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EFFECTS OF SILVER NANO-PARTICLES ON MEAT PRESERVATION

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Abstract

Nano packaging is currently one of the most important topics in food packaging technologies. The aim of the application of this technology in food packaging is increasing shelf life of foods by preventing internal and external deterioration and microbial contaminations. Hence this study investigate the effects of silver nano-particles on meat preservation. The silver nanoparticle was sourced from Sigma Aldrich. Meat samples were treated with solutions containing 10%, 15% and 20% silver nanoparticle solution and were kept for 24, 72 and 120 hours. Bacterial count, oraganoleptic characteristics and chemical properties were determined using standard methods. The effects of silver nano-particles on microorganisms revealed that six (6) microorganism were isolated. The microbial isolates are Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Clostridium perfringens, Listeria monocytogenes and Salmonella cholerabuis. The organoleptic characteristics showed that the meat texture, odour and colour change was after 72 hours. Proximate composition of the meat showed that the highest crude protein is recorded in the control group with 83.75% and the least was in group C with 69.21%. Also control had the highest percentage of ether extract with 22.43% and group D recorded the least with 16.09. The highest ash and fat content were recorded in the control group and the least is recorded in group C. in conclusion Meat treated with 20% of silver nanoparticle concentration for 24 hours had the best quality.

Key Words: *Silver, Nano-particles, Meat, Preservation*

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 and Langsrud*,* 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). Nanotechnology is emerging as a rapidly growing field with its wide application in science and technology for manufacturing of new materials at nanoscale level (Albrecht *et al.,* 2006). This technology gained a tremendous impetus due to its capability of reformulating metals into new nanosized particles, with dimension less than 100 nm in size. Due to nanoparticle size, their physio-chemical properties drastically changes leading to broad spectrum of new applications.

In recently years, huge advances in nanotechnology open up a new era in industrial technology, majority of nanoparticles incorporated into products related to several fields (Antonio *et al.,* 2014). Nanoparticles have been used in disinfection of textile fabrics, water disinfection, medicine, and food packaging/preservation (Mihindukulasuriya and Lim, 2014). Due to their high surface area to volume ratio and the unique chemical and physical properties, nanoscale materials have emerged up as novel antimicrobial agents (Morones *et al.,* 2005; Kim *et al.,* 2007). Metal nanoparticles with their potent antimicrobial properties are therefore used as "active packaging". Emerging metal nanoparticles with biocidal properties are Cu, Zn, Au, Ti, Ag (Toker *et al*., 2013). Among them silver nanoparticles (AgNPs) demonstrated to have the most effective bactericidal properties against a wide range of pathogenic microorganisms, including bacteria, yeasts, fungi and viruses (Rai *et al*., 2009; Martinez-Abad *et al*., 2012). AgNPs showed better antimicrobial properties compared to metallic silver thanks to their extremely large surface area which can provide a better contact with the microorganism (Toker *et al*., 2013). Furthermore, they exhibit low volatility and stability at high temperatures (Youssef and Abdel-Aziz, 2013). 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).

Fresh meat is a highly perishable commodity because of its biological composition. At the time of slaughter, meat obtained from a normal, healthy animal may be regarded as essentially bacteria-free nevertheless, contamination of the meat may originate from the animal, hands of personnel and equipment. Meat spoilage mainly results from microbial growth (Cho *et al.,* 2005). As shelf life is one of the problems of preserving meat, research to improve the methods of transporting, packaging and storing food products continues (Cho *et al.,* 2005). Under normal aerobic packaging conditions, the shelf life of refrigerated meat is limited by the growth and activities of aerobic bacteria. In

the meat industry, the possibility of increasing the shelf life of meat by additional control methods is one of the main objectives of research. Silver nanoparticles (Ag-NPs) have recently attracted increasing interest because among heavy metal nanoparticles, Ag-NPs are known to possess inhibitory and bactericidal effects against both gram-positive and gram-negative bacteria (Cho *et al.,* 2005). Therefore this study is undertaken to determine the effects of silver nano-particles on meat preservation.

Methodology

Sample Collection

A fresh beef meat was purchased from the retailer at "Monday market" in New-bussa, Niger state, Nigeria. The sample was collected into a clean polythene bag and sealed to prevent contamination. The sample was taken to the laboratory within 1hour of purchase for analysis. A stock solution of silver nano-particle was purchase from sigma Aldrich in Lagos.

Sterilization of Materials

All glass wares were thoroughly washed with detergents, rinsed with distilled water and subsequently allowed to drain, after which they were wrapped with aluminum foil and were sterilized in the oven at 170^0c for 1 hour before use. All media were sterilized by autoclaving at 121^0c for 15 minutes. Inoculating loops were heated to red hot in the Bunsen flame. The work bench was swabbed with 70% alcohol and all inoculation, pour platting and sub culturing were done near naked flame to enhance aseptic condition.

Sample Preparation and Treatment

The meat was cut into dimensions of 3×3 cm thick using a sharp stainless steel knife. The sample was divided into four groups and placed in petri dish labeled A, B, C and D. Each of the four samples were subdivided into three portions to represent a replicate. The pure silver nanoparticle solution 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 samples in group A were kept in laboratory cabinet without any treatment. Samples in group B were immersed in 10% for 24, 72 and 120 hours. Samples in Group C were immersed in 15% silver nano-particles for 24, 72 and 120 hours while the group E were immersed in 20% silver nano-particles for 24, 72 and 120 hours. All the treated samples were joined with the untreated sample in the laboratory cabinet for storage.

Isolation and Enumeration of Bacteria in the Samples

The total bacteria counts were determined by standard plate count using pour plate method. One gram of sample was weighed using weighing balance and then put in a test tube containing 9mL of sterile distilled water, making 10^{-1} dilution. The sample was marshed properly and 1mL of this suspension was transferred using sterile syringe into another test tube containing 9mL of sterile distilled water, making 10^{-2} dilution. This was repeated to obtain 10^{-4} dilutions. From each of 10^{-3} & 10^{-4} diluents, 0.1mL was transferred onto Nutrient Agar (NA) plates and spread using flame sterilized glass rod. Colonies were counted using Cole Parmer colony counter and described following a guide by Fawole and Oso, (2007). The bacterial identification were characterized on the basis of their colonial morphology, pigmentation, staining and microscopy and biochemical tests.

Organoleptic Analysis

The organoleptic properties of the beef sample were determined. The texture was

determined by feeling the sample between fingers to determine whether it is hard, soft or slippery, the change in colour was determined by comparing the sample colour after treatment and storage with the sample original colour before treatment and storage while the change in odour was determined by bringing the sample close to the nostrils to smell to determine whether it is pleasant, slightly pleasant, moderately bad, bad or very offensive. The changes in organoleptic properties were conducted by four individuals in order to ascertain the change in smell, odour and texture by individuals with different sense of smell, touch and sight.

Determination of Proximate Composition

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

Data Analysis

Data obtained was analysed using SPSS (Version 20.0) statistical package. Data were subjected to descriptive statistics in form of tables.

Results

The effects of silver nano-particles on microorganisms is reveal in table 1, in which six (6) microorganism was isolated. The microoganisms are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes* and *Salmonella cholerabuis*. Highest number of microorganism were recorded among group A and lowest number are recorded among group D. For 24 hours in Group A, four organism was isolated which are *Staphylococcus aureus, Escherichia coli, Clostridium perfringens* and *Salmonella cholerabuis.* While 72-120 hours contains all the microorganism and they are too numerous to be counted.

Table 1: Effect of Silver Nano-particle against Microorganisms Load in Meat Sample

Period of Preservation	Microorganisms count (cfu/mL) x 10^3							
	Control (A)		10% Silver Nano-silver Particles (B)		15% Silver Nano-silver Particles (C)		20% Silver Nano-silver Particles (D)	
	Organism	No	organism	No	Organism	No	organism	No
24 hour	A	TNTC	A	11	A	1	D	1
	C	21	B	8	C	6		
	D	TNTC	D	17	F	3		
	F	39						
72 hours	A	TNTC	A	31	A	28	A	17
	B	TNTC	B	27	C	31	C	23
	C	TNTC	C	4	D	13	D	17
	D	TNTC	D	12	F	3		
	E	TNTC	F	9				
	F	TNTC						
120 hours	A	TNTC	A	TNTC	A	49	A	52
	B	TNTC	B	58	C	TNTC	B	21
	C	TNTC	C	41	D	38	C	35
	D	TNTC	D	TNTC	F	29	D	29
	E	TNTC	F	TNTC				
	F	TNTC						

Key; TNTC- Too Numerous to be counted; A- *Staphylococcus aureus*; B - *Pseudomonas aeruginosa*; C- *Escherichia coli*; D- *Clostridium perfringens*; E- *Listeria monocytogenes* F- *Salmonella cholerabuis*

Table 2 shows the change in texture of the sample during storage. The texture across 24 hours remains soft throughout the storage period while the texture at 72 hours at 10 and 15% are hard while 20% still retain soft. The texture at 120 hours at 10 and 15% are slippery while 20% remains hard.

Table 2: Change in Texture of Silver nano-particle treated sample during storage

Period of Preservation	10%	15%	25%
24 hours	Soft	Soft	Soft
72 hours	Hard	Hard	Soft
120 hours	Slippery	Slippery	Hard

Table 3 shows the change in odour that occurred during storage of the samples. The odour of the control sample changed to moderately bad after 24 hours while 10% change to slightly pleasant and 15 and 20% are pleasant. At 72 hours the control sample had become bad while 10% had change to moderately bad and 15% had change to slightly pleasant while 20% still retains pleasant. At 120 hours, control had change to bad and offensive, 10% had change to bad, 15% had change to moderately bad while 20% had change to slightly pleasant.

Table 3: Changes in Odour of Silver Nano-particle Treated Beef sample During Storage

Period of Storage	Control	10%	15%	20%
24 hours	Moderately bad	Slightly pleasant	Pleasant	Pleasant
72 hours	Bad	bad	Slightly pleasant	Pleasant
120 hours	Bad and offensive	bad	Moderately Bad	Slightly Pleasant

Change in colour is presented in Table 4 and it shows deterioration in colour that occurred during the storage period.

Table 4: Change in colour of Silver Nano-particle Treated Beef sample During Storage

Period of Storage	Control	10%	15%	20%
24 hours	Dark Brown	Brown	Brown	brown
72 hours	Black	Dark Brown	Brown	Brown
120 hours	Black	Black	Dark brown	Brown

Proximate composition of the beef meat is revealed in table5, the highest crude protein is recorded in the control group with 83.75% and the least is in group C with 69.21%. Also control had the highest % of ether extract with 22.43% and group D recorded the least with 16.09. The highest ash content and fat content is also highest in the control group and the least is recorded in group C.

Table 5: Chemical composition of Silver Nano-particle Treated Beef sample During Storage

Period of Storage	Control	10%	15%	20%
Crude Protein (%)	83.75±0.21	71.26±0.28	70.12±0.32	69.21±0.39
Ether Extract (%)	22.43±0.11	19.42±0.19	17.05±0.22	16.09±0.27
Ash content (%)	17.10±0.32	21.19±0.21	28.34±0.17	33.02±0.12
Fat content (%)	44.23±0.24	41.03±0.29	39.09±0.37	38.49±0.42

Discussion

The multiplication of microorganisms in food is greatly influenced by the intrinsic factors and environmental characteristics of the food (Onyenekwe *et al*., 2012). In general, microorganisms multiply most rapidly in moist, nutritionally-rich, pH - neutral and warm, oxygen rich environment. The result showed that the effectiveness of Silver nanoparticles preservation of meat increased with increase in concentration. The total plate count value of 1×10^3 cfu·g−1 was considered as a microbiological limit for good fresh meat quality, as defined by Dainty and Mackey, (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 24hours in 15 and 20 per cent concentrations had the least microbial populations, and extension of the retention time at a contact period of 72 and 120 hours resulted in an increase in microbial population. This might be due to reduction in the efficacy of the silver 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.

The effects of the silver nanoparticles solution on proximate compositions revealed that protein, fat, ether extract and ash contents of the samples ranged from 69.21 to 83.75 per cent, 38.49 to 44.23 per cent, 16.09 to 22.43 and 11.34 to 17.10 per cent respectively. The protein and fat content decreased as the concentration of the silver nanoparticles increased. Limited reduction in protein and fat contents was observed from the sample treated with 15 and 20% concentration of silver nanoparticles for 24h. 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 nano-particles 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 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 nanoparticles increased. 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.

Conclusion

The result of the study showed that qualities of meat were positively affected by silver nanoparticle treatment. Based on the results of this study, nano-silver packaging could be one of the preferred choices for preserving the overall quality of beef and increase its shelf life. Nano-silver packaging reduce microbial growth and compared with control treatment, the silver concentration of 20 per cent, 24 h immersion could be said to be the optimum condition for the nanoparticle treatment in terms of quality parameters considered. It is recommended that silver nano-silver packaging should be combined with other preserving/package methods such as freezing, the spoilage time may be significantly increased compared with solely pure nano-silver packaging.

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