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Effects of silver bio-nanoparticle treatment on the wet preservation, technological, and chemical qualities of meat

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Abstract

Background/Objective: Nanotechnology is a recent technology, but its application to meat preservation is limited.
Materials and Methods: The silver bio-nanoparticle was sythensized from the extract of pawpaw and 1 mM solution of silver nitrate using standard method. Meat samples were treated with solutions containing 10%–20% silver bio-nanoparticle suspension and were kept for 2, 4 and 6 h. Protein, crude fat, ash, weight loss, water loss, solid gain, absorbed silver ion, bacterial count and sensory characteristics were determined using standard methods.
Results: The protein, crude fat, ash, weight loss, water loss, solid gain, absorbed silver ion and total plate count varied from 21.63%–30.89%, 3.71%–4.21%, 1.55%–3.98%, 0.04 to 0.25 g, 0.42–0.84, 0.38–0.62, 18.00–48.42 µg/mL and 2.74×10^5–1.39×10^{11} cfu/g respectively. The results showed that qualities of meat were positively affected by silver bionanoparticle treatment.
Conclusion: Meat treated with10% of silver bio-nanoparticle concentration for 4 h had the best quality.

Key words: bio-nanoparticle; technological properties; chemical properties; sensory properties.

Introduction

Meat is a good source of protein, fat, and minerals, and it also contains high percentage of moisture which has a significant impact on its physico-chemical, sensory, and technological properties (Barat *et al.*, 2009). Several meat preservation methods have been reported by many researchers. Kozempel *et al.* (2003) reported on preservation of meat using steaming method and concluded that it reduced the meat nutrient quality, most especially the vitamin B complex which soluble in water. During low temperature preservation of meat, the freezing and thawing cycles have deleterious effect on meat quality (Kondratowicz *et al.*, 2008). However, the use of chemical preservatives for meat preservation is limited by reduction in its sensory quality (Schirmer *et al.*, 2010). Therefore, there is a need to develop sound science or technologically based preservation methods with the possibility retaining meat quality.

Nanotechnology is an inter-disciplinary science that connects knowledge of biology, chemistry, physics, engineering, and material science (Islam and Miyazaki, 2009) and deals with the design, production, and application of nanoparticles with a size below 100 nm (Nowack, 2010). Silver nanoparticles (Ag-NPs or nanosilver) have attracted increasing interest due to their unique physical, chemical, and biological properties compared with their macro-scaled counterparts (Sharma *et al.*, 2009). Ag-NPs exhibit broad-spectrum bactericidal and fungicidal activities (Ahamed *et al.*, 2010), which makes it extremely popular in a diverse range of consumer products, including plastics, soaps, pastes, food, and textiles (Garc´ıa-Barrasa *et al.*, 2011).

Ag-NPs can be synthesized using different methods. The physical and chemical methods used to synthesize Ag-NPs are expensive and often raise questions of environmental risk because it involves the use of toxic and hazardous chemicals (Tripathy *et al.*, 2010). The prospect of

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exploiting natural resources for metal nanoparticle synthesis has become an environmentally benign approach (Bhattacharya *et al.*, 2005). The synthesis of Ag-NPs, by reducing the silver ions present in the solution of silver nitrate in aqueous extract of papaya fruit, was reported by Maqdoom *et al.*, (2013) and discovered that the biologically synthesized nanoparticles were found to be highly toxic against different multi-drug-resistant human pathogens. This suggests that biologically synthesized nanoparticles can also be used for food preservation. Therefore, applying bio-nanoparticles synthesized from an antimicrobial silver nitrate and a meat tenderizer papain extract for meat treatment might have a positive effect on the quality of treated meat. The aim of this research is to investigate the preservative potential of silver bio-nanoparticles from papaya fruit extract and silver nitrate solution.

Materials and Methods

Materials

Raw materials and sample preparation

The cow meat was purchased at Bodija Market in Ibadan, Nigeria. The meat was cut into dimensions of 3 × 3 × 2 cm using a sharp stainless steel knife. The preparation of extract and synthesis of silver bio-nanoparticles were done according to the method of Maqdoom *et al.* (2013) described below.

Preparation of extract

Unripe papaya fruits of 25 g in weight were thoroughly washed in distilled water, drained, and cut into pieces, and the pieces were ground into slurry using a blender. The slurry was then poured into 100 ml sterile distilled water and filtered through Whatman No. 1 filter paper (pore size 11 µm). The filtrate was further filtered through 6 µm-sized filters, and the free biomass residue that was not the capping ligand of the nanoparticles was removed from the solution obtained and centrifuged at 5000 rpm for 10 min. The resulting suspension was dispersed into 10 ml sterile distilled water. The centrifugation and dispersion processes were repeated three times (Maqdoom *et al.*, 2013).

Synthesis of silver bio-nanoparticles

One millimolar (1 mM) of aqueous solution of silver nitrate ($AgNO_3$) was prepared and used for the synthesis of Ag-NPs. Papaya fruit extract (10 ml) was added into 90 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ ions and the mixture was kept at room temperature for 24 h (Maqdoom *et al.*, 2013).

Methods

The pure silver bio-nanoparticle suspension produced was measured into 10, 15, and 20 ml and made up to 100 ml by adding sterile distilled water in order to achieve 10, 15, and 20 per cent concentrations, respectively. The meat sample was immersed into each solution and left without agitation for a period of 2, 4, and 6 h for each sample (Figure 1).

Determination of UV-vis spectra of the prepared silver bio-nanoparticles solution

The reduction of pure Ag+ ions was monitored by measuring the UV-vis spectrum of the reaction medium. UV-vis spectral analysis was done using a double-beam UV-vis spectrophotometer (AOAC, 2005).

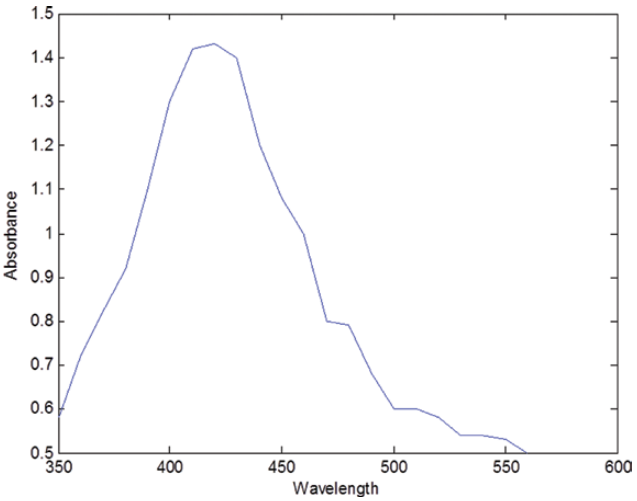


Figure 1 Flow chart for the production of bio-nanoparticle-treated meat.

Determination of technological properties

Percentage weight reduction

Five grams of each sample was weighed and immersed in a bio-nanoparticles solution. The weight observed was recorded before and after treatment. The weight reduction (WR) was determined according to the following equation (AOAC, 2005):

$$\%WR = \frac{w_o - w}{w_o} x \frac{100}{1},$$

where w_o is the initial sample weight (g) and w is the sample weight after osmotic dehydration (g).

Percentage solid gained

Five grams of fresh and bio-nanoparticle-treated samples were placed in an oven already set at 100°C to dry to constant weight for 8 h. The change in weight observed was recorded. The solid gained (SG) was determined from the following equation (AOAC, 2005):

$$\%SG = \frac{u - u_o}{w_o} x \frac{100}{1},$$

where u_o is the initial solid content in the fresh sample (g) and u is the solid content in the sample after osmotic dehydration (g).

Determination of absorbed silver ion in bio-nanoparticle-treated meat: wet oxidation of the sample

Five grams (5 g) of the samples were weighed into digestion flask, and 10 ml of 2:1 by volume of nitric/perchloric acid was added to the sample and digested until dense white fumes appeared. The digest was allowed to cool and some quantity of distilled water was added to the digest. The solution was then filtered into 50 ml volumetric flask and diluted to volume (Hossner, 1996).

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Ag analysis using the atomic absorption spectrophotometer

Nanosilver was analysed using model 210VGP of the Buck Scientific Atomic Absorption Spectrometer series with air-acetylene gas mixture as oxidant. Extract from the digestion were aspirated and the equipment calibrated for silver (Ag). The results were recorded as mg l^{-1} of solution and calculated as mg kg^{-1} of sample (Hossner, 1996).

Calculation

$$mgkg^{-1} = digest - conc\, x\, dilution\ factor,$$

$$digest - conc = analyte\ reading\ on\ ASS,$$

$$dilution\ factor = \frac{vol.of\ digest\ x\ aliquot}{weight\ of\ the\ sample}.$$

Determination of wet preservation effect of silver bio-nanoparticle-treated meat

The wet preservative effect of silver bio-nanoparticles was determined using the method of Hossner (1996). Each treated sample was wrapped in polythene nylon and stored without refrigeration for 7 days. The microbial quality was determined for day one, three, and seven by conducting an aerobic mesophilic plate count test on the samples.

Total plate count

The total plate count for microbial enumeration was determined using standard procedure established by Prescott (2005).

Determination of chemical composition

The protein, ash, and crude fat contents of the meat samples were evaluated using the standard AOAC (2005) procedure.

Sensory evaluation

Cooking was carried out on the samples at 80°C for 50 min in a water bath. Each sample was properly wrapped and labelled before cooking. After the cooking process, the samples were provided to the judges. Twenty trained panelists were selected from the staff and students of the University of Ibadan, Ibadan, Nigeria. The panelists evaluated the characteristics of bio-nanoparticle-treated meat for aroma, texture, taste, juiciness, and overall acceptability, using a 9-point Hedonic scale where 1 and 9 represent dislike extremely and like extremely, respectively.

Statistical analysis

Data obtained were analysed using SPSS (Version 17.0, 2002) statistical package. Data analysed with SPSS were subjected to analysis of variance (ANOVA) and their means were separated with Duncan's Multiple Range Test (DMRT) according to Larmond (1977) with a statistical significance number of $P < 0.05$.

Results and Discussion

UV-vis spectra analysis of silver bio-nanoparticles solution

Figure 2 shows the UV-vis spectra recorded from the reaction medium after 24 h. Absorption spectra of Ag-NPs formed in the reaction media had absorbance peak at 430 nm, and broadening of peak indicated that the particles were polydispersed.

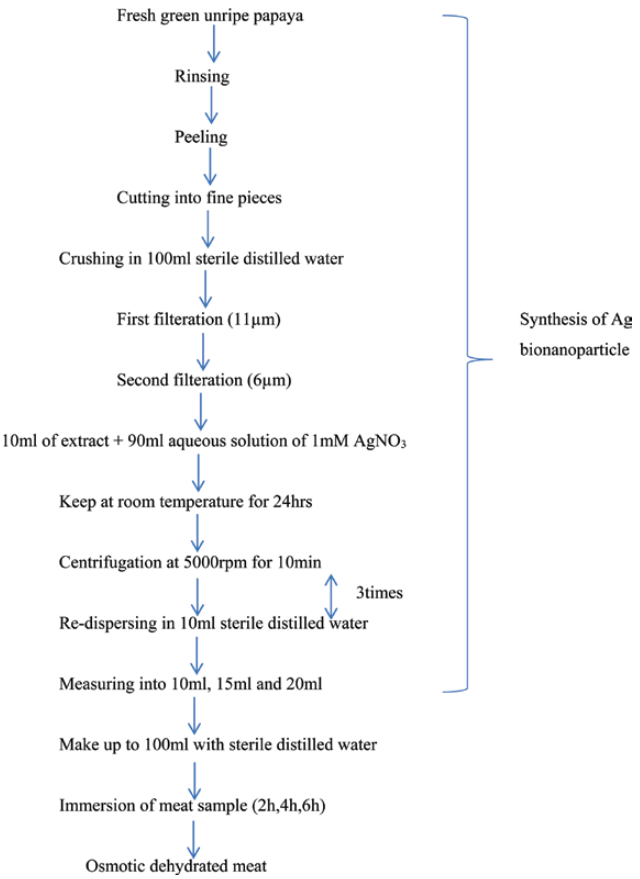


Figure 2 UV-vis absorption spectrum of silver nanoparticles synthesized.

The size of Ag-NPs is very important because it is connected with their antimicrobial properties as reported by Fernandez *et al.* (2009). However, when their size increases, the activity of silver nanaoparticles decreases (Nabikhan *et al.*, 2010). The particle sizes were below 100 nm and their optimum wavelength under UV spectrophotometer was at 430 nm (Figure 2). The particle sizes obtained fell within the range reported by Fernandez *et al.* (2009).

Technological properties of silver bio-nanoparticle-treated meat

The effects of the bio-nanoparticles solution on technological properties (water reduction, solid gained, and water loss) of the samples were presented in Tables 1–3, respectively. All parameters measured showed significant difference ($P < 0.05$) except for solid gained with concentration contact period of 4 h. The highest weight reduction was 0.25 g at a contact period of 6 h in 20 per cent concentration, whereas the lowest was 0.04 g at a contact period of 2 h in 10 per cent concentration. The highest solid gained was 0.62 g at a contact period of 6 h in 20 per cent concentration, whereas the lowest was 0.38 g at a contact period of 2 h in 10 per cent concentration. The highest water loss was 0.84 g at a contact period 6 h in 20 per cent concentration, whereas the lowest was 0.42 g at a contact period of 2 h in 10 per cent concentration. The effect could be related to pH and the salt concentration. The pH value of 5 could result in weight loss, which was probably due to swelling that occurs in the myofibrillar proteins and connective tissue (Barat *et al.*, 2009). The technological properties increased with increase in concentration and

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Table 1. Weight reduction (wr)

Time (h)	Concentration		
	10	15	20
	wr (g)	wr (g)	wr (g)
2	$0.04^{g} \pm 0.00$	$0.07^{f} \pm 0.00$	$0.10^{e} \pm 0.01$
4	$0.16^{d} \pm 0.01$	$0.21^{b} \pm 0.00$	$0.22^{b} \pm 0.00$
6	$0.15^{d} \pm 0.01$	$0.19^{c} \pm 0.01$	$0.25^{a} \pm 0.00$

Mean values having different superscripts are significantly different ($P < 0.05$).

Table 2. Solid gained (g)

Time (h)	Concentration		
	10%	15%	20%
2	$0.38^{c} \pm 0.02$	$0.51^{b} \pm 0.02$	$0.55^{b} \pm 0.02$
4	$0.51^{b} \pm 0.01$	$0.50^{b} \pm 0.01$	$0.50^{b} \pm 0.01$
6	$0.42^{c} \pm 0.02$	$0.52^{b} \pm 0.01$	$0.62^{a} \pm 0.02$

Mean values having different superscripts are significantly different ($P < 0.05$).

Table 3. Water loss (g)

Time (h)	Concentration		
	10%	15%	20%
2	$0.42^{e} \pm 0.01$	$0.58^{d} \pm 0.02$	$0.65^{c} \pm 0.02$
4	$0.67^{bc} \pm 0.01$	$0.72^{ab} \pm 0.02$	$0.75^{b} \pm 0.02$
6	$0.58^{d} \pm 0.02$	$0.71^{ab} \pm 0.01$	$0.84^{a} \pm 0.02$

Mean values having different superscripts are significantly different ($P < 0.05$).

Table 4. Silver ion (Ag) concentration in samples of bio-nanoparticle-treated meat (μg/ml)

Time (h)	Concentration		
	10%	15%	20%
2	$18.00^{e} \pm 1.15$	$21.06^{d} \pm 0.58$	$21.89^{d} \pm 0.58$
4	$36.84^{b} \pm 0.58$	$36.78^{b} \pm 0.58$	$39.41^{b} \pm 1.15$
6	$25.94^{c} \pm 0.58$	$39.86^{b} \pm 1.15$	$48.42^{a} \pm 1.73$

Mean values having different superscripts are significantly different ($P < 0.05$).

