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Application of zinc oxide nanoparticles to enhance quality and shelf life of chilled chicken panne

Safa, M. H. El Din[1, 2], Abo Bakr, M. Edris[1], Nahla, A. Shawky[3]

[1]*Department of Food Control, Faculty of Veterinary Medicine, Benha University, Egypt*

[2]*Pharco Pharmaceuticals Company*

[3]*Department of Food Hygiene, Animal Health Research Institute, Shebin El –Kom Branch, Agriculture Research Center, Egypt*

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ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) have garnered a lot of interest in the food sectors as a way to lessen the activity of microorganisms. So, the present investigation aimed to study the effectiveness of ZnO-NPs to enhance quality and shelf life of chilled chicken panne. Zinc oxide nanoparticles (ZnO-NPs) suspension in chicken panne at varying concentrations (2, 5, and 10 mM) was applied. Physicochemical parameters (TBA, TVB-N and pH), bacteriological indices (Total aerobic plate count, Staphylococcus aureus count and coliform count) and sensory attributes of treated chicken panne group differ significantly ($P < 0.05$) from the untreated control group. Among the treated samples, ZnO-NPs (10mM) showed the highest reduction in aerobic plate count, coliform count, and *Staphylococcus aureus* count compared to other concentrations (2 and 5mM) and it increased the shelf-life of the chicken panne, which lasted up to 21 days when kept properly refrigerated (4 °C) compared to the control group, which became spoiled by the ninth day of storage. It is concluded that 10 mM ZnO nanoparticles have the best antibacterial effect in chicken panne stored at 4 °C for 21 days.

1. INTRODUCTION

Chicken meat is often chosen as a source of protein worldwide due to its lower production costs, lower fat content, higher nutritional value, ease of preparation, lighter texture, and acceptance in various cultures and religions (Allam et al., 2022). There has been a change in meat consumption toward poultry of 2023, 139.68 million tons of chicken meat produced worldwide, up from 17.61 million tons in the previous year (Kleyn and Ciacciariello, 2021). The creation and application of materials with nanoscale spatial dimensions is the main goal of nanotechnology (Islam et al., 2022). The Food and Drug Administration (FDA) has authorized zinc oxide (ZnO) as a generally regarded as safe (GRAS) substance for both humans and animals (FDA, 2023). Nanotechnology evolved as a creative substitute that is being used more often in the meat production chain to guarantee a longer shelf life with better food safety, quality, and traceability (Bayda et al., 2019). At very low concentrations, nano-sized materials are more effective at reaching their target because of their high ratio of surface area to volume (Khan et al., 2019). Zinc oxide (ZnO-NPs) provide zinc to the body, as it is a crucial trace element (Wiesmann et al., 2021), in addition to their low-concentration antibacterial action (Khatami et al., 2018). The physicochemical properties of zinc oxide nanoparticles are unique that make them extremely efficient against a variety of pathogens, such as viruses, bacteria, and fungi (Mohd et al., 2019). The antimicrobial activity of ZnO NPs arises from their ability to induce oxidative stress, disrupt cell membrane integrity, and interfere with cellular functions of the microorganisms (Bahrami et al., 2023). The size, shape, and surface properties of ZnO nanoparticles (NPs) are some of the features that make them potent antibacterial agents (Arakha et al., 2015). The aim of the present study is adding Zinc oxide nanoparticles with different concentrations (2 mM, 5 Mm and 10mM) and assess their impact on the chicken pane's sensory qualities, bacteriological quality and physicochemical characteristics.

2. MATERIAL AND METHODS

Ethical Approval

This work was approved by the Scientific Research Ethics Committee of the Faculty of Veterinary Medicine, Benha University, with ethical approval number (BUFVTM03-04-24).

2.1 Preparation of zinc oxide nanoparticles

It was performed according to Mottaki et al. (2014).
Zinc oxide nanoparticles were prepared by dissolving 11 g zinc acetate hydrate with 99.9% purity (Zn (Ac)2•2H2O, Sigma-Aldrich) in 500 ml ethanol. Then, 2.9 g sodium hydroxide was added into the solution through ultra-sonication, and a transparent solution was obtained. The conical flask containing the transparent solution was put into a water tank with a constant temperature of 60 °C. After that, 10 ml of distilled water was added to the solution into the conical flask. The solution was stirred for 30 min at 60

* Correspondence to: sfhsm@yahoo.com

°C. The prepared ZnO nanoparticles were collected by centrifuging and drying at 60 °C.

2.2 Chicken panne

The experiment was carried out in the Shibin Elkom lab of the Animal Health research institute. Four kilograms of randomly selected fresh pane samples were bought from same store and supermarkets. Without excessive delay, the samples will be kept in individual plastic bags and brought straight to the lab in an insulated ice box under aseptic conditions. All of the samples were separated into four equal groups in the lab (each weighing 1000 grams), and kept at 4 ºC until they were needed.

2.3Experimental design

Four equal sets of chicken panne were created from the samples. The first group was dipped in sterile distilled water (control), the second group in 2 mM ZnO NPs suspension, the third group in 5 mM ZnO NP suspension, and the fourth group in 10 mM ZnO NPs suspension (Abd El-Aziz et al., 2020). The samples were tagged, individually packed in polyethylene bags, and kept at 4 ±1ºC. The samples were analyzed for bacteriological, physicochemical and sensory properties during chill storage period (at 1st, 3rd, 6th, 9th, 12th, 15th, 18th and 21th days) and the experiment was conducted in triplicate.

2.4. Sensory Evaluation

Sensory evaluation was performed under the controlled condition of temperature (28 °C), humidity (65%), and light by five well-trained female panelists of 30 to 35 years of age, who were selected according to ISO (2012). The panelists were able to perform descriptive sensory analysis for treated samples and control one and give reliable comparative judgments. The criteria used as the basis of the organoleptic descriptive assessment and the samples were rated on a continuous hedonic scale (ISO, 2003). The panel received a list of descriptors (odor, color, and texture) to score on numerical and continuous scales from 0 (the lowest score for each attribute, very bad) to 10 (the highest score for each attribute, very good) according to Cullere et al. (2018). Every one of panelists took disposable dish containing three samples (two identical and another different) in triangle form randomly coded with four numbers and worksheet to give the score for each point. A mean score of lower than 5 indicated unacceptable quality.

2.5. Bacteriological quality:

The Aerobic Plate Count (APC) of chicken pane samples was assessed using the pour plate method on plate count agar (HIMEDIA, M091S) at 35 °C during the storage period (ISO 4833-1, 2013), count of *Staphylococcus aureus* was done on Baