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Influence of Silver Nanoparticles on Microbiological and Freshness Quality Attributes of Beef Sausage and Fish Patties During Cryopreservation

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Abstract

Among the most recent developments in nanotechnology. Due to their antibacterial action, silver nanoparticles have lately gained growing interest in food applications. Traditional food preservation techniques have been shown to have several drawbacks and restrictions on their ability to effectively reduce microbial loads and maintain food quality, increase the shelf life, and improvement of physicochemical and microbiological quality in beef sausage and fish patties, such as silver nanoparticles have been deemed to be very promising replacements. These techniques could serve as an effective substitute for the food industry. The most important features of the mechanisms of action under microbiological and physicochemical as well as the effectiveness of nanoparticles used in meat and fish products are covered in this article. A significant capacity for microorganism inactivation is found in silver nanoparticles. Antimicrobial activity of AgNPs showed higher antifungal activity against (*A. flavus and A. niger*) followed that antibacterial activity against gram-positive bacteria (*S. aureus and B. subtilis*) and against gram-negative bacteria (*E coli and S. typhimurium*), respectively. Antioxidant activity of AgNPs ranged from 5.52% to 27.42% with increasing their concentrations from 5 to 100 µg/ml. The changes in total volatile nitrogen (TVN), thiobarbituric acid (TBA), and microbiological quality in AgNPs calculations were made, and the results were compared to the control sample ($p = 0.05$). Results claimed that AgNps 40.0µg/ml treatment improved microbiological quality, TVN, TBA decrease, pH, and other aspects of quality preservation ($p = 0.05$). compared to the control and lengthened their shelf-life from 8 to 16 days in cryopreservation for beef sausage and 8 to 12 days in cryopreservation for fish patties.

Keywords: Silver nanoparticles, Shelf life, Beef sausage, Fish patties**.**

1. Introduction

In recent years, the food industry has paid more attention to preservation methods for various types of processed foods containing bioactive compounds to increase the shelf life and quality of food. Biotechnological methods are being used to overcome the disadvantages of these processes, which lead to reduced nutrient levels and physicochemical properties [1].

The characterization, creation, and usage of systems, tools, and structures enable the design and implementation of nanotechnologies for a variety of objectives by controlling shape and size on a nanometer scale. Nanotechnologies can enhance food properties, easily transport nutrients and flavors into the body, extended its shelf-life, and improved production processes, [2].

Nanotechnology is one of the six key technologies that the European Commission is considering adequate to initiate sustained growth and competition between nations and industries.

Although industry experts emphasize an increasing number of articles claim that agricultural nanotechnology does not provide a substantial economic return to offset the expensive initial production costs. Patents related to nanotechnology in the fields of agriculture, food, nutrition, and medicine encourage the European Commission. [3,4,5].

However, the scientific involvement goes beyond the rise in publications and patents connected to nanotechnology and the rapid progress of

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nanotechnology key industries are convincing arguments for the EC to support nano research engagement and to explore possibilities of how the bulk of publications and patents could be transferred into marketable and profitable agricultural products over time.

Silver nanoparticles become a powerful weapon against the re-emergence of multidrug-resistant bacteria [6]. Utilizing silver-based compounds for antimicrobial applications against foodborne pathogens as well as antioxidants to maintain the quality of the food is a great advantage because silver has long been known to exhibit strong toxicity to a wide range of microorganisms with low systemic toxicity toward humans [7].

Hence metal nanoparticles could potentially be an alternative and effective approach for enhancing the safety of meat products. Regarding the regulations for using nanomaterials and nanoparticles, it should be mentioned that some salts of silver have been approved by the FDA for use in packaging materials of food products [8]**.** According to European Food Safety Authority (EFSA), the overall migration of silver nanoparticles should not exceed 0.05 mg/kg in food products, [9]**.**

Chicken breast fillet shelf life was affected by the combination of an LDPE film packaging with AgNPs and a changing environment. AgNPs were included in the creation of two films and assessed for their compatibility with polymer (0.5 and 1% polymer weight, w/w).antibacterial action against different types of bacteria. Compared to bacterial growth in the control film (without AgNPs) Ag/LDPE nanocomposite film was considerably hindered until day 6 (up to a decrease of 22.5%), which greatly boosted the chicken breast fillet's shelf life [10].

The results suggest that chitosan-silver nanoparticles could be used in food preservation as antimicrobial agents and for shelf-life extension [11]**.**

Melt mixing was used to create films comprising silver, clay, and titanium dioxide nanoparticles. The findings showed that chicken wrapped in film terms had a logarithmic decline in the number of microorganisms during the chicken's shelf life. As a result, this study suggests using sheets with silver and titanium dioxide nanoparticles for chicken packing [12].

Muscle foods, such as meat and fish, are good sources of high-quality proteins, essential amino acids, B vitamins, minerals, and various other micronutrients [13, 14].

Muscle food spoils easily as well, lowering the quality and sustainability of the food supply. Due to these factors, several researchers are creating novel strategies to increase the nutritional value and shelf life of muscle meals by altering their nutritional composition or adding natural preservatives [15].

Unlike other muscle meals, fish is quite prone to chemical and microbiological degradation. Fish are quickly perishable due to their high moisture content, close composition, and natural pH, frequently spoiling soon after harvest [16].

The purpose of this study is applying of nanoscale materials that have emerged as novel antimicrobial agents, where nanoparticles of silver were effective against tested pathogenic microorganisms. The application of AgNPs appears to be highly promising in the field of food processing for extending the shelf life of beef sausage and fish patties during storage at 4 °C.

2. Experimental work

2.1.1. Raw material:

Fresh beef meat was obtained from the local market at Dokki Square, Giza, Egypt, and immediately transported in an ice box to the laboratory, then carefully cut into fillets and finally weighed until use. Packaging materials natural casings of 20 ± 2 mm diameter were purchased at the neighborhood market for Attaba in Cairo, Egypt. Spices mixture, was purchased at the neighborhood market for Cairo, Egypt. Other ingredients such as texturized soy, salt, bread crust, ground onion, and polyethylene bags were purchased from the local market, Giza, Egypt. While Fresh Nile tilapia (*Oreochromis niloticus*) fish were purchased from the local market, Giza, Egypt, and rapidly transported in iceboxes to the laboratory of Food Technology Research Institute, Agriculture Research Center, Giza, Egypt.

2.1.2. Chemicals:

Silver nitrate (99.8%) was obtained from Sigma Aldrich Company, Germany. Sodium hydroxide was obtained from RANKEM Company, New Delhi, India. Maize starch was supplied from Egyptian Starch and Glucose Company, Cairo, Egypt. 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH) and Tween 20 all were obtained from Sigma-Aldrich Chime, Steinheim, Germany.

2. 1.3. Bacterial strains:

Bacterial strains were obtained from Cairo MIRCEN, Faculty of Agriculture, Ain Shams University, Egypt. The test microorganisms were *Bacillus cereus* DSM 351, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 12600, *Salmonella typhimurium* ATCC14028. The plant pathogenic fungi strains: *Aspergillus niger* ATCC 16404 and *Aspergillus flavus* EMCC 125, were assessed for experiments of antimicrobial activity.

2.1. 4. Microbiological media:

Nutrient agar medium, VRBA medium, and chloramphenicol glucose yeast extract agar medium were obtained from Biolife Company, Italy, and the agent in Egypt Al-Badr Engineering Company.

2.1.5 Preparation:

2.1.5.1. Preparation of silver nanoparticles:

Silver nanoparticles were prepared according to the method described by [17]. Starch was dissolved in alkali solution (1 g starch in 80 ml of distilled water containing 2 g sodium hydroxide) by using high speed homogenizer. After complete dissolution the temperature of the reaction medium was raised to the desired degree (60°C). In this moment, 20 ml silver nitrate solution (10 mM) was added dropwise. The reaction medium was kept under continuous stirring for 60 minutes. After complete reaction, the solution was allowed to cool down slowly to 25°C. Then AgNPs were precipitated using absolute ethyl alcohol under high speed homogenizer. The powder precipitate was collected by centrifugation at 4,500 rpm for 15 min, washed twice with 80/20 ethanol/water to remove the unreacted materials and impurities, and then finally washed with absolute ethanol. The collected powder was dried and identified as AgNPs.

2.1.5.2. Preparation of beef sausage and Nile tilapia fish patties:

Beef sausages were processed as described by [18, 19]. formulation: 60% frozen beef, 18% fat tissue, 9% Ice water, 5% rehydrated texturized soy, 1.4% bread crust, 1.7% skimmed milk, 1.5% fresh onion, 1.9% salt, and 1.5% spices. Beef meat was cut into approximately 5 cm cubes. Beef meat and fat tissues were minced twice with the ice flakes by using the mincer. The other ingredients were added and mixed. The mixture was ground for a third time using a laboratory emulsifier (Hobbart Kneading machine) for 10 minutes. The obtained emulsion was then stuffed into a nature casing which was hand linked at about 15 cm intervals then divided into two equal portions. The first portion was used as control and the second portion were immersed for 1 min in the AgNPs solution (40 µg/mL) and, after that, they were kept over a grid to remove the excess solution[20].All beef sausages were aerobically packaged in a foam plate, wrapped with polyethylene film, and stored at 4±1˚C for up to 16 days. The samples were taken for analysis every 4 days periodically.While Nile tilapia patties were prepared without seasonings using a simple traditional formulation: 77.3% minced Nile tilapia, 6.36% corn flour, 3.63% wheat flour, 2% bread crumbs, and 1.63% salt were added as ingredients, afterward, 9.08 % ice water was added, thoroughly mixed by hand for five minutes, then divided into two equal portions and separately comminuted again through the mincer steel plate. The first portion was used as control and the second portion with adding AgNPs 40.0µg/ml. After treatments, each group was separately mixed well to ensure uniform distribution of silver nanoparticles, and the obtained pastes were formed into 50±3 g Nile tilapia patties as described and modified by [21]. All Nile tilapia patties were aerobically packaged in a foam plate, wrapped with polyethylene film, and stored at 4±1˚C for up to 16 days. The samples were taken for analysis every 4 days periodically.

2.2. Methods:

2.2.1. Transmission electron microscope of the nanoparticles:

Transmission electron microscope characterization is performed using (JEOL, JEM-1230, Japan) instrument with an acceleration voltage of 120 kV. For the TEM measurements, on a copper grid that has been coated with carbon, a drop of a solution containing nanoparticles is applied. Five minutes later, the excess drips were wiped off the film using blotting paper and a grid. allows drying before the measurements as reported by [22].

2.2.2. Antioxidant activity of nanoparticles by DPPH assay:

The (DPPH) 1,1-diphenyl-2-picrylhydrazyl is a stable free radical and is widely used to assess the radical scavenging activity of the antioxidant component. The reduction of DPPH radical was made following the methodology reported by [23] with some modifications. Briefly, DPPH solution was prepared in methanol (Exactly 0.00394 g DPPH was weighed and diluted with 100 mL of 95 % methanol to obtain 0.1 mmol/100 mL solution) and 1 ml of this solution was added to 3.0 ml of synthesized nanoparticles solution of 5,10, 25, 50, 75 and 100 µg / ml). The solution was incubated for 30 min in dark conditions at room temperature and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Butylated hydroxytoluene (BHT) and ascorbic acid were used as a standard. The control solution was prepared by mixing ethanol and DPPH radical solution. The reduction of the DPPH radical was calculated as a percentage of inhibition by equation (1):

$$\% \text{ Inhibition} = (A_0 - A_1 / A_0) \times 100 \quad (1)$$

Where: A_0 is the absorbance of the control and A_1 the absorbance of samples solution.

2.2.3. Total volatile nitrogen, thiobarbituric acid value, and pH value:

Total volatile nitrogen (T.V.N) was measured according to the method of [24]. T.V.N as mg/100g.The TBA as an indication for Lipid oxidation was measured using the technique outlined by [25]. T.B.A as mg malonaldehyde /kg sample. The pH using the technique outlined by, a pH-meter (Jenway 3510 pH meter) was used to measure the prepared sample [26].

2.2.4. Microbiological examinations:

2.2.4.1. Antimicrobial activity of nanoparticles:

The effect of different concentrations of nanoparticles (25, 50, 75, and 100 ppm) on bacteria

growth was studied by using the well-diffusion method, according to [27] by measuring the diameter of the inhibition zone.

2.2.4.2. Microbiological examination:

10 gm was added to a culture medium (1: 10^{-1} to 1: 10^{-6} and homogenized in a stomacher for 2 minutes), Total bacterial count was determined using nutrient agar medium. Incubation was carried out at 37°C for 48 hrs. The counts were then calculated per gram of samples as reported by the methodology of [28], coliforms group [29], and yeasts and molds count [30].

2.2.5. Statistical analysis:

The obtained results were analyzed using a comparison of variance (ANOVA) and least significant difference (L.S.D) at the 5% level of probability; as reported by [31].

3. Results and Discussion

3.1. Transmission Electron Microscope (TEM) of AgNPs:

Transmission electron microscope imaging showed the morphological properties and surface appearance of AgNPs which have a nearly spherical shape and smooth surface. As illustrated in **Fig. (1)**, Observations revealed that the typical particle size of the produced nanoparticles is about 10.4 to 20.9 nm. Furthermore, these nanoparticles are well dispersed with no sign of aggregation.

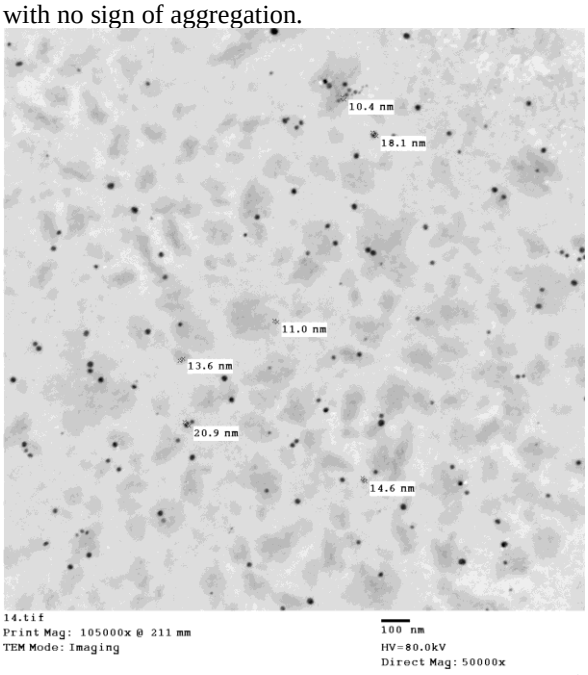

Figure **(1)** Transmission electron microscopy micrograph of AgNPs.

3.2. Antimicrobial activity of AgNPs:

Antimicrobial activity of AgNPs at different concentrations (25, 50, 75, and 100 ppm) against microorganisms strains, expressed as the diameters of inhibition zones (mm) are presented in **Table (1)**, AgNPs showed various degrees of inhibition against the tested microorganisms. The highest inhibition zone was obtained for *S. typhimurium* (21.5 mm), while the lowest (17.0 mm) was recorded for *E. coli* at 100 ppm of AgNPs. On other hand, the highest inhibition zone was obtained for *S. aureus* (22.5 mm) , while the lowest (20.5 mm) was recorded for *B. subtilis* at 100 ppm of AgNPs. Generally, AgNPs showed higher antibacterial activity against gram-positive bacteria (*S. aureus* and *B. subtilis*) than gram-negative bacteria (*E coli* and *S. typhimurium*). These outcomes are consistent with those observed by, [27]. The silver cations produced by AgNPs, which serve as reservoirs for the bactericidal agent Ag^+, are undoubtedly the cause of the high bactericidal activity of AgNPs [32]. [33], reported that, the antimicrobial activity of AgNPs against *Salmonella Typhimurium*, and stated that the activity is controlled by an inner membrane dysfunction involving reactive oxygen species (ROS)-independent Ca^{2+} imbalance. For antifungal activity, AgNPs showed various degrees of inhibition against the growth of *A. flavus* and *A. niger*. Where, the inhibition zones caused by AgNPs were 24.5 and 23.5 mm for *A. flavus* and *A. niger*, respectively as shown in **Table (1)** at 100 ppm of AgNPs. The antifungal mechanism of AgNPs has been explained by the following mechanistic pathways as reported by [34]: (1) owing to their small size, AgNPs can easily uptake by the fungal cells through the disruption of fungal cell walls; (2) AgNPs act as a reservoir for releasing of Ag^+ ion, causing to cease the ATP production and to stop DNA replication by ROS and hydroxy radicals. As a result, the biochemical cycle of fungal cells was ceased to induce fugal cells death.

Table.1. Diameter of inhibition zones (mm) of silver nanoparticles at different concentrations against some selected microorganisms.

Microbial strains	***Diameter of inhibition zones (mm)***				***L.S.D***
	silver nanoparticles				
	25 ppm	**50 ppm**	**75 ppm**	**100 ppm**	
Escherichia coli ATCC 6933	**5.50 ^{B} ^{d}±0.30**	**9.50 ^{B} ^{c}±0.50**	**12.50 ^{B} ^{b}±0.50**	**17.00 ^{B} ^{a}±0.60**	**1.683**
Salmonella typhimurium ATCC14028	**7.50 ^{A} ^{d}±0.50**	**11.50 ^{A} ^{c}±0.50**	**18.50 ^{A} ^{b}±0.50**	**21.50 ^{A} ^{a}±0.50**	**1.385**
L.S.D	**1.385**	**1.099**	**1.385**	**1.910**	

Bacillus subtilis ATCC 33221	**6.00 ^{A} ^{d}±0.00**	**8.50 ^{B} ^{c}±0.50**	**17.50 ^{A} ^{b}±0.50**	**20.50 ^{B} ^{a}±0.50**	**1.200**
Staphylococcus aureus ATCC 20231	**6.50 ^{A} ^{d}±0.50**	**11.50 ^{A} ^{c}±0.50**	**15.00 ^{B} ^{b}±0.50**	**22.50 ^{A} ^{a}±0.50**	**1.833**
L.S.D	**0.980**	**1.385**	**1.191**	**1.385**	
Aspergillus flavus ATCC 10124	**7.50^{A} ^{d}±0.50**	**12.50 ^{A} ^{c}±0.50**	**17.50 ^{A} ^{b}±0.50**	**24.50 ^{A} ^{a}±0.50**	**1.400**
Aspergillus niger ATCC 16404	**8.50^{A} ^{d}±0.50**	**12.00 ^{A} ^{c}±0.50**	**15.50 ^{B} ^{b}±0.50**	**23.50 ^{A} ^{a}±0.50**	**1.863**
L.S.D	**1.385**	**1.533**	**1.099**	**1.385**	

Where: A,B,C,D in the same columns are not significantly different($p>0.05$) while a,b,c,d in the same rows are not significantly different ($p>0.05$), L.S.D: Least significant differences at ($p>0.05$). (Mean ± standard error).

3.3. Antioxidant activity of AgNPs:

The activity of scavenging free radicals of nanoparticles was evaluated on the potency to scavenge the synthetic DPPH. The standard ascorbic acid and BHT were used to compare with preparations of AgNPs. AgNPs antioxidant capacity was assessed using DPPH scavenging tests. An overview of the antioxidant activity values was provided in **Fig 2.** The DPPH reducing the ability of AgNPs was converted from purple to yellow in the DPPH and measured spectrophotometrically. From the obtained results, it could be evidenced that, The activity of scavenging free radicals it could be observed that the antioxidant activity increased from 5.52 to 27.42 % for AgNPs and from 12.47 to 81.55 % for BHT, and from 10.33 to 68.41% for ascorbic acid with increasing their concentrations from 5 to 100 µg/ml. Also, AgNPs antioxidant was lower than antioxidant activity at all concentrations. Generally, the data obtained from **Fig 2,** exhibited good antioxidant potency and suggested the possibility that AgNPs could be effectively employed as antioxidant materials for application in the field of food. In this sense proved AgNPs to be very effective scavengers [7]**.** Outcomes appear plausible based on the huge number of articles reporting both sorts of results. The chemical makeup of the extract generally affects the silver nanoparticles' antioxidant effects, which typically get better as the AgNPs concentration rises. If the extract contains a lot of flavonoids and phenolic chemicals, the nanoparticles have a high level of scavenging action [35].

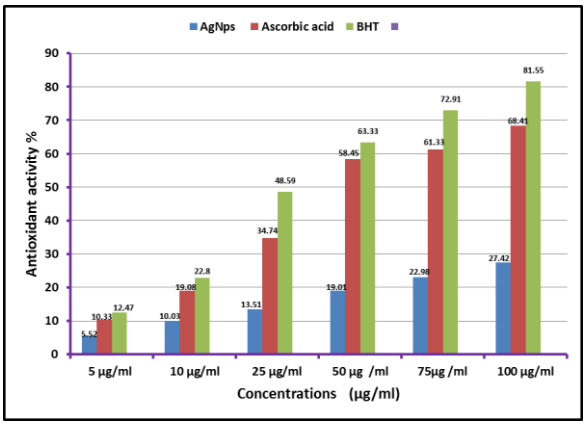

Figure (2) DPPH-free radical scavenging activity (%) of silver nanoparticles at different concentrations compared to ascorbic acid and BHT

3.4. Effect of AgNPs on freshness properties of beef sausage during cold storage at 4±1ºC:

3.4.1. Total volatile basic nitrogen (mg/100 g sample):

A perishable good is meat, and the activity of microbes and natural enzymes during storage leads to changes in chemical composition. Utilized extensively as an indicator of the degradation of proteins and amines is (TVB-N), [36].

Results given in **Table (2)** showed the effect of AgNPs on the total volatile nitrogen of beef sausage throughout cold storage at 4±1ºC for 16 days. From statistical analysis of these data, it could be observed that there were significant differences ($p\leq0.05$) in total volatile nitrogen between all beef sausage control samples or beef sausage with AgNPs at zero time, while significant differences were recorded between all above-mentioned samples during cold storage. Total volatile nitrogen of all beef sausage samples ranged from 9.85 to 9.99 mg/100g at zero time. Total volatile nitrogen values of all beef sausage were significantly affected by cold storage time. By increasing the storage period, TVBN of all beef sausage samples significantly increased ($p\leq0.05$). Control samples had significantly higher total volatile nitrogen than beef sausage samples with AgNPs at any time of storage for 4 and 8 days. This could be due high antimicrobial effect of AgNPs. After the 8th-day control sample was not evaluated because it exceeded the permissible limits of TVN and showed off odor, while total volatile nitrogen values of 16.76 mg N/100g were observed for beef sausage with AgNPs were range of permissible levels reported by [37]. Also, on the 16th day, beef sausage with AgNPs had the lowest total volatile nitrogen 19.51 mg N/100g; these values did not exceed the permissible level. These results reflex the strong antimicrobial effect of AgNPs. [38] reported that, the result of the study showed that the qualities of meat were positively affected by silver bio-nanoparticle

treatment. The mechanisms of antimicrobial activities are many potential theories that have been reported. A study on cell membrane dissolution theory states that Ag ions released from AgNPs would dissolve cell membranes and affect transcriptional responses [39]. [40] studied that, the NPs would adhere to the surface of bacterial cells, alter their membrane properties and damage the DNA, followed by affecting the protein functions. [41] showed that, AgNPs might stop the energy supply and cause the death of the cell.

3.4.2. Thiobarbituric acid (mg alonaldehyde/kg) (TBA):

The amount of time that meat products may be stored without losing quality is highly conditioned by oxidation reactions, especially those related to lipid oxidation [42]. The effect of AgNPs on TBA of beef sausage samples throughout cold storage at 4±1ºC for 16 days was presented in **Table (2)**. According to statistical analysis of these data, it could be observed that there were significant differences ($p>0.05$) in TBA values between all beef sausage samples whether the control sample or beef sausage with AgNPs sample at zero time, and significant differences were observed between them along cold storage periods. Thiobarbituric acid of all beef sausage samples ranged from 0.300 to 0.305 mg malonaldehyde/kg sample at zero time. Furthermore, the thiobarbituric acid of all beef sausage samples was significantly affected by cold storage time. Throughout the cold storage, TBA values progressively increased ($p\leq0.05$) as the period of storage increased for all beef sausage samples. This rise in TBA value during cold storage may be a sign of ongoing lipid oxidation and the subsequent formation of oxidative byproducts [18]. Beef sausage with AgNPs sample has a significantly lower TBA value than the control sample at any time of storage 4 and 8 days due to the antioxidant effect of AgNPs. On the 8th day, the highest TBA value was (0.876 mg malonaldehyde/kg) for the control sample. The spillover of prooxidants and oxidative enzymes from numerous damaged cellular organelles may be the cause of the decrease in the fat contents of the samples treated with silver bio nanoparticles [43]. On the contrary, the lowest TBA value (0.674 mg malonaldehyde/kg) was observed for beef sausage with AgNPs sample. Also, on the 16th day, TBA values of beef sausage with AgNPs sample was 0.893 mg malonaldehyde/kg sample. TBA value of beef sausage with AgNPs did not exceed the range of permissible level reported by [37], being not more than 0.9 mg malonaldehyde/kg sample.

3.4.3. pH value:

The pH of the beef sausage sample affected by with AgNPs throughout cold storage at 4±1ºC for 16 days was presented in **Table (2)**. From these results, it could be noticed that pH values of the beef sausage sample were decreased insignificantly ($p\leq0.05$) from 5.74 for the control sample to 5.66 for the beef sausage sample with AgNPs, respectively. Also, from statistical analysis of these data, it could be observed that there were significant differences ($p\leq0.05$) in pH values between all beef sausage samples whether the control sample or beef sausage sample with AgNPs at any time of cold storage. Furthermore, the pH of all beef sausage samples was significantly affected by cold storage time at 4±1ºC. With the advancement of cold storage time, the pH values were significantly decreased ($p\leq0.05$) for all. This might be caused by microbial development and acid production, which lowers pH during storage, [44]. After the 12th day, there were significant differences ($p\leq0.05$) in pH values between beef sausage samples and beef sausage samples with AgNPs, while at the end of cold storage the pH value (5.01) was recorded for beef sausage samples with AgNPs. These results reflect the strong antimicrobial effect of AgNPs.

3.5. Effect of AgNPs on microbiological quality attributes of beef sausage throughout cold storage at 4°±1C:

3.5.1. Total bacterial count (CFU/g):

Data presented in **Table (3)**, illustrated the changes in TPC of beef sausage as affected by AgNPs throughout cold storage at 4±1ºC for 16 days. The total bacterial count of all beef sausage samples ranged from 5.60×10^3 to 9.20×10^3 CFU/g immediately after processing at zero time. The highest total bacterial count (9.20×103 CFU /g) was in the control sample, while the lowest TBC (5.60×103 CFU /g) was observed for beef sausage treated with 40.0µg/ml AgNPs. TBC of all beef sausage samples was also affected by cold storage time. The total bacterial count of all beef sausage samples was increased by increasing the cold storage period at 4±1°C. [45], they found that microbial growth increased during the storage period. The increase in the total bacterial count was lower for beef sausage samples treated with AgNPs compared with beef sausage control. This indicates the effectiveness of silver nanoparticles in preventing the spread of bacteria [27]**.** Also, the total bacterial count of the control sample reached 1.65×10^6 CFU /g, on the 8th day of cold storage exceeding the maximum allowable limit of 10^6 CFU / gm for the total bacterial count for fresh and frozen sausage (Egyptian standard specifications, 2005), while the TBC of beef sausage treated with 40.0µg/ml AgNPs reached 9.30×10^5
CFU / g, at 16th day. Therefore, processing beef sausage with 40.0µg/ml AgNPs was more effective to maintain high microbiological enhancing the quality and shelf life. [38] reported that, the effectiveness of bio-nanoparticles in wet preservation of meat increased with an increase in concentration.

Table.2. Effect of silver nanoparticles on physicochemical properties of beef sausage during cold storage at 4±1ºC up to 16 days.

Storage period	*Total volatile nitrogen (mg/100 g sample)*		*L.S.D*	*Thiobarbituric acid (mgmalonaldehyde/kg)*		*L.S.D*	*pH values*	*L.S.D*	
	Control	AgNps 40.0µg/ml		Control	AgNps 40.0µg/ml			AgNps 40.0µg/ml	
0 day	9.99^{Ca}±0.017	9.85^{Eb}±0.011	0.041	0.305^{Ca}±0.000	0.300^{Eb}±0.000	0.002	5.74^{Aa}±0.015	5.66^{Ab}±0.018	0.047
4 th day	13.93^{Ba}±0.108	11.74^{Db}±0.085	0.270	0.463^{Ba}±0.010	0.376^{Db}±0.002	0.021	5.41^{Bb}±0.005	5.61^{Ba}±0.005	0.016
8 th day	20.56^{Aa}±0.290®	16.76^{Cb}±0.029	0.572	0.876^{Aa}±0.022®	0.674^{Cb}±0.004	0.045	4.98^{Cb}±0.014®	5.42^{Ca}±0.017	0.044
12 th day	®	18.57^{B}±0.076	--	®	0.821^{B}±0.027	--	®	5.16^{D}±0.005	--
16 th day	®	19.51^{A}±0.075	--	®	0.893^{A}±0.005	--	®	5.01^{E}±0.111	---
L.S.D	0.497	0.156		0.040	0.034		0.034	0.036	