Table 5. Wet preservation effect for 10 per cent concentration (cfu/g)

No. of days	0	3	7
Immersion time (h)	Microbial count		
0	3.77×10^{5}	9.54×10^{9}	1.39×10^{11}
2	3.55×10^{5}	2.70×10^{8}	7.44×10^{9}
4	2.86×10^{5}	1.72×10^{7}	4.54×10^{8}
6	3.02×10^{5}	9.89×10^{7}	1.12×10^{9}

Table 6. Wet preservation effect for 15 per cent concentration (cfu/g)

No. of days	0	3	7
Immersion time (h)	Microbial count		
0	3.77×10^{5}	9.54×10^{9}	1.39×10^{11}
2	3.42×10^{5}	2.56×10^{8}	6.52×10^{9}
4	2.91×10^{5}	1.68×10^{7}	4.25×10^{8}
6	2.74×10^{5}	1.43×10^{7}	3.19×10^{8}

residence time. This could be related to the effect of mass transfer which is affected by pH of sample and behaviour of salt during the osmotic process. According to Koprivca *et al.* (2010), mass transfer is caused by a difference in osmotic pressure, water outflow from product to solution, solute transfer from solution into the product, and leaching out of the products own solutes.

Silver ion concentration of bio-nanoparticle-treated meat

The result of concentration of silver ion absorbed is shown in Table 4. The concentration measured showed significant difference ($P < 0.05$) for every sample except for sample with a contact period of 4 h. The result obtained was within the range of most reported toxicology and microbial inhibition concentration of 1–100 μg/ml (Morones *et al.*, 2005). However, the size used in this study is small compared with most in the literature and is expected to be of high-dose range. In Table 4, the maximum value recorded was 48.42 μg/ml for a contact period of 6 h in 20 per cent concentration, whereas the lowest value recorded was 18.00 μg/ml for a contact period of 2 h immersion at 10 per cent concentration.

Effect of treatment on microorganisms

The results are shown in Table 5–7 for 10, 15, and 20 per cent concentrations. The result showed that the effectiveness of bio-nanoparticles wet preservation of meat increased with increase in concentration. The total plate count value of 7×10^{5} cfu·g^{-1} was considered as an upper microbiological limit for good fresh meat quality, as defined by Dainty *et al.* (1992). Therefore, all meat samples stored beyond a day without treatment were found unsafe for consumption due to heavy microbial load. However, samples with a contact period of 6 h in 15 and 20 per cent concentrations had the least microbial populations, and extension of the retention time at a contact period of 6 h beyond 6 days resulted in an increase in microbial population. This might be due to reduction in the efficacy of the bio-nanoparticles over time. This implies that microbial growth inhibition in the samples was dependent on concentration and retention time. Similar findings were observed by Shrivastava *et al.* (2007) who discovered that Ag-NPs antimicrobial activities were influenced by the dosage applied.

Chemical composition of silver bio-nanoparticle-treated meat

The effects of the bio-nanoparticles solution on chemical compositions (protein, fat, and ash) of the samples were studied and the results are presented in Table 8. The concentration of the silver bio-nanoparticles significantly influenced the protein, fat, and ash contents of the meat samples ($P < 0.05$).

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The protein, fat, and ash contents of the samples ranged from 21.63 to 37.19 per cent, 3.71 to 5.00 per cent, and 1.55 to 3.98 per cent, respectively. The protein and fat content decreased as the concentration of the silver bio-nanoparticles increased. Limited reduction in protein and fat contents (30.88% and 4.21%, respectively) was observed from the sample treated with 10 ml concentration of silver bio-nanoparticles for 2 h. Protein denaturation can be explained as the changes in the protein structures due to the disruption of chemical bonds and by secondary interactions with other constituents (Alizadeh, 2009). The reduction in crude protein of the meat samples during treatment with solutions containing silver bio-nanoparticles could be attributed to the gradual degradation of the initial crude protein to more volatile products as total volatile bases (TVB), trimethyl amine (TMA) hydrogen sulphide, and ammonia. Also, it might be due to a decrease in salt-soluble and water-soluble proteins (Chomnawang *et al.*, 2007) or to autolytic deterioration associated with the actions of endogenous enzymes and bacteria (Hultman and Rustard, 2004). Fats play protective roles in the body system (Olusanya, 2008) and some important fatty acids such as omega-3-fatty acid, etc., that are derived from fats played significant roles in the proper functioning of body system (Obidoa *et al.*, 2010). The reduction in fat content indicates an increase in lipid oxidation. The reduction in the fat contents of the silver bio-nanoparticle-treated samples could be due to the release of oxidative enzymes and prooxidants from various rupture cellular organelles (Boonsumrej *et al.*, 2007).

Ash content is an index of mineral contents in biota (Akubugwo *et al.*, 2007). The ash contents of the samples increased as the concentration of the silver bio-nanoparticles increased (Table 8). Reductions in other chemical components (protein and fat) might result into corresponding increase in ash contents due to the concentration of soluble solids with relatively chemically stable products.

Table 7. Wet preservation effect for 20 per cent concentration (cfu/g)

No. of days	0	3	7
Immersion time (h)	Microbial count		
0	3.77×10^{5}	9.54×10^{9}	1.39×10^{11}
2	3.42×10^{5}	2.54×10^{8}	6.48×10^{9}
4	2.77×10^{5}	1.55×10^{7}	4.32×10^{8}
6	2.58×10^{5}	1.36×10^{7}	3.02×10^{8}

Table 8. Chemical composition of silver bio-nanoparticle-treated meat (%)

Time (h)	Concentration		
	10%	15%	20%
Protein content			
2	$30.89^{a} \pm 1.73$	$29.88^{ab} \pm 1.15$	$29.25^{ab} \pm 1.73$
4	$26.85^{bc} \pm 0.58$	$25.63^{cd} \pm 1.15$	$26.44^{bc} \pm 1.15$
6	$28.44^{ab} \pm 1.15$	$22.25^{de} \pm 1.15$	$21.63^{e} \pm 0.58$
Fat content			
2	$4.21^{a} \pm 0.035$	$4.13^{ab} \pm 0.023$	$4.11^{b} \pm 0.035$
4	$3.88^{d} \pm 0.046$	$3.88^{d} \pm 0.023$	$3.86^{de} \pm 0.023$
6	$4.02^{c} \pm 0.029$	$3.78^{ef} \pm 0.012$	$3.71^{f} \pm 0.029$
Ash content			
2	$1.55^{d} \pm 0.06$	$1.76^{cd} \pm 0.12$	$1.87^{c} \pm 0.17$
4	$3.19^{b} \pm 0.11$	$3.22^{b} \pm 0.06$	$3.26^{b} \pm 0.03$
6	$1.90^{c} \pm 0.06$	$3.90^{a} \pm 0.06$	$3.98^{a} \pm 0.05$

Mean values having different superscripts are significantly different ($P < 0.05$).

Sensory evaluation

The result of the sensory evaluation as displayed in Table 9 indicated the concentration of solutions (10%, 15%, and 20%) and time of immersion (2, 4, and 6 h) of meat samples. All parameters measured showed significant differences ($P < 0.05$). The highest value for aroma (8.10) was recorded for samples treated with 20 per cent concentration for a period of 6 h, whereas the lowest value (5.90) was recorded for samples treated with 20 per cent concentration for a period of 2 h. Considering the texture of the treated meat samples, samples treated with 15 per cent concentration for a period of 2 h had the highest value of 8.15, whereas samples treated with 15 per cent concentration for a period of 6 h had the lowest value of 3.95. For taste, samples treated with 10 and 15 per cent concentrations for 2 h had the highest value of 8.25, whereas samples treated with 15 per cent for 6 h had the lowest value of 5.95. For juiciness, samples treated with 10 per cent concentration for a period of 2 h had the highest value of 8.15, whereas samples treated with 15 per cent for 6 h had the lowest value of 3.95, respectively. The value for colour showed that samples treated with 15 per cent concentration for 6 h had the highest value of 8.10, whereas samples treated with 15 per cent concentration for 2 h had the lowest value of 3.90, respectively. However, samples in 10 per cent concentration and with 4 h immersion time had all their sensory attribute scores above 5, giving this time as the optimum immersion time.

