

**Original Paper****Nano technological improvement of minced fresh Beef quality**Mohamed A. Hassan¹, Nahla A. Abo EL-Roos², Gehan El tanany¹, Manal A. Esam¹¹Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Egypt²Department of Food Hygiene, Animal Health Research Institute, shebin El -Kom branch, Egypt.**ARTICLE INFO****ABSTRACT****Keywords**

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Food safety is considered the most important concern of consumers and the food industry. This work was carried out to evaluate the efficacy of zinc oxide nanoparticles in improving minced quality meat while being in cold storage and determination of the antibacterial and antioxidant activities of the ZnO nanoparticles. Using various concentrations (10, 20, and 30 ppm) of Zinc oxide (ZnO) NPs (20-30 nm). Treated groups with ZnO nanoparticles showed a reduction in the Aerobic Plate, Total coliform, total *staphylococcus*, and *Bacillus cereus* (count) have been decreased. The physical (PH) and chemical attributes (TVB-N, and TBA) of treated samples were significantly different ($p \leq 0.05$) from untreated group. ZnO nanoparticles with size 30 ppm have bacterial and antioxidant activity extended the shelf-life of fresh minced beef by keeping food safe from spoilage and food poisoning bacteria.

1. INTRODUCTION

Since bacterial contamination continues to be a serious food safety concern, consumers and the food industry place a high priority on food safety and security. As a result, significant efforts are made on a daily basis to meet customer demands and enhance quality (Nikolic et al., 2021). Meat and its products are considered one of the most nutrient food in human diet while, beef acts as a suitable medium for different types of pathogens as it contain high level of proteins, fat as well as water (Lin et al., 2018). Therefore, during all stages of processing, meat products, especially minced meat, are highly susceptible to spoiling (Lorenzo et al., 2018).

Food spoilage with microorganisms is not the only problem for human life but also, constitutes a huge economic loss for meat industries (Lin et al., 2019) as, foodborne diseases incidence are increasing all over the world (Jevnsnik et al., 2013).

Physio-chemical characteristics of nanoparticles frequently differ dramatically from those of their macroscale counterparts. Their optical, chemical, and electrical properties reflect these distinctions. (Kimber et al., 2018). By protecting food from dangerous bacteria, fungus, and viruses that can cause food to degrade and by maintaining freshness throughout longer storage times, nanoparticles with an inherent antimicrobial activity help products last longer on store shelves (Gaikwad et al., 2020).

ZnO NPs were employed to combat Staph and Salmonella typhimurium, two well-known foodborne pathogens. At various doses, and were found to be very efficient against both of them (Ali and Anil, 2014).

The purpose of the current study was to assess the of ZnO NPS on fresh minced beef quality (bacteriologically and physico-chemically).

2. MATERIAL AND METHODS**2.1. Preparation of sample:**

From a nearby butcher shop, four groups of fresh minced beef were divided. First group was the untreated control group, followed by the ZnO (10 ppm), ZnO (20 ppm), and ZnO (4 ppm) groups (30 ppm). The samples were then sealed in plastic film and kept at 4°C for 18 days. At days 0, 3, 6, 9, 12, 15, and 18, samples were collected for examination.

2.2. Zinc oxide nanoparticles (Wang, et al., 2007):

To assess NPs physical characteristics, they were studied under a TEM (Fig. 1).

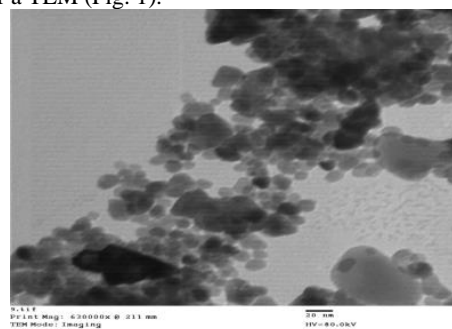


Figure 1 Transmission electron microscopic (TEM, 300000×) image of ZnO nanoparticles showed that the Shape (Spherical shape), the Size was (20 ±5 nm) and Purity (99.9%).

2.3. Bacteriological assay:**Preparation of samples (ISO 4833-1, 2013):**

Exactly, 25 grams of each sample were homogenized aseptically with 225 ml of 0.1% sterile peptone water in a stomacher for 1.5 minutes from which tenfold serial dilution was prepared.

Aerobic Plate Count (ISO 4833-1, 2013).

Total Coliform count (ISO 4832, 2006).

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Total Staphylococcal count (FDA, 2001).
 Bacillus cereus count (ISO7932, 2004).
 Physico-chemical examination
 PH measurement (Zenebon et al., 2008).
 Total volatile base nitrogen (TVB-N) (AOAC, 2005).
 Thiobarbituric acid (TBA) (AOAC, 2005).

2.4. Statistical analysis:

Using the SPSS application for Windows, analysis of variance (ANOVA) was performed on all data (Version 22). To eliminate result diversity, means and SD were computed. Significant F-values were found at the $P \leq 0.05$ level (Feldman et al., 2003).

3. RESULTS

The results recorded in table (1) cleared that APC in control group increased from 6.15 on zero to 7.6 (log₁₀ cfu/gm) on 18th day of cold storage. While APC (log₁₀ cfu/g) of the treated groups with different concentration of ZnO (10, 20 and 30 ppm) decreased throughout the cold storage period from 6.15 to 4.89, 4.3 and 3.95 (log₁₀ cfu/g), respectively. Results in table (2) revealed that the mean values of total coliform counts in the control group on zero day was 3.9 log₁₀ cfu/g which increased gradually to become 4.1 log₁₀ cfu/g at 6th day before reaching 5.1 (log₁₀ cfu/g) at the end of storage period. On contrary, in the treated group as the total coliform counts in zero day was 3.9 log₁₀ cfu/g then decreased gradually to 2.6, 2.21, 2.01 (log₁₀ cfu/g) for group treated with 10, 20 and 30 (ppm ZnO), respectively.

Table (3) showed that the total staphylococcal count of control group was significantly differed from treated ones as p value was less than 0.05. The count of control increased with the storage time however, the count of treated groups declined as the storage proceeded.

Table (4) revealed high significant differences ($p \leq 0.05$) in B. cereus count between control and treated groups. B. cereus count increased in control group with storage time while, it decreased in treated groups as it became not detected (ND) on 12th day of storage in group treated with 30 ppm ZnO, and completely inhibited with 20 ppm on 15th day of storage while it completely inhibited on 18th day of storage with using ZnO with concentration 10 ppm. The physical criteria of minced meat samples explained in table (5) as pH values increased during cold storage period it was 5.63 on zero day for all groups while, it became 7.5, 6.9, 6.81 and 6.55 for control, ZnO (10 ppm), ZnO (20 ppm) and ZnO (30 ppm), respectively.

The main chemical criteria TVB-N explained in table (6) that showed significance difference ($p \leq 0.05$) between control and other treated groups. At 9th day of storage TVB-N was 20.5, 14.88, 13.14 and 11.43 (mg/100gm).

High significant differences in TBA values between control and treated groups appeared in table (7) ($p \leq 0.05$). As the values of TBA increased rapidly from 0.25 at zero day to 1.45 (mg malonadhyde/ kg) at the end of storage period. While values were 0.25 at zero then increased to 0.99, 0.89 and 0.81 for groups treated with ZnO 10, 20, 30 ppm, respectively.

Table 1 Influence of Zn O NPs on Aerobic plate count of the examined control and treated groups of fresh minced beef during cold storage at4°C.

Groups/storage period	Zero day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
control	6.15±0.1 ^a	6.45±0.3 ^a	6.7±0.2 ^a	6.95±0.4 ^d	7.01±0.2 ^d	7.3±0.3 ^d	7.6±0.5 ^d
ZnO (10ppm)	6.15±0.1 ^a	6.01±0.1 ^a	5.73±0.2 ^b	5.55±0.2 ^{bc}	5.23±0.1 ^c	5.04±0.2 ^c	4.89±0.1 ^c
ZnO (20ppm)	6.15±0.1 ^a	5.95±0.2 ^a	5.5±0.2 ^b	5.2±0.3 ^c	4.95±0.2 ^{ce}	4.64±0.1 ^c	4.3±0.1 ^f
ZnO (30ppm)	6.15±0.1 ^a	5.91±0.1 ^a	5.25±0.1 ^c	4.97±0.2 ^c	4.75±0.3 ^c	4.5±0.1 ^c	3.95±0.2 ^f

The results are represented as Mean ± standard deviation (SD) of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).

Table 2 Influence of ZnO NPs on coliforms count of the examined control group and treated groups of fresh minced beef during cold storage at4°C.

Groups/storage period	Zero day	3 th day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	3.9±0.12 ^a	3.97±0.14 ^a	4.1±0.11 ^a	4.43±0.4 ^a	4.85±0.6 ^d	4.91±0.2 ^d	5.1±0.3 ^d
ZnO (10ppm)	3.9±0.12 ^a	3.55±0.12 ^b	3.4±0.09 ^b	3.2±0.3 ^b	3.05±0.1 ^c	2.99±0.3 ^c	2.6±0.2 ^e
ZnO (20ppm)	3.9±0.12 ^a	3.41±0.14 ^b	3.22±0.05 ^b	3.01±0.3 ^c	2.94±0.11 ^c	2.78±0.2 ^c	2.21±0.14 ^e
ZnO (30ppm)	3.9±0.12 ^a	3.35±0.16 ^b	3.1±0.03 ^c	2.96±0.2 ^c	2.64±0.2 ^{ce}	2.35±0.1 ^e	2.01±0.11 ^f

The results are represented as Mean ± standard deviation (SD) of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).

Table 3 Influence of ZnO NPs on staphylococcal count of the examined control group and treated groups of fresh minced beef during cold storage at4°C.

Groups/ storage period	Zero day	3 th day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	3.2±0.12 ^a	3.57±0.4 ^b	3.91±0.1 ^b	3.99±0.5 ^b	4.14±0.1 ^d	4.37±0.2 ^d	4.51±0.15 ^d
ZnO (10ppm)	3.2±0.22 ^a	3.01±0.31 ^a	2.95±0.12 ^c	2.7±0.22 ^c	2.5±0.14 ^c	2.2±0.3 ^c	1.95±0.11 ^f
ZnO (20ppm)	3.2±0.3 ^a	2.97±0.11 ^a	2.79±0.2 ^c	2.3±0.1 ^c	2.1±0.12 ^c	1.85±0.25 ^f	1.53±0.1 ^f
ZnO (30ppm)	3.2±0.11 ^a	2.88±0.14 ^a	2.55±0.3 ^c	2.01±0.11 ^c	1.94±0.2 ^c	1.3±0.19 ^f	1.01±0.2 ^g

Table 4 influences of ZnO NPs on B. cereus count of the examined control group and treated groups of fresh minced beef during cold storage at4°C.

Groups/storage period	Zero day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
control	2.01±0.1 ^a	2.5±0.5 ^a	2.97±0.3 ^a	3.1±0.22 ^d	3.97±0.4 ^d	4.03±0.6 ^f	4.2±0.3 ^f
ZnO (10ppm)	2.01±0.1 ^a	1.99±0.13 ^{ab}	1.7±0.21 ^b	1.45±0.13 ^c	1.35±0.2 ^c	1.2±0.11 ^e	ND*
ZnO (20ppm)	2.01±0.1 ^a	1.9±0.16 ^{ab}	1.52±0.11 ^b	1.3±0.15 ^c	1.21±1.22 ^c	ND*	ND*
ZnO (30ppm)	2.01±0.1 ^a	1.84±0.10 ^{ab}	1.31±0.13 ^c	1.2±0.12 ^c	ND*	ND*	ND*

The results are represented as Mean ± standard deviation (SD) of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).

Table 5 Changes in physical quality attribute (pH) of fresh minced beef after treatment with Zn O NPs during cold storage at4°C.

Groups/storage period	Zero day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	5.63±0.01 ^a	6.2±0.02 ^b	6.52±0.01 ^b	6.65±0.03 ^d	6.95±0.06 ^g	7.1±0.05 ^g	7.5±0.06 ^g
ZnO (10ppm)	5.63±0.01 ^a	5.8±0.01 ^a	5.9±0.02 ^c	6.2±0.01 ^e	6.4±0.02 ^f	6.8±0.03 ^h	6.9±0.01 ⁱ
ZnO (20ppm)	5.63±0.01 ^a	5.75±0.03 ^a	5.8±0.01 ^c	6.17±0.02 ^f	6.32±0.01 ^e	6.6±0.03 ^f	6.81±0.02 ^j
ZnO (30ppm)	5.63±0.01 ^a	5.73±0.02 ^a	5.76±0.03 ^c	6.12±0.01 ^{ef}	6.2±0.02 ^h	6.41±0.01 ^e	6.55±0.03 ^k

The results are represented as Mean ± standard deviation (SD) of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).

Table 6 Changes in chemical quality attribute TVB-N (mg/100 gm) of fresh minced beef after treatment with ZnO nanoparticles during cold storage at4°C.

Groups/Storage period	Zero day	3 rd day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	3.4±0.3 ^a	8.1±0.2 ^b	14.14±0.6 ^{ab}	20.5±0.4 ^{abc}	24.6±0.2 ^c	26.7±0.6 ^c	28.3±0.4 ^d
ZnO (10ppm)	3.4±0.3 ^a	6.15±0.1 ^c	10.1±0.4 ^d	14.88±0.2 ^f	18.1±0.3 ^g	19.11±0.5 ^h	20.1±0.4 ⁱ
ZnO (20ppm)	3.4±0.3 ^a	6.01±0.2 ^d	9.45±0.3 ^c	13.14±0.1 ^f	13.11±0.2 ^h	15.8±0.11 ⁱ	19.5±0.2 ^g
ZnO (30ppm)	3.4±0.3 ^a	5.95±0.1 ^c	8.19±0.1 ^{cd}	11.43±0.3 ^g	12.42±0.1 ⁱ	14.16±0.2 ^h	18.25±0.1 ^k

The values are represented as Mean ± standard error (SE) of three experiments. Means within a column and rows followed by different letters are significantly different ($P \leq 0.05$).

Table 7 Changes in chemical quality attribute TBA (mg malonaldehyde/kg) of fresh minced beef after treatment with ZnO nanoparticles during cold storage at 4°C.

Groups/Storage period	Zero day	3 th day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	0.25±0.05 ^a	0.46±0.04 ^a	0.65±0.01 ^c	0.95±0.02 ^e	1.2±0.01 ^e	1.33±0.03 ^{ec}	1.45±0.05 ^{ec}
ZnO (10ppm)	0.25±0.05 ^a	0.4±0.02 ^{ab}	0.52±0.01 ^b	0.76±0.03 ^d	0.81±0.02 ^g	0.89±0.01 ^g	0.99±0.04 ^j
ZnO (20ppm)	0.25±0.05 ^a	0.38±0.01 ^{ab}	0.46±0.02 ^b	0.63±0.01 ^f	0.75±0.01 ^g	0.85±0.01 ^h	0.89±0.02 ^k
ZnO (30ppm)	0.25±0.05 ^a	0.35±0.2 ^{ab}	0.43±0.01 ^d	0.56±0.02 ^f	0.64±0.1 ^h	0.79±0.02 ⁱ	0.81±0.01 ^k

4. DISCUSSION

Due to its enormous potential and numerous applications in a variety of industries, including the meat industry, the field of nanotechnology focuses on the engineering and implementation of materials in the scale range of 1 to 100 nanometers (Olga *et al.*, 2022). Also, ZnO NPs have antibacterial effect and improve the quality of meat and meat products (Abd El-Aziz *et al.*, 2020).

In table (1) using different concentrations (10, 20, 30 ppm) of ZnO with size (20-30 nm) affect the APC that present in minced meat as there were significance difference ($P \leq 0.05$) between control and treated groups. Also there were significance difference ($P \leq 0.05$) between group treated with high concentration of ZnO (30 ppm) and group treated with ZnO (10 and 20 ppm). Similar results obtained by Abd El-Aziz *et al.* (2020) who reported that ZnO NPs decrease the APC of meat balls. APC is considered as an indication for sanitary conditions that occurred during mincing of minced meat furthermore, APC determine the freshness of meat (Huh *et al.*, 2021).