Where: A ,B,C,D in the same columns are not significantly different(p>0.05) while a,b,c,d in the same rows are not significantly different (p>0.05), L.S.D: Least significant differences at (p>0.05). (Mean ± standard error). Ag Nps: sliver nanoparticles ®: Rejected

3.5.2. Psychrophilic bacterial count (CFU/g):

Data presented in **Table (3)**, psychrophilic bacteria count of all beef sausage samples ranged from 6.50×10 to 7.65×10^2 CFU/g immediately after processing at zero time. The highest total bacterial count (7.65×10^2 CFU /g) for the control sample, while the lowest TBC (6.50×10 CFU /g) was observed for beef sausage treated with 40.0µg/ml AgNPs. The psychrophilic bacteria count of all beef sausage samples was also affected by cold storage time. The increase in psychrophilic bacteria count was lower for beef sausage samples treated with AgNPs compared with beef sausage control. This indicates the effectiveness of silver nanoparticles in preventing the germination of bacteria [27]. Also, the psychrophilic bacterial count of the control sample reached 4.00×10^4 CFU /g, at the 8th day of cold storage, while the psychrophilic bacterial count of beef sausage treated with 40.0µg/ml AgNPs reached 2.10×10^4 CFU / g, at 16th day. Generally, psychrophilic bacteria count was decreased with AgNPs at zero time and at any time of cold storage, but psychrophilic bacteria counts were increased by increasing cold storage time. Similarly, Films coated with AgNP were also said to increase the shelf life of sausages [20].

3.5.3. Coliform group count (CFU/g):

From the results in **Table (3)** it could be observed that all beef sausage samples whether untreated samples or treated with 40.0µg/ml AgNPs were completely free from coliform, either at zero time or along cold storage period, which means that all samples were prepared and stored under good sanitary conditions, also coliform bacteria were not detected in beef sausage with 40.0µg/ml AgNPs, but found in control sample at 8th day with low counts (5.50×10 CFU /g). These results go parallel with [37] for coliform group counts.

3.5.4. Yeasts and molds count (CFU/g):

Yeasts and mold counts of all beef sausage samples as affected by AgNPs throughout cold storage at 4±1ºC for 16 days were tabulated in **Table (3)**. From these data it could be observed all samples were completely free from yeasts and molds counts, either at zero time which means that all samples were prepared and stored under good sanitary conditions, also yeasts and molds counts were not detected in beef sausage with 40.0µg/ml AgNPs due to the effectiveness of silver nanoparticles in preventing the spread of bacteria, [27, 32], but found in control sample at 4th day with low counts (4.00×10 CFU /g). After the 8th day of cold storage, yeasts and molds counts of all samples ranged from 1.50×10 to 9.30×10^2 CFU /g. The lowest count (1.50×10 CFU/g) was recorded for beef sausage with 40.0µg/ml AgNPs. While the highest (9.30×10^2 CFU /g) was observed for the control sample. The reduction

of yeasts and molds of beef sausage with AgNPs is probably due to the antimicrobial activity of AgNPs. In the end, the yeasts and mold count of beef sausage with AgNPs reached 9.50×10 CFU /g.

Table. 3. Effect of silver nanoparticles on microbiological quality attributes of beef sausage during cold storage at 4±1ºC up to 16 days.

Storage period	*Total bacterial counts (CFU/ g)*		*Psychrophilic bacterial counts (CFU / g)*		*Coliform group counts (CFU / g)*		*Total yeasts and molds counts (CFU / g)*	
	Control	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**
0 day	**9.20×10^{3}**	**5.60×10^{3}**	**7.65×10^{2}**	**6.50×10**	**N.D**	**N.D**	**N.D**	**N.D**
4 th day	**4.05×10^{5}**	**9.10×10^{3}**	**5.10×10^{3}**	**2.30×10^{2}**	**N.D**	**N.D**	**4.00×10**	**N.D**
8 th day	**1.65×10^{6}®**	**4.65×10^{4}**	**4.00×10^{4}®**	**1.60×10^{3}**	**5.50×10®**	**N.D**	**9.30×10^{2}®**	**1.50×10**
12 th day	**®**	**2.70×10^{5}**	**®**	**7.30×10^{3}**	**®**	**N.D**	**®**	**3.00×10**
16 th day	**®**	**9.30×10^{5}**	**®**	**2.10×10^{4}**	**®**	**N.D**	**®**	**9.50×10**

Where: Ag Nps: sliver nanoparticles ®: Rejected N.D: Not detected (CFU/ g): colony forming unit /gram.

3.6. Effect of AgNPs on freshness properties of fish patties throughout cold storage at 4±1ºC:

3.6.1. Total volatile basic nitrogen (mg/100 g sample):

The volatile basic nitrogen value is among the most commonly used quality index for fish foods [46]. Results in **Table (4)** showed that through statistical analysis of these data it could be observed that there were significant differences ($p\leq0.05$) in total volatile nitrogen between all fish patties control sample or fish patties with AgNPs at zero time, while significant differences were recorded between all above-mentioned samples during cold storage. Total volatile nitrogen of all fish patties ranged from 10.26 to 11.20 mg/100g at zero time. Total volatile nitrogen values of all fish patties samples were significantly affected by cold storage time. By increasing the storage period, TVBN of all fish patties samples significantly increased ($p\leq0.05$). The control sample had significantly higher total volatile nitrogen than fish patties with AgNPs at any time of storage for 4 and 8 days. This could be a result of the high antimicrobial effect of AgNPs. After the 12th day control sample was not evaluated because it exceeded the permissible limits of TVN and showed off odor, while total volatile nitrogen values of 22.86 mg N/100g were observed for fish patties with AgNPs within the acceptable level range reported by [47]. Also, on the 16th day, fish patties with AgNPs had not evaluated because they exceeded the permissible limits of TVN and showed off odor, [48] reported that, by ending the storage period, the control showed a significantly higher TVB-N value than the other treated samples ($p < .05$), and NCN2 showed the significantly lowest TVB-N compared to the other treatments. [16], reported that different species with different acceptability limits for TVB-N value was 25 mg/100 g.

