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Antibacterial effect of zinc oxide nanoparticles in fresh meat

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ABSTRACT

Practical application of ZnO-NPs (20nm) suspension with different concentrations (5mM, 8mM, and 10mM) were investigated to evaluate its antibacterial effect in fresh meat. A total number of 12 samples of fresh meat were collected from different abattoirs (150 gm. of each) in Gharbia Governorate, Egypt, under complete aseptic conditions and transferred without undue delay to the Lab to evaluate the efficacy of ZnO NPs as antibacterial agents in fresh meat. The obtained results indicated that ZnO-NPs had a significant inhibitory effect on the growth of APC and *Staphylococcus aureus* during 6 days of refrigerator storage at 4 0C. At zero day, the mean values of APC in the control group was $3.27 \times 10^7 \pm 6.05 \times 10^6$ cfu/g, while after treatment with ZnO-NPs 5mM, 8mM and 10mM the mean values were decreased to $1.67 \times 10^7 \pm 9.11 \times 10^6$, $1.55 \times 10^7 \pm 9.73 \times 10^6$, and $9.41 \times 10^6 \pm 7.67 \times 10^5$ cfu/g, respectively. By the 3rd day of refrigeration storage the control group showed complete spoilage. While the mean values of *Staphylococcus aureus* at zero day in control group was $3.09 \times 10^7 \pm 2.33 \times 10^7$ cfu/g, while after treatment with ZnO-NPs 5mM, 8mM and 10mM the mean values were highly decreased to $1.40 \times 10^7 \pm 9.56 \times 10^6$, $1.79 \times 10^6 \pm 4.70 \times 10^5$, and $8.05 \times 10^5 \pm 9.51 \times 10^5$ cfu/g, respectively. Accurately, ZnO-NPs with concentration 10mM showed the highest reduction percentage 99.5% and 99.85% to APC and *Staphylococcus aureus*, respectively, from $3.27 \times 10^7 \pm 6.05 \times 10^6$ to $1.64 \times 10^5 \pm 2.47 \times 10^4$ and $3.09 \times 10^7 \pm 2.33 \times 10^7$ to $4.61 \times 10^4 \pm 4.44 \times 10^4$ respectively, compared to other concentrations (5mM and 8mM). Thus the best sensory quality was obtained by ZnO-NP 10mM indicating to the fact that the antibacterial activity of ZnO-NPs is concentration dependent.

1. INTRODUCTION

Meat is considered an important source of protein, fat, vitamins and minerals, but low in carbohydrate content and with sufficient water activity that may supports the growth of both spoilage and pathogenic bacteria. A great diversity of microbes inhabits fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage, temperature, and transportation (Adu-Gyamfi *et al.*, 2012). The raw meat may harbor many important pathogenic microbes such as *Salmonella spp.*, *E. coli*, and *Staph. aureus*, making a risk for human health, as the improper handling and control of these pathogens, foodborne illnesses may occur (Nørnung *et al.*, 2009). World Health Organization (WHO), and Food and Agricultural Organization (FAO) of the United Nations stated that the illness due to contaminated food is considered the most widespread health problem and an important cause of reduced economic productivity (Käferstein, 2003). The bacterial contamination of meat could be decreased by strict hygienic measure, but the total elimination of food borne pathogens is very difficult. As a result of these concerns, researchers are interested in new technologies to decrease the microbial load of raw meat through alternative

compounds with antimicrobial properties, and to control the food-borne illnesses.

Recently, nanotechnology invades the world and has become increasingly important in the biomedical and pharmaceutical areas. This brought great opportunities for the development of materials with new properties for use as antimicrobial agents (Roco, 1999). Nanotechnology is engineering branch of recent well-established technology referring at the nano scale, i.e. anything measures between 1 and 100 nm. (Willard *et al.*, 2004). Nanomaterials are broadly grouped into inorganic and organic materials, but in both cases, they have different properties than larger particles of the same type (Cushen *et al.*, 2012). Most antibacterial inorganic compounds are metallic nanoparticles and metal oxide nanoparticles such as silver, copper, titanium oxide, and zinc oxide (ZnO) (Bradley *et al.*, 2011). ZnO nanoparticles have been extensively used in many industrial areas such as pharmaceutical, cosmetic and food industries (Deng *et al.*, 2008). Recently, zinc oxide is incorporated into packaging materials as antimicrobial agent. They can play an important role in reducing the risk of pathogen contamination and extending the shelf life of food (Espitia *et al.*, 2012). ZnO is one of the five zinc compounds that are listed as a generally recognized as safe (GRAS) material by U.S. Food and Drug Administration (FDA, 2011). ZnO nanoparticles are nontoxic and they have

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bactericidal effects against both Gram-positive and Gram-negative bacteria (Arabi *et al.*, 2012).

Therefore, the present study aimed at evaluating the antibacterial effect of ZnO nanoparticles in fresh beef meat.

2. MATERIAL AND METHODS

2.1. Preparation of Zinc Oxide nanoparticles:

Zinc oxide nanoparticles with size of 20 nm were purchased from Nano. Tech. Egypt for Photo-Electronics according to NT-ZONP brand with certificate of analysis. To obtain a homogenous solution of nanoparticles at different concentrations, including concentrations (5 mM, 8 mM and 10 mM), 200 ml distilled water was added to each concentration of nanoparticles in glass containers. The resulting homogenous suspensions were autoclaved for 30 minutes to be sterilized (Mottaki *et al.*, 2014).

2.2. Collection of samples:

A total number of 12 samples of fresh meat were collected from abattoir (150 g each) in Gharbia Governorate, Egypt. The collected samples were packed in separate sterilized plastic bags and transferred directly to the laboratory in an insulated ice-box under complete aseptic condition without undue delay to evaluate the efficacy of ZnO NPs as antibacterial agents in fresh meat samples by using ZnO NPs (20 nm) with different concentrations (5 mM, 8 mM and 10 mM) then sensorial analysis of treated samples including color, odor and texture were applied.

2.3. Application of Zinc Oxide Nanoparticles:

Fresh meat samples were divided into 4 groups. 1st: control group was dipped in sterile distilled water (0 mM ZnO-NPs), 2nd: was dipped in 5 mM ZnO-NP suspension, 3rd: was dipped in 8 mM ZnO-NP suspension, and 4th was treated with 10 mM ZnO-NP suspension at room temperature (25 °C) for 10 min. Meat samples were removed thereafter and properly packed in polyethylene bags, labeled and stored at 4 °C until sensory analysis and bacteriological examinations. The experimental trials were repeated 3 times all over the experimental period.

2.4. Sensory evaluation of the treated groups:

Overall acceptability of all samples was carried out using nine-point standardized numerical scale, where ten corresponded to components characteristic of the highest quality. The panelist consisted of 9 members of the staff who were familiar with meat characteristics was conducted during storage according to Kanatt *et al.* (2010)

2.5. Preparation of samples:

Preparation of samples for bacteriological examination was conducted according to FDA (2001). Briefly, ten grams of sample were taken from each treatment and homogenized with 90 ml of buffered peptone water (0.1%) in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. Then the homogenate was transferred into a sterile test tube and one ml was transferred into a sterile test tube containing 9 ml of 0.1% peptone water from which ten-fold serial dilutions up to 10⁷ were prepared.

2.6. Bacteriological Examination:

Aerobic Plate Count was conducted every day, and *S. aureus* count was conducted every 48 hrs during the period of refrigeration at 4 °C

2.6.1. Aerobic plate count:

For aerobic plate count, one ml from each of the previously prepared serial dilutions was poured into two separate sterile petri dishes, using pour plate method, to which approximately 15 ml of sterile melted and tempered plate count agar (45 °C) were poured. After thorough mixing, the inoculated and control plates were allowed to solidify at room temperature before being incubated in an inverted position at 37 °C for 24 hrs. Total aerobic plate count (cfu/g) was calculated on plates containing 30-300 colonies and each count was recorded separately (ISO, 2013).

2.6.2. Staphylococcus aureus count:

Black shiny colonies with narrow white margins surrounded by a clear halo zone extending into the opaque Baird parker medium were counted and expressed as colony forming unite (cfu/g) (FDA, 2001).

2.7. Statistical analysis:

The obtained data were statistically analyzed using one-way ANOVA under significance level of ($P < 0.05$) for the obtained results using SPSS package (SPSS 19.0, Chicago, IL, USA). Duncan's post-hoc test was used to determine the significance of the differences between mean values. The results were presented as means \pm SD. (Feldman *et al.*, 2003).

3. RESULTS AND DISCUSSION

The results obtained in table (1) showed that the scores of overall acceptability in case of using 5 mM ZnO-NPs was 9, 8, 7, 6 and 5 at zero, 1st, 2nd, 3rd and 4th day, respectively. While in case of using 8 mM ZnO-NPs was 9, 9, 8, 7, 6 and 5, at zero, 1st, 2nd, 3rd, 4th and 5th day, respectively. Moreover, in case of using 10 mM ZnO-NPs was 9, 9, 9, 7, 7, 6 and 5 at zero, 1st, 2nd, 3rd, 4th, 5th and 6th day of storage period at 4 °C, respectively, comparing with the score of overall acceptability in the control samples which were 9, 7 and 4 at zero, 1st and 2nd day, respectively.

Table 1 Overall acceptability of the examined fresh beef samples treated with various concentrations of ZnO-NPs during storage at 4 °C.

Day	Control	5 mM ZnO-NP	8 mM ZnO-NP	10 mM ZnO-NP
0	9	9	9	9
1	7	8	9	9
2	4	7	8	9
3	Spoiled	6	7	7
4	Spoiled	5	6	7
5	Spoiled	Spoiled	5	6
6	Spoiled	Spoiled	Spoiled	5

Score system for sensory evaluation (Kanatt *et al.*, 2010). 9: Excellent. 8: Very very good. 7: Very good. 6: Good. 5: Medium. 4: Fair. 3: Poor. 2: Very poor. 1: Very very poor

Nanotechnology is one of new technologies which have been implemented in the meat chain, promising more efficient safety and better quality for consumers. The application of nanoparticles in meat industry successfully enhanced the quality and safety of meat. In the same time, the use of new technologies in meat production chains may affect consumers' opinion of meat products. Future investigations involving the incorporation of functional nanoparticle ingredients containing substances such as antimicrobials, antioxidants as well as flavors and colors will certainly benefit the meat industry.

From the obtained results there was a decline of sensorial characters after the 1st day of storage with clear reduction of overall acceptability values in the control samples and showed complete spoilage at 3rd day of the storage period at 4 °C. Furthermore, the best sensory quality was obtained by 10 mM ZnO-NP which combat against APC, *Enterobacteriaceae*, *Coliform*, *total staphylococcal* and *Staph. aureus* till the 6th day of the storage period at °C. This may be due to the fact that the action of ZnO-NP is concentration dependent. These results were nearly similar to those reported by Rezk-Heba (2018).

Table 2 and 3 showed that the mean values of APC (cfu/g) in the examined beef samples during 6 days of refrigerator storage at 4 °C. At zero day, the mean values of APC in the control group was $3.27 \times 10^7 \pm 6.05 \times 10^6$, while after treatment with ZnO-NPs at 5 mM, 8 mM and 10 mM the mean values decreased to $1.67 \times 10^7 \pm 9.11 \times 10^6$, $1.55 \times 10^7 \pm 9.73 \times 10^6$, and $9.41 \times 10^6 \pm 7.67 \times 10^5$, respectively, with highly significant difference ($P < 0.05$) between the different concentration, and with high reduction percent (99.5%) in the treated group with 10 mM ZnO-NP than other groups treated with 5 mM and 8 mM. ZnO-NP by the 3rd day of refrigeration, the mean values of APC slightly decreased to $1.63 \times 10^6 \pm 5.20 \times 10^5$, $1.02 \times 10^6 \pm 6.93 \times 10^5$ and $5.53 \times 10^5 \pm 3.11 \times 10^5$ cfu/g after treatment with ZnO-NP 5 mM, 8 mM and 10 mM, respectively, as well as they were acceptable from aesthetic points without off odor or discoloration compared with the control group which showed extreme discoloration and off-odor on the 3rd day of storage. By the 5th day of refrigeration storage, the mean values of APC slightly decreased to $1.64 \times 10^5 \pm 2.47 \times 10^4$ cfu/g in the treated group with ZnO-NP 10 mM, while the other groups treated with ZnO-NP 5 mM and 8 mM showed extreme discoloration and off-odor on the 3rd day of storage. Furthermore, the obtained results indicated that ZnO-NP have greater antibacterial activity against APC with high reduction percent (99.5%) in the treated group with ZnO-NP 10 mM than other groups as the antibacterial activity increased with increasing concentration. These results were nearly similar to those reported by Raghupathi *et al.* (2011), Espitia *et al.* (2013) and Rezk (2018).

Antibacterial properties of ZnO-NPs depends on the physicochemical properties of NPs including their size, charge, surface morphology, and crystal structure, which are significant elements that regulate the actions of NPs on bacterial cells. Moreover, environmental conditions, the bacterial strain, and the exposure time are other major factors that influence the antibacterial effects of NPs (Çalışkan *et al.*, 2014). Particles size and concentration of ZnO-NP play important roles in the antibacterial activity, as the antibacterial activity directly correlates with their concentration as reported by several studies, larger surface area and higher concentration are accountable for ZnO-NPs antibacterial activity (Peng *et al.*, 2011).

From the results achieved in tables (4) and (5), it was obvious that the mean values of *S. aureus* markedly decreased after treatment with 5 mM, 8 mM and 10 mM ZnO-NPS at zero day to be $1.40 \times 10^7 \pm 9.56 \times 10^6$, $1.79 \times 10^6 \pm 4.70 \times 10^5$, and $8.05 \times 10^5 \pm 9.51 \times 10^5$, respectively, with highly significant difference ($P < 0.05$) between the different concentrations, comparing with the control sample which was $3.09 \times 10^7 \pm 2.33 \times 10^7$ cfu/g, with high reduction percent (99.85%) in the group treated with 10 mM ZnO-NP than other groups treated with 5 mM and 8 mM. ZnO-NPS. The

obtained results were nearly similar to those reported by Espitia *et al.* (2013), Mostafa (2015), while lower results were obtained by Amin and Eleiwa (2017), who reported that *S. aureus* was sensitive to 8 mM ZnO-NP, as indicated by the population reductions (9.63 to 3.97 log cfu/g) (58.77%) in broiler chicken fillet samples, and (Rezk, 2018), 15% reduction % of *S. aureus* by concentration 10 mM.

Table 2 The effects of different concentrations of ZnO-NP on APC (cfu/g) in the examined fresh beef samples

Day	Control	5 mM ZnONP	8 mM ZnO-NP	10 mM ZnO-NP
0	$3.27 \times 10^7 \pm 6.05 \times 10^6$	$1.67 \times 10^7 \pm 9.11 \times 10^6$	$1.55 \times 10^7 \pm 9.73 \times 10^6$	$9.41 \times 10^6 \pm 7.67 \times 10^5$
1	$1.30 \times 10^7 \pm 8.49 \times 10^6$	$3.34 \times 10^6 \pm 2.10 \times 10^6$	$2.71 \times 10^6 \pm 1.16 \times 10^6$	$5.55 \times 10^5 \pm 4.09 \times 10^5$
2	$5.94 \times 10^6 \pm 1.06 \times 10^6$	$1.89 \times 10^6 \pm 1.56 \times 10^6$	$1.33 \times 10^6 \pm 6.93 \times 10^5$	$1.76 \times 10^5 \pm 9.86 \times 10^4$
3	Spoiled	$1.63 \times 10^6 \pm 5.20 \times 10^5$	$1.02 \times 10^6 \pm 6.93 \times 10^5$	$5.53 \times 10^5 \pm 3.11 \times 10^5$
4	Spoiled	$8.30 \times 10^5 \pm 7.42 \times 10^5$	$2.34 \times 10^5 \pm 2.30 \times 10^4$	$5.45 \times 10^5 \pm 2.48 \times 10^4$
5	Spoiled	Spoiled	Spoiled	$1.64 \times 10^5 \pm 2.47 \times 10^4$

The values represent Mean \pm SD of three experiments. Means within a row followed by different letters are highly significantly different ($P < 0.05$).

Table 3 Reduction % of APC (cfu/g) in the examined fresh beef samples treated with different concentrations of ZnO-NP

Day	5 mM ZnO-NP	8 mM ZnO-NP	10 mM ZnO-NP
0	48.93	52.6	71.22
1	89.79	91.71	83.03
2	94.22	95.93	94.62
3	95.02	96.88	98.3
4	97.46	99.28	98.3
5	spoiled	spoiled	99.5

Table 4 The effects of different concentrations of ZnO-NP on *Staphylococcus aureus* count (cfu/g) in the examined fresh beef samples