As seen in table (2) the coliforms count also decrease throughout our experiment while using different concentrations of ZnO especially 30 ppm with $P \leq 0.05$ from control group. Similar results obtained by Zazouli *et al.* (2021) who used ZnO NP at concentration 20 ppm to decrease the total coliforms and prevent water pollution.

Total coliform is used as indicator for pollution with fecal matter (Paulsen *et al.*, 2006). The hydroxyl radicals and super oxides that the ZnO NP create react with the bacterial cell surface and harm the cell's contents, including its protein, lipids, and DNA (Bajpai *et al.*, 2012).

Table (3) revealed that the total staphylococcal count in control group increase with storage time that exceed 4 log₁₀ cfu/g at 12th day of cold storage. While the total staphylococcal count decrease with storage time with using ZnO NP. There were significance difference appeared between different treated groups as ZnO NP showed great antibacterial action against staphylococcal group similar results were recorded by (Akbar and Anal, (2014) and Abd El-Aziz *et al.* (2020)).

Data in table (4) revealed that *B. cereus* count was affected by ZnO NP as there were significance difference between control and treated groups ($P \leq 0.05$). But, *B. cereus* count more than 3 log cfu/g may be enough to motivate food poisoning (Stenfors *et al.*, 2008). The results were similar to Ahmed *et al.* (2021) who examined the antibacterial effect of ZnO NPs against *Bacillus* spp. and reported that using 50 ppm inhibits *Bacillus* completely. The count of control group increase ~2 log cfu/g so, it covert from safe to unsafe food as *B. cereus* count less than 3 log cfu/g of food is considered acceptable Health Protection Agency (2009).

The treated and control groups in Table 5, the pH values of the fresh minced beef increased over the course of the storage period. The increase in alkaline volatile chemicals like ammonia and trimethylamine produced by microbial and endogenous enzymes is likely to blame for the rise in pH levels (Badee *et al.*, 2013). Additionally, at the ninth day of cold storage, the treated group received ZnO (10 ppm) and ZnO (30 ppm). These results are remarkably comparable to those of Abd El-Aziz *et al.* (2020).

ZnO NPs' impact on microbial activity caused a considerable delay in the increase in pH values of minced beef during cold storage, which is what caused the delay in pH values (Suo *et al.*, 2017).

Data in table (6) indicated that TVB-N (mg/100gm) in the four groups of minced meat showed increasing during cold storage but, this increase slowed down through using ZnO NP. The results of untreated groups significantly differ from treated ones. The maximum permissible limit(MPL) for TVB-N is 20 mg/100mg according to EOS (2005) so that, control group is rejected at 9th day of cold storage 4^c while sample treated with ZnO 10 ppm was accepted till 15th day of cold storage 4^c followed by ZnO NPs (20 ppm and 30ppm) which still accepted till 18th day. Moreover, the P value between ZnO 20 ppm and ZnO 30 ppm was less than 0.05. The amino acids in meat are decomposed with bacteria and enzymes producing volatile nitrogenous compounds alkaline in nature as ammonia and amines. The increase of the TVB-N level normally indicates changes in amino acid contents and over increase indicates decomposition of meat protein Suo *et al.*, (2017). So, the TVB-N value is determined as one of the most important index that reflecting the degree of chilled meat spoilage. Similar results obtained by Suo *et al.*, (2017) and Abd El-Aziz *et al.* (2020).

The results present in table (7) revealed that TBA (mg malonaldehyde/kg) values increased throughout the storage period with $P \leq 0.05$ between untreated and treated groups. Malonaldehydes are toxic compounds that o formed due to oxidation of fatty acid as the value of TBA. Aldehydes are compounds that have rancid odor which speed the rate of lipid oxidation (Abdel-Hamied *et al.*, 2009). The rise in TBA levels during storage could be a sign of ongoing oxidation of meat's lipids and the development of oxidation byproducts (Abdou *et al.*, 2018). For beef products, the TBA allowed maximum is 0.9 mg malonaldehyde/kg (EOS, 2005).

5. CONCLUSION

ZnO Nanoparticles have bacterial and antioxidant activity extended the shelf-life of fresh minced beef by keeping food safe from spoilage and food poisoning bacteria also, harmful substances so, providing freshness during cold storage time.

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