3.6.2. Thiobarbituric acid (mg malonaldehyde/kg) (TBA):

The effect of AgNPs on thiobarbituric acid of fish patties samples at 4±1ºC for 16 days was presented in **Table (4)**. According to statistical analysis of these data, it could be observed that there were significant differences ($p\leq0.05$) in TBA values between all fish patties samples whether the control sample or beef sausage with AgNPs sample at zero time and significant differences were observed between them along cold storage periods. Thiobarbituric acid of all fish patties samples ranged from 0.488 to 0.595 mg malonaldehyde/kg sample at zero time. Furthermore, thiobarbituric acid of all fish patties samples was significantly affected by cold storage time. Throughout the cold storage, TBA values progressively increased ($p\leq0.05$) as the period of storage increased for all fish patties samples. This increase throughout cold storage could be indicating Lipids are continuously oxidized, which results in the generation of oxidative byproducts [18]. Fish patties with AgNPs sample have significantly lower TBA values than the control sample at any time of storage 4 and 8 days due to the antioxidant effect of AgNPs. On the 8th day, the highest TBA value was (0.876 mg malonaldehyde/kg) for the control sample. The spillover of prooxidants and oxidative enzymes from numerous damaged cellular organelles may be the cause of the decrease in the fat contents of the samples treated with silver bio nanoparticles. [43]. Also, on the 12th day, TBA values of fish patties with AgNPs sample was 1.22 mg malonaldehyde/kg sample. TBA value of fish patties with AgNPs does not exceed the range of permissible levels reported by [47], being not more than 4.5 mg malonaldehyde/kg sample.

Table. 4. Effect of silver nanoparticles on physicochemical properties of fish patties during cold storage at 4±1°C up to 16 days (Means ± SE).

Storage period	*Total volatile nitrogen (mg/100 g sample)*		*L.S.D*	*Thiobarbituric acid (mgmalonaldehyde/kg)*		*L.S.D*	*pH values*		*L.S.D*
	Control	**AgNps 40.0µg/ml**		**Control**	**AgNps 40.0µg/ml**		**Control**	**AgNps 40.0µg/ml**	
0 day	**11.20^{Ca} ±0.72**	**10.26^{Da}±0.46**	**1.293**	**0.595^{Ca} ±0.00**	**0.488^{Db}±0.00**	**0.005**	**6.39^{Ca}±0.00**	**6.30^{Db}±0.01**	**0.029**
4 th day	**18.66^{Ba} ±0.56**	**16.33^{Cb}±0.51**	**1.372**	**0.883^{Ba}± 0.00**	**0.686^{Cb} ±0.00**	**0.002**	**6.50^{Ba}±0.01**	**6.42^{Cb}±0.01**	**0.036**
8 th day	**23.33^{Aa} ±0.59®**	**20.53^{Bb}±0.57**	**1.660**	**1.153^{Aa}± 0.00®**	**0.856^{Bb}±0.00**	**0.002**	**6.97^{Aa}±0.01®**	**6.62^{Bb}±0.01**	**0.033**
12 th day	**®**	**22.86^{A}±0.61®**	**--**	**®**	**1.22^{A}±0.01®**	**--**	**®**	**6.81^{A}±0.00®**	**--**
16 th day	**®**	**®**	**--**	**®**	**®**	**--**	**®**	**®**	**--**
L.S.D	**1.137**	**1.126**		**0.003**	**0.016**		**0.033**	**0.031**	

Where: A ,B,C,D in the same columns are not significantly different(p>0.05) while a,b,c,d in the same rows are not significantly different (p>0.05), L.S.D: Least significant differences at (p>0.05). (Mean ± standard error). Ag Nps: sliver nanoparticles ®: Rejected

3.6.3. pH value:

pH may use as a fish freshness indicator because it starts with a low value with the good nutritional state of fish at initial storage and increased with a certain storage period [49]. pH values of fish patties sample affected by with AgNPs at 4±1ºC for 16 days were presented in **Table (4)**. From these results, it could be noticed that pH values of the fish patties sample were decreased insignificantly (p>0.05) from 6.39 for the control sample to 6.30 for the fish patties sample with AgNPs, respectively. Also, from statistical analysis of these data, it could be observed that there were significant differences (p≤0.05) in pH values between all fish patties samples whether the control sample or fish patties with AgNPs at any time of cold storage. Furthermore, the pH values of all fish patties samples were significantly affected by cold storage time. With the advancement of cold storage time, the pH values were significantly increased (p≤0.05) for all. This elevation might be due to the microbial breakdown of proteins and some other nutrients that lead to the production of alkaline derivatives [50]. After the 12th day, the pH value was 6.81 for fish patties with AgNPs, while at the end of cold storage the pH value was not evaluated because it show the off odor.