Table 9. Sensory evaluation of bio-nanoparticle osmotic–treated meat

Parameters	Aroma	Texture	Taste	Juiciness	Colour	Overall acceptability
s102	$6.10^{d} \pm 0.06$	$7.9^{b} \pm 0.06$	$8.25^{a} \pm 0.06$	$8.13^{a} \pm 0.07$	$4.25^{d} \pm 0.06$	$7.16^{a} \pm 0.10$
s152	$6.06^{d} \pm 0.14$	$8.15^{a} \pm 0.06$	$7.96^{b} \pm 0.06$	$7.95^{b} \pm 0.06$	$3.90^{e} \pm 0.06$	$7.10^{a} \pm 0.06$
s202	$5.92^{d} \pm 0.07$	$7.97^{ab} \pm 0.07$	$7.93^{b} \pm 0.06$	$6.92^{de} \pm 0.07$	$3.97^{ef} \pm 0.07$	$7.37^{a} \pm 0.23$
s104	$7.20^{b} \pm 0.06$	$6.95^{d} \pm 0.06$	$7.11^{c} \pm 0.11$	$7.10^{c} \pm 0.06$	$6.10^{b} \pm 0.06$	$6.94^{a} \pm 0.07$
s154	$7.15^{b} \pm 0.06$	$6.9^{d} \pm 0.06$	$7.15^{c} \pm 0.06$	$6.90^{e} \pm 0.06$	$6.11^{b} \pm 0.06$	$6.90^{a} \pm 0.06$
s204	$6.79^{c} \pm 0.13$	$7.26^{c} \pm 0.08$	$6.94^{c} \pm 0.11$	$6.98^{de} \pm 0.60$	$5.75^{c} \pm 0.22$	$6.91^{a} \pm 0.06$
s106	$7.90^{a} \pm 0.06$	$4.25^{e} \pm 0.06$	$6.00^{d} \pm 0.06$	$4.10^{f} \pm 0.06$	$7.97^{a} \pm 0.06$	$5.49^{b} \pm 0.61$
s156	$7.96^{a} \pm 0.06$	$3.96^{f} \pm 0.06$	$5.95^{d} \pm 0.61$	$3.95^{f} \pm 0.06$	$8.10^{a} \pm 0.06$	$5.91^{b} \pm 0.06$
s206	$8.10^{a} \pm 0.06$	$4.06^{ef} \pm 0.11$	$6.05^{d} \pm 0.06$	$3.95^{f} \pm 0.06$	$7.97^{a} \pm 0.06$	$5.94^{b} \pm 0.06$

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Conclusions

The result of the study showed that qualities of meat were positively affected by silver bio-nanoparticle treatment. The silver concentration of 10 per cent, 4 h immersion having absorption of 36.84 µg/ml could be said to be the optimum condition for the bio-nanoparticle treatment in terms of quality parameters considered. The sensory evaluation was within the highest range (7) of the overall acceptability in the degree of preference which indicated 'like moderately'. The microbial count was within the lowest colony obtained (10^8) after 7 days. The chemical composition (protein 29%, fat 3.88%, and ash 3.19%) and technological properties (weight reduction 0.21 g, solid gained 0.51 g, and weight loss 0.67 g) were also observed to be well preserved at the concentration when compared with others. Since meat processing is a daily activity that is not limited to the operations in abattoir, therefore, people should therefore be enlightened on the advantage of the use of AgNPs solution in meat processing. The use of the solution should be included in the SOPs of large-scale meat processing industry and butchery.

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