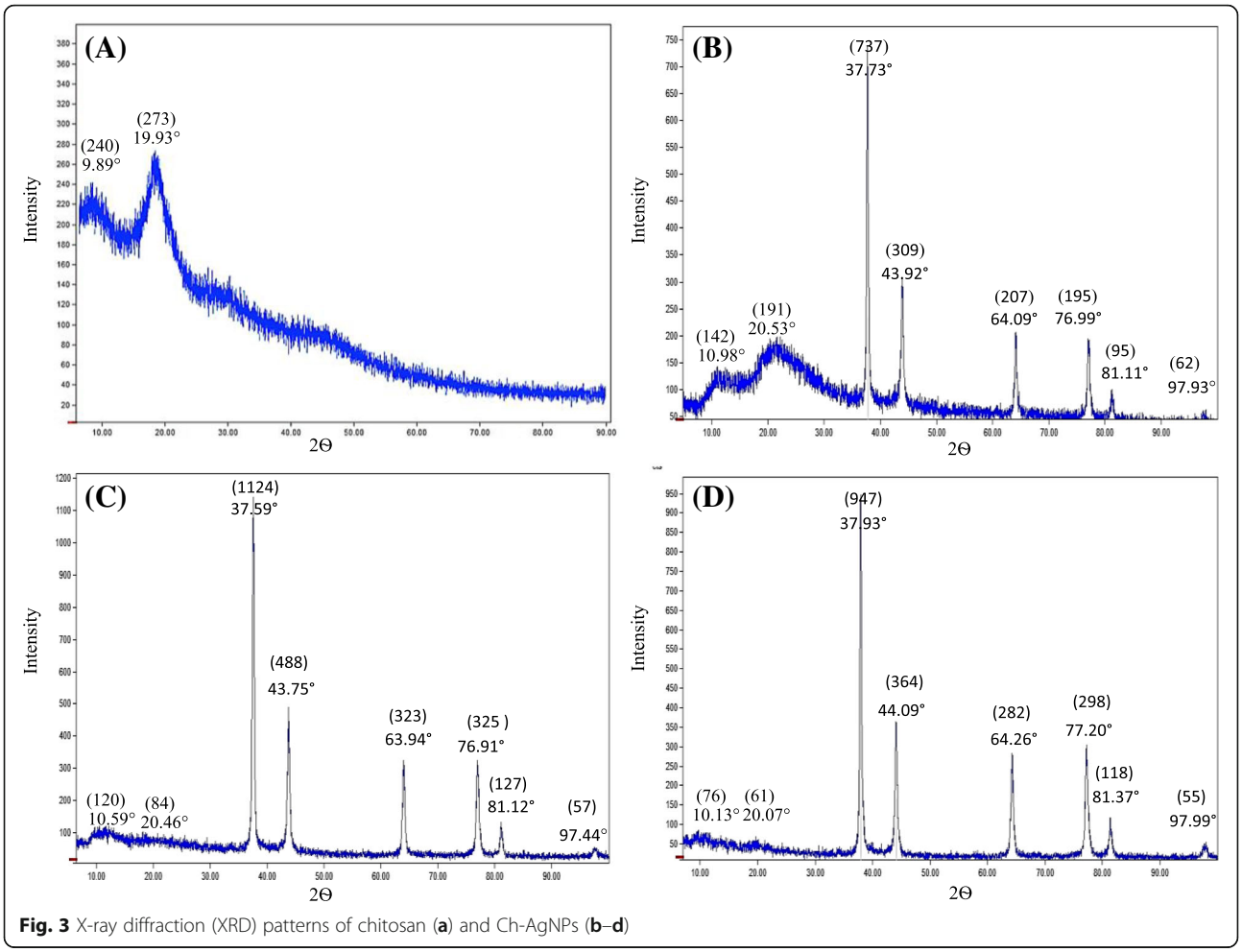


Fig. 3 X-ray diffraction (XRD) patterns of chitosan (**a**) and Ch-AgNPs (**b**–**d**)

respectively. The data showed a little variation between all tested treatments of Ch-AgNPs and Ch or control treatments. The soluble protein ranged between 0.08 and 0.13 mg/g; however, the total protein content ranged from 23.33 to 23.99%.

Fat content

Fat contents in treated minced meat samples after storage for 10 days at 4 °C are shown in Table 4. A little variation in fat content was found between all treatments of Ch-AgNPs whereas the range of fat content was 20.30–26.09% compared with 20.46% and 22.65% in control+ and control−, respectively. Moreover, no significant differences in fat content were observed between the two tested concentrations or between the times of storage.

Table 2 In vitro antibacterial activity of with chitosan (Ch) and chitosan-silver nanoparticles (Ch-AgNPs) against *E. coli* and *S. typhimurium*

Treatments	MIC (mg/L)	
	E. coli	*S. typhimurium*
Ch	500	650
Ch-AgNP1	200	250
Ch-AgNP2	180	225
Ch-AgNP3	150	200

MIC is the minimum inhibitory concentration
Ch chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), *Ch-AgNP3* (0.3% Ag)

Antioxidant activity

Changes in the antioxidant activity (%) in minced beef meat products treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C are shown in Table 5. The antioxidant activity of control + treated with *E. coli* was the lowest compared with control− (without *E. coli*) and other treatments. The antioxidant values of the three Ch-AgNPs were high and closed to each other whereas the average of the antioxidant activities during the storage periods were 39.25, 40.80, and 43.63 at 1000 μg/g and 43.76%, 46.14%, and 49.62% at 2500 μg/g, respectively, for the three tested products compared. The data confirmed that the antioxidant values were increased with the increasing of the concentration of silver content in the synthesized products. Meanwhile, the antioxidant

Table 3 In vivo antibacterial activity against *E. coli* (10^4 cfu/g) in minced beef meat products treated with chitosan (Ch) and chitosan-silver nanoparticles (Ch-AgNPs) after storage for 10 days at 4 °C

Treatment	Concentration (μg/g)	Antimicrobial activity against *E. coli* (10^4 cfu/g) ± SE at time (day)			Average ± SE
		3	7	10	
Control+	0.00	295.00 ± 2.89	˃ 300	˃ 300	˃ 300
Control–	0.00	137.50 ± 1.44	295.00 ± 2.89	298.5 ± 0.87	243.67^{ed} ± 53.09
Ch	1000	75.00 ± 2.89	132.50 ± 1.44	172.50 ± 1.44	126.67^{d} ± 28.30
	2500	62.50 ± 1.44	85.00 ± 2.89	105.00 ± 2.89	84.17^{c} ± 12.28
Ch-AgNP1	1000	8.50 ± 0.29	14.50 ± 0.29	21.00 ± 0.58	14.67^{c} ± 3.61
	2500	1.50 ± 0.29	11.00 ± 0.58	14.50 ± 0.29	9.00^{b} ± 3.88
Ch-AgNP2	1000	5.50 ± 0.29	12.50 ± 0.87	16.50 ± 0.87	11.50^{b} ± 3.21
	2500	2.00 ± 0.00	4.50 ± 0.29	11.00 ± 1.73	5.83^{a} ± 2.68
Ch-AgNP3	1000	1.50 ± 0.29	9.50 ± 0.29	13.50 ± 0.87	8.17^{a} ± 3.53
	2500	1.50 ± 0.29	2.50 ± 0.29	7.00 ± 0.58	3.67^{a} ± 1.69
Mean		85.25^{a}	121.4^{b}	129.75^{c}	Between treatment for 1000 = 2.38
$LSD_{0.05}$		Between time = 1.23			Between treatment for 2500 = 2.60

Values are mean of three replicates ± standard error (SE). Different letters in the same column indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)
Before treatment, 130×10^4 cfu/g
Control (+) minced meat treated with *E. coli*, *Control (–)* minced meat without *E. coli*, *Ch* chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), Ch-AgNP3 (0.3% Ag)

activity was significantly reduced with the increase of storage time.

Peroxide value

The changes in peroxide value in minced beef meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C are shown in Table 6. The results showed that control+ had greater values than control– and all treatments of Ch-AgNPs. The PV values of the samples treated with Ch-AgNPs were 18.84, 16.25, and 14.50 meq. O_2/kg meat for treatments of Ch-AgNP1, Ch-AgNP2, and Ch-AgNP3 at 1000 μg/g, respectively. However, at 2000 μg/g of AgNP1, Ch-AgNP2, and Ch-AgNP3, the values were 16.25, 14.33, and 12.33 meq. O_2/kg, respectively. In general, the results showed that the treatments reduced the oxidation of minced beef meat and that might be because the meats are

Table 4 Fat content (%) in minced beef meat products treated with chitosan (Ch) and chitosan-silver nanoparticles (Ch-AgNPs) after storage for 10 days at 4 °C

Treatment	Concentration (μg/g)	Fat content (%) ± SE at time (day)			Average ± SE
		3	7	10	
Control+	0.00	21.00 ± 0.58	20.25 ± 0.14	20.13 ± 0.07	20.46^{ab} ± 0.27
Control–	0.00	22.70 ± 0.06	22.62 ± 0.01	22.61 ± 0.01	22.65^{c} ± 0.03
Ch	1000	21.00 ± 0.58	19.85 ± 0.09	19.92 ± 0.04	20.26^{a} ± 0.37
	2500	24.20 ± 0.12	24.25 ± 0.09	24.32 ± 0.04	24.26^{d} ± 0.04
Ch-AgNP1	1000	22.50 ± 0.87	20.76 ± 0.14	20.58 ± 0.24	21.28^{b} ± 0.61
	2500	20.90 ± 0.17	20.39 ± 0.12	20.36 ± 0.14	20.55^{b} ± 0.18
Ch-AgNP2	1000	20.50 ± 0.64	20.47 ± 0.23	20.28 ± 0.15	20.41^{a} ± 0.07
	2500	20.50 ± 0.29	20.49 ± 0.30	20.46 ± 0.31	20.48^{b} ± 0.01
Ch-AgNP3	1000	21.00 ± 0.58	19.94 ± 0.04	19.97 ± 0.02	20.30^{a} ± 0.35
	2500	26.80 ± 0.46	25.83 ± 1.02	25.66 ± 0.89	26.09^{e} ± 0.36
Mean		21.98^{b}	21.4^{a}	21.34^{a}	Between treatment for 1000 = 0.59
$LSD_{0.05}$		Between time = 0.310			Between treatment for 2500 = 0.65

Values are mean of three replicates ± standard error (SE). Different letters in the same column indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)
Before treatment, 24%
Control (+) minced meat treated with *E. coli*, *Control (–)* minced meat without *E. coli*, *Ch* chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), *Ch-AgNP3* (0.3% Ag)

Table 5 Changes in antioxidant activity (%) in minced beef meat products treated with chitosan (Ch) and chitosan-silver nanoparticles (Ch-AgNPs) after storage for 10 days at 4 °C

Treatment	Concentration (μg/g)	Antioxidant activity (%) ± SE at time (day)			Average of ± SE
		3	7	10	
Control+	0.00	36.88 ± 0.58	17.84 ± 0.19	16.15 ± 0.26	23.62^{a} ± 6.6
Control−	0.00	38.12 ± 0.24	21.42 ± 0.66	18.90 ± 0.21	26.15^{b} ± 6.03
Ch	1000	43.76 ± 0.16	27.29 ± 0.29	23.67 ± 0.42	31.57^{c} ± 6.18
	2500	52.89 ± 0.24	28.62 ± 0.16	24.68 ± 0.32	35.40^{c} ± 8.82
Ch-AgNP1	1000	45.23 ± 0.11	42.95 ± 0.18	29.56 ± 0.33	39.25^{d} ± 4.89
	2500	55.83 ± 0.13	45.07 ± 0.44	30.39 ± 0.62	43.76^{d} ± 7.37
Ch-AgNP2	1000	47.84 ± 0.03	43.25 ± 0.36	31.31 ± 0.25	40.80^{e} ± 4.93
	2500	57.39 ± 0.29	47.19 ± 0.79	33.86 ± 0.13	46.14^{e} ± 6.81
Ch-AgNP3	1000	51.74 ± 0.37	45.29 ± 0.47	33.86 ± 0.37	43.63^{f} ± 5.23
	2500	60.69 ± 0.29	52.60 ± 0.22	35.58 ± 0.06	49.62^{f} ± 7.40
Mean		47.1^{c}	34.23^{b}	26.08^{a}	Between treatment for 1000 = 0.57
$LSD_{0.05}$		Between time = 0.240			Between treatment for 2500 = 0.63

Values are mean of three replicates ± standard error (SE). Different letters in the same column indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)
Before treatment, 28.9%
Control (+) minced meat treated with *E. coli*, *Control (−)* minced meat without *E. coli*, *Ch* chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), *Ch-AgNP3* (0.3% Ag)

rich in iron, which activate the self-oxidation. Moreover, the peroxide value of most treatments along the time of storage (10 days) does not exceed the recommended value (100 meq. O_2/kg meat).

Ash content

Ash content (%) in minced beef meat samples treated with Ch and Ch-AgNPs after storage for 10 days at 4 °C is shown in Additional file 1: Table S5. The results showed little variations between all tested treatments of Ch-AgNPs whereas the range of ash content was between 1.09 and 1.18% at the two tested concentrations compared with 1.04% and 1.07% in control+ and control−, respectively. Moreover, no significant differences in the percentage of ash contents were observed between the two tested treatments concentrations or between the three storage times.

Table 6 Changes in peroxide value (meq O_2/kg fat) in minced beef meat samples treated with chitosan (Ch) and chitosan-silver nanoparticles (Ch-AgNPs) after storage for 10 days at 4 °C

Treatment	Concentration (μg/g)	Peroxide value (meq O_2/kg fat) ± SE at time (day)			Average of ± SE
		3	7	10	
Control+	0.00	60.15 ± 0.58	76.32 ± 0.19	92.17 ± 0.26	76.21^{d} ± 9.24
Control−	0.00	54.78 ± 0.24	66.06 ± 0.66	88.32 ± 0.21	69.72^{c} ± 9.85
Ch	1000	14.66 ± 0.16	24.33 ± 0.29	36.37 ± 0.42	25.12^{b} ± 6.28
	2500	12.61 ± 0.24	20.16 ± 0.16	34.36 ± 0.32	22.38^{b} ± 6.38
Ch-AgNP1	1000	10.27 ± 0.11	19.25 ± 0.18	27.00 ± 0.33	18.84^{a} ± 4.80
	2500	8.24 ± 0.13	16.50 ± 0.44	24.00 ± 0.62	16.25^{a} ± 4.60
Ch-AgNP2	1000	8.00 ± 0.03	18.12 ± 0.36	23.50 ± 0.25	16.54^{a} ± 4.50
	2500	7.00 ± 0.29	15.50 ± 0.79	20.50 ± 0.13	14.33^{a} ± 3.90
Ch-AgNP3	1000	7.00 ± 0.37	16.00 ± 0.47	20.50 ± 0.37	14.50^{a} ± 4.00
	2500	6.00 ± 0.29	13.50 ± 0.22	17.50 ± 0.06	12.33^{a} ± 3.40
Mean		26.04^{c}	37.1^{b}	52.63^{c}	Between treatment for 1000 = 0.34
LSD 0.05		Between time = 0.16			Between treatment for 2500 = 0.297

Values are mean of three replicates ± standard error (SE). Different letters in the same column indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)
Before treatment, 8 meq O_2/kg fat
Control (+) minced meat treated with *E. coli*, *Control (−)* minced meat without *E. coli*, *Ch* chitosan, *NP* nanoparticle, *Ch-AgNP1* (0.1% Ag), *Ch-AgNP2* (0.2% Ag), *Ch-AgNP3* (0.3% Ag)

Color of minced meat treated with Ch-AgNPs

The color of minced beef meat samples treated with Ch and Ch-AgNPs was determined at 3, 7, and 10 days of storage at 4 °C, and the data are presented in Additional file 1: Table S6. The chroma of control+ treated with *E. coli* has the lowest value compared with the other treatments. No significant difference was observed between the two tested concentrations of each product of Ch-AgNPs. Ch has a low value of chroma compared with control− that showed the highest value of chroma. Samples with Ch-AgNPs showed high chroma values with a range of 4.78–7.28.

The hue values of minced beef meat samples treated with Ch-AgNPs were determined at 3, 7, and 10 days of storage at 4 °C, and the data are presented in Additional file 1: Table S7. The values ranged from 2.39 to 2.87 at the two tested concentrations of three products of Ch-AgNPs. No significant difference was observed between the two tested concentrations of each product of Ch-AgNPs.

Discussion

Preparation and characterization of Ch-AgNPs

Many researchers report that Ch has a strong affinity for metal ions because there are many amino and hydroxyl groups (Aggarwal et al. 2004; Ngah et al. 2011; Varma et al. 2004). Under alkaline condition, Ch reacted with Ag^+ ions to form Ch-AgNPs (Sanpui et al. 2008). The current results are in agreement with some previous data that reported the formation of nanoparticles contains silver ions (Gupta and Tripathi 2011; Honary et al. 2011), in which the Ch-AgNPs are spherical in shape under SEM. In addition, the Ch-AgNPs were found to be spherical with an average size of 7–30 nm as observed from TEM (Yoksan and Chirachanchai 2009).

Zeta potential of nanoparticles is a useful tool for understanding and predicting interactions between particles. The zeta potential is the surface charge value of nanoparticles that influences particle stability or speeding the particle flocculation which are important features of adsorbents (Honary and Zahir 2013b; Patil et al. 2007). The previous study informed that when $AgNO_3$ was mixed with Ch solution, Ag^+ ions probably bound to Ch macromolecules via electrostatic interaction between the electron-rich oxygen atoms of the polar hydroxyl and ether groups of Ch and the electropositive transition cations (Ag^+) (Shao et al. 2015).

The results of XRD are in agreement with other studies which reported that a strong reflection was exhibited around 33° and 40° of the silver nanoparticles (Govindan et al. 2012; Hajji et al. 2019; Suteewong et al. 2018). Therefore, this gives a clear evidence for the preparation of Ch-AgNPs in the current study. The characteristic peaks found at $2\theta = 9.89°$ and $2\theta = 19.93°$ refer to the inter- and intra-molecular hydrogen bonds in the chitosan molecule (Haas and Franz 2009; Pereira et al. 2017). A number of Bragg reflections that was observed with 2θ of 37.73°, 43.92°, 64.09°,76.99°, 81.11°, and 97.93° may be indexed as the band for face-centered cubic structure of silver nanoparticles (Kalaivani et al. 2018; Venkatesham et al. 2014). However, the peaks at 2θ of 37.73°, 43.92°, and 64.09° are in perfect agreement to that of the JCPDS card no. 89-3722 (Raffi et al. 2008). The obtained results showed that silver-loaded chitosan nanoparticle exhibits a sharp peak at 2θ at ~ 37.5° which is due to the face-centered cubic crystalline structure of silver, corresponding to the crystal face of silver (Vimala et al. 2010).

Antimicrobial activity

The action of NPs through trapping facilitates releasing of bioactive particles to cover a large surface area of treated samples (Bouwmeester et al. 2009). In NPs production, bioactive compounds are loaded into different polymeric nano-carriers. The antibacterial effect of AgNPs was more potent than that of other products described in earlier reports. This effect was concentration dependent and was more effective against gram-negative bacteria than gram-positive organisms (Rai et al. 2009). Fernandez et al. (2010) reported that the Ag nanotechnology achieved a reduction of spoilage related to microflora in absorbent during the modified atmosphere packaging of beef meat. The authors reported that the levels of *Pseudomonas* spp. were significantly reduced in the presence of silver ions. Moreover, the color of meat is not affected by the presence of silver. Ch, lysozyme, and nano-silver were applied as antimicrobial agents as edible protective hydrosols on the surface of the meat (Zimoch-Korzycka and Jarmoluk 2015). In addition, Ch and nano-silver exhibited a potent inhibiting effect of the growth of *E. coli*, *Pseudomonas fluorescens*, *Bacillus cereus*, and *Staphylococcus aureus* in minced meat stored in refrigerator (Taylor et al. 2012; Zimoch-Korzycka and Jarmoluk 2015).

Chemical analysis of treated minced meat

The moisture content of meat products is considered as an important factor to consumers and producers. In certain products, the high limits of water and fat content are regulated by the Meat Inspection Division, Consumer and Marketing Service, U. S. Department of Agriculture (1965). The manufacturer desires to maintain the optimal level of fat and water in meat products, so there is a need for rapid accurate analysis. The results are in agreement with Kamruzzaman et al. (2012), who reported no difference between the percentage of moisture contents in all tested samples of minced meat.

The results of the pH values are in agreement with that reported by Soriyi et al. (2008) in which the pH values of various samples of meat were between 6.50

and 6.90, and this is within the normal range (5.6–7.0). Ramanathan et al. (2009) elucidated that the glycogen was converted into lactic acid in the aging process of meat and the produced lactic acid lowers the pH of the muscle from about 7.0 in the living animals to 5.6 in the carcasses after a period of time. However, the protein content in meat tends to neutralize the pH (Tarrant et al. 1971), and these conditions favor bacterial survival and growth. Moreover, at certain pH, the growth of the following bacteria was limited: *E. coli* (pH 5.0), *Salmonella* spp. (pH 4.6), *S. aureus* (pH 4.9), and *B. cereus* (pH 5.0) (Claus and Fritze 1989; Cowan and Steel 1965). Furthermore, proteolysis may produce nitrogenous compounds that cause an increase in the pH values (Aksu et al. 2005). Minced meat is not suitable for consumption when the pH is over 6.4 or below 5.6 (Skrökki 1997). However, Ahmad et al. (2015) reported a reduction in pH value of minced spent hen treated with *P. granatum* after storage for 6 weeks.

The little variation of soluble or total protein between tested treatments might be due to the little homogeneity of minced meat samples or might be due to some denaturation of sarcoplasmic proteins (Chan et al. 2011; Marcos et al. 2010). Denaturation of proteins leads to a loss in their ability to bind water. Proteins form about one fifth of the wet weight of meat and bind the amount of water about half of its weight. Scopes (2013) reported that the bound water in meat protein is about 10% of the muscle weight. The remaining water must be held within the meat structure by capillarity forces (Tornberg 2013). In fact, water that lost from meat cannot be solely attributable to the differences for water bound by the meat proteins (Havugimana et al. 2012; Pearce et al. 2011; Shahidi 2012).

The little variations of fat contents within the tested Ch and Ch-AgNPs or between all tested treatments might be due to the less homogeneity of tested samples. However, a decrease in fat contents was reported in beef meat and beef burger after storage for 6 months (Muela et al. 2010; Shah et al. 2014). They explained that the little reduction of fat content by increasing storage periods might be due to the activity of fat hydrolyzed enzymes or oxidative enzymes. There is considerable evidence that vitamin E reduces lipid oxidation as well as myoglobin oxidation of meats (Falowo et al. 2014; Leygonie et al. 2012). In contrary, Al-Mossawi and Al-thary (2017) reported an increase in the free fatty acids in minced meat after storage for 100 days.

Numerous studies have indicated that lipid oxidation in meat and meat products may be organized or reduced by using antioxidants (Faustman et al. 2010; Karre et al. 2013). Thyme extracts have a potential antioxidant activity that makes them suitable to be used as possible substitutes for synthetic antioxidants for food industry (Mihailovic-Stanojevic et al. 2013). Ch has a great potential for a wide range of food applications due to its biocompatibility, non-toxicity, and low cost of application. Wan et al. (2013) suggested that the higher molecular weight of Ch may have antioxidant activity. Flavor is an important consideration that may limit the use of some antioxidants in meat and meat products. The development of natural preservatives with both antioxidant and antibacterial activities prolong the shelf life of meat and prevent meat spoilage. Ch, lysozyme, and the nano-silver had biological activity as antimicrobial agents of edible protective hydrosols that were applied onto the surface of meat. The addition of lysozyme to sol composition significantly increases antioxidant activity (Zimoch-Korzycka and Jarmoluk 2015).

This result of peroxide value was consistent with the study done by Jin et al. (2012) who reported that the peroxide value of the raw ground pork reached the maximum after 7 days of storage and that may be because the decomposition rate of the peroxides was faster after the induction period than the production rate. A significant difference among the samples treated with Ch-NPs of different sizes was observed over the storage for 15 days, in the sample with 527–250 nm. Meanwhile, Ch as a preservative could improve the oxidative stability of meat for chelating with the free iron released from the hemoproteins (Hu et al. 2015).

The data of color are in agreement with that reported by other studies in which Ch added individually or in combination with either rosemary or a-tocopherol had also a noteworthy effect on the burgers' appearance as it contributed to red color retention for a much longer period compared with other treatments and the controls (Doolaege et al. 2012; Rodríguez et al. 2012). The data of chroma and hue might attribute to the gradual oxidation of myoglobin and accumulation of metmyoglobin with time (Aroeira et al. 2017; Chakanya et al. 2017).

In general, AgNPs are becoming increasingly prevalent in consumer products as antibacterial agents. However, an adequate assessment of the long-term effects of AgNPs' exposure on human physiology and their release into the environment is lagging behind the rapid increase in the commercialization of AgNP products (Durán et al. 2010; Stensberg et al. 2011). Many of these studies have shown that AgNPs have significant toxicity in several cell lines as well as in a number of aquatic organisms, but the mechanistic basis for these toxic effects is under investigation (Antony et al. 2015). Particularly, the bioavailability of the silver ions (Ag^+) of the AgNPs, considered by many to be a major factor of Ag-mediated toxicity, remains poorly understood (Lubick 2008; Stensberg et al. 2011).

Conclusion

The results showed that Ch-AgNPs with well-controlled shape and size had little aggregation with the protection

of Ch colloids and they dispersed well in the matrix. The SEM picture detected that the nanoparticles exhibited a regular spherical shape with the size of less than 80 nm and AgNPs were encapsulated in the Ch particles. Moreover, zeta potential values were negative and were decreased by the increase of the ratio of Ag. The microbial experiment indicated that the Ch-AgNPs exhibited excellent antimicrobial properties against *E. coli* and *S. typhimurium* with AgNPs participating in the activities. In addition, the in vivo antibacterial activity of Ch-AgNPs against *E. coli* in minced beef meat samples showed excellent activity compared with either controls or Ch alone. The resultant nanoparticles could be suitable for biological applications in food preservation and medical treatments. However, toxicological studies are needed.

Additional file

Additional file 1: Table S1. Moisture content (%) in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch-AgNPs) after storage for ten days at 4 °C. **Table S2.** Changes in pH value in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch Ag NPs) after ten days storage at 4 °C. **Table S3.** Changes in soluble protein (Lowery) in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch Ag NPs) after ten days storage at 4 °C. **Table S4.** Changes in total protein Kjeldahl's content (%) in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch Ag NPs) after ten days storage at 4 °C. **Table S5.** Ash content (%) in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch-AgNPs) after storage for ten days at 4 °C. **Table S6.** Changes in Color parameters (L*, a*, b*,) Chroma in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch Ag NPs) after storage for ten days at 4 °C. **Table S7.** Changes in Color parameters (L*, a*b*,) Hue in minced beef meat products treated with chitosan (Ch), chitosan- silver nanoparticles (Ch Ag NPs) after storage for ten days at 4 °C. (DOCX 36 kb)

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Availability of data and materials
All data generated or analyzed during this study are included in this article. In addition, the related datasets are available from the corresponding author on reasonable request.

Authors' contributions
MEI Badawy, TMRL, and SMSS contributed to the design and implementation of the research, analysis of the results, and writing of the manuscript. All authors performed and shared in the experiments. All authors read and approved the final manuscript.

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