3.7. Effect of AgNPs on microbiological quality attributes of fish patties throughout cold storage at 4°±1C:

3.7. 1. Total bacterial count (CFU/g):

Data presented in **Table (5)**, illustrated the changes in TBC of fish patties as affected by AgNPs throughout cold storage for 16 days. TBC of all fish patties samples ranged from 2.83×10^4 to 3.65×10^4 CFU/g immediately after processing at zero time. The highest total bacterial count (3.65×10^4 CFU /g) for the control sample, while the lowest TBC (2.83×10^4 CFU /g) was observed for fish patties with 40.0µg/ml AgNPs. TBC of all fish patties samples was also affected by cold storage time and increased by increasing the cold storage period at 4±1°C. Results are in the same direction by [21, 48] they found that microbial growth increased during the storage period. The increase in the total bacterial count was lower for fish patties with AgNPs compared with fish patties control. This indicates the effectiveness of silver nanoparticles in inhibiting microorganisms [27]. Also, the TBC of the control sample reached 1.25×10^6 CFU /g, on the 8th day of cold storage, exceeding the maximum allowable limit of 10^6 CFU / gm for the total bacterial count for chilled fish [47]. While the TBC of fish patties with 40.0µg/ml AgNPs reached 1.11×10^6 CFU / g, on the 12th day. Therefore, processing fish patties with 40.0µg/ml AgNPs was more effective to maintain high microbiological enhancing the quality and the shelf life. [38] reported that, The concentration of bio-nanoparticles improved the efficacy of meat preservation.

3.7. 2. Psychrophilic bacterial count (CFU/g):

Data presented in **Table (5)**, psychrophilic bacteria count of all fish patties samples ranged from 4.15×10^3 to 4.95×10^3 CFU/g immediately after processing at zero time. The highest total bacterial count (4.95×10^3 CFU /g) for the control sample, while the lowest TBC (4.15×10^3 CFU /g) was observed for fish patties with 40.0µg/ml AgNPs. The psychrophilic bacteria count of fish patties samples was also affected by cold storage time. The increase in psychrophilic bacteria count was lower for fish patties with AgNPs compared with fish patties control. This indicates the effectiveness of silver

nanoparticles in inhibiting microorganisms [27]. Also, the psychrophilic bacterial count of the control sample reached 1.09×10^5 CFU /g, on the 8th day of cold storage, while the psychrophilic bacterial count of fish patties with 40.0µg/ml AgNPs reached 1.07×10^5 CFU / g, at 12th day. Generally, psychrophilic bacteria count was decreased with AgNPs at zero time and at any time of cold storage, while extending the cold storage period boosted psychrophilic bacteria numbers [21].

Table. 5. Effect of silver nanoparticles on microbiological quality attributes of fish patties during cold storage at 4±1ºC up to 16 days.

Storage period	*Total bacterial counts (CFU / g)*		*Psychrophilic bacterial counts (CFU / g)*		*Coliform group counts (CFU / g)*		*Total yeasts and molds counts (CFU / g)*	
	Control	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**	**Control**	**AgNps 40.0µg/ml**
0 day	**3.65×10^4**	**2.83×10^4**	**4.95×10^3**	**4.15×10^3**	**1×10**	**ND**	**3×10**	**ND**
4 th day	**2.51×10^5**	**1.87×10^5**	**1.5×10^4**	**9.95×10^3**	**9×10**	**1×10**	**9×10**	**2×10**
8 th day	**1.25×10^6®**	**8.15×10^5**	**1.09×10^5®**	**4.85×10^4**	**1.43×10^2®**	**5×10**	**1.5×10^2®**	**5×10**
12 th day	®	**1.11×10^6®**	®	**1.07×10^5®**	®	**9×10®**	®	**1.1×10^2®**
16 th day	®	®	®	®	®	®	®	®

Where: Ag Nps: sliver nanoparticles ®: Rejected N.D: Not detected (CFU/ g): colony forming unit /gram.

3.7. 3. Coliform group count (CFU/g):

From the results in **Table (5)** it could be observed that fish patties with 40.0µg/ml AgNPs were completely free from the coliform group at zero time, but the fish patties' control sample was 1×10 CFU /g, Coliform group count of all fish patties samples ranged from 1×10 to 9×10 CFU/g immediately after processing at 4th day. The coliform group count of fish patties samples was also affected by cold storage time. The increase in coliform group count was lower for fish patties with AgNPs compared with fish patties control. Also, the coliform group count of the control sample reached 1.43×10^2 CFU /g, on the 8th day of cold storage, while the coliform group count of fish patties with 40.0µg/ml AgNPs reached 9×10 CFU / g, on the 12th day [21]. These results go parallel with [47] for coliform group counts.

3.7. 4. Yeasts and molds count (CFU/g):

From the results in **Table (5)** it could be observed that fish patties with 40.0µg/ml AgNPs were completely free from yeasts and molds numbers at zero time, but the fish patties control sample was 3×10 CFU /g, yeasts and molds of all fish patties samples ranged from 2×10 to 9×10 CFU/g immediately after processing at 4th day. Yeasts and molds of fish patties samples were also affected by cold storage time. The increase in yeasts and mold count was lower for fish patties with AgNPs compared with fish patties control. Also, the yeasts and molds count of the control sample reached 1.5×10^2 CFU /g, on the 8th day, while the yeasts and molds numbers of fish patties with 40.0µg/ml AgNPs reached 1.1×10^2 CFU / g, at the 12th day, results are in the same direction by [21]. The reduction of yeasts and mold numbers of fish patties with AgNPs is probably due to the antimicrobial activity of AgNPs [27].

4. Conclusions

Our research was analyzing silver nanoparticles (AgNPs). This approach is simple, quick, and cost-effective has no toxic chemicals and is environmentally safe. To our knowledge, no studies have evaluated the physicochemical and microbiological characteristics of AgNPs on beef sausage and fish patties but it would also be a good idea to give healthier alternatives to enhance quality. Therefore, our study is the first to report on this topic, thus making a significant contribution to the scientific field. This entails modifying formulations and integrating cutting-edge technologies that reduce the need for synthetic additives. As a result, companies are more prepared to spend on creating nanostructured systems that enhance their goods, and nanotechnology is becoming more widely accepted. It is also possible to advance the technology used to monitor chemical reactions in meat and fish products. This will extend the goods' shelf life and marketing times while preserving important physicochemical and microbiological properties. Finally, despite the need for much more research, both scientists and producers are interested in creating and funding practical, affordable methods that will improve their businesses and benefit their operations and customers by supplying goods of exceptional quality and great nutritional value.

5. Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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