Day	Control	5 mM ZnO-NP	8 mM ZnO-NP	10 mM ZnO-NP
0	$3.09 \times 10^7 \pm 2.33 \times 10^7$	$1.40 \times 10^7 \pm 9.56 \times 10^6$	$1.79 \times 10^6 \pm 4.70 \times 10^5$	$8.05 \times 10^5 \pm 9.51 \times 10^5$
1	$7.83 \times 10^5 \pm 3.88 \times 10^5$	$7.75 \times 10^5 \pm 6.40 \times 10^5$	$7.34 \times 10^5 \pm 1.07 \times 10^5$	$2.78 \times 10^5 \pm 9.12 \times 10^4$
2	$3.59 \times 10^5 \pm 2.13 \times 10^5$	$2.48 \times 10^5 \pm 1.25 \times 10^5$	$2.31 \times 10^5 \pm 1.08 \times 10^5$	$2.11 \times 10^5 \pm 1.05 \times 10^5$
3	Spoiled	$2.07 \times 10^5 \pm 5.33 \times 10^4$	$1.93 \times 10^5 \pm 5.67 \times 10^4$	$1.82 \times 10^5 \pm 3.06 \times 10^4$
4	Spoiled	$1.64 \times 10^5 \pm 6.47 \times 10^4$	$9.58 \times 10^4 \pm 9.71 \times 10^4$	$7.37 \times 10^4 \pm 7.39 \times 10^4$
5	Spoiled	Spoiled	$8.85 \times 10^4 \pm 7.95 \times 10^4$	$4.81 \times 10^4 \pm 4.43 \times 10^4$
6	Spoiled	Spoiled	Spoiled	$4.61 \times 10^4 \pm 4.44 \times 10^4$

The values represent Mean \pm SD of three experiments. Means within a row followed by different letters are highly significantly different ($P < 0.05$).

Table 5 Reduction % of *Staph. aureus* count (cfu/g) in the examined fresh beef samples treated with different concentrations of ZnO-NP

Day	5 mM ZnO-NP	8 mM ZnO-NP	10 mM ZnO-NP
0	54.69	94.21	97.39
1	97.49	97.62	99.10
2	99.20	99.25	99.32
3	99.33	99.38	99.41
4	99.46	99.69	99.76
5	Spoiled	99.71	99.84
6	Spoiled	Spoiled	99.85

The bacterial cell wall plays an important role in maintaining the bacterium's natural shape. The components of the cell membrane produce different adsorption pathways for NPs (Lesniak *et al.*, 2013). Studies have shown that ZnO-NPs have greater activity against Gram-positive bacteria than Gram-negative one, because the cell wall of Gram-negative bacteria is composed of LPS, lipoproteins and phospholipids which form a penetration barrier that allows the entrance of only macromolecules. In contrast, the cell wall of Gram-

positive bacteria includes a thin layer of peptidoglycan and its amino acid, surface proteins (e.g. adhesions) and teichoic acids plus lipoids (forming lipoteichoic acids), which act as chelating agents and also execute certain types of adherence (Tayel *et al.*, 2011) and abundant pores that allow foreign molecules to penetrate, resulting in cell membrane damage and cell death. In addition, compared with Gram-negative bacteria, Gram-positive bacteria have a high negative charge on the cell wall surface, which can attract NPs (Sarwar *et al.*, 2015).

The results obtained in tables (2) and (3) showed that the mean values of APC (cfu/g) in the examined fresh beef samples during six days of refrigeration. At zero day, the mean values of APC in the control group was $3.27 \times 10^7 \pm 6.05 \times 10^6$, while after treatment with 5 mM, 8 mM and 10 mM ZnO-NPs the mean values decreased to $1.67 \times 10^7 \pm 9.11 \times 10^6$, $1.55 \times 10^7 \pm 9.73 \times 10^6$, and $9.41 \times 10^6 \pm 7.67 \times 10^5$, respectively, with highly significant difference ($P < 0.05$) between the different concentration.

4. CONCLUSION

The antibacterial activity of ZnO-NPs is concentration dependent, as the best sensory quality was obtained by ZnO-NP 10 mM which extend the shelf life of fresh meat samples till 6 days comparing with the control sample which reached 2 days only stored at 4 °C. ZnO is one of the five zinc compounds that are listed as a generally recognized as safe (GRAS) material by the U.S. Food and Drug Administration so can be used in food industry. In future, more research should be focused on that aspect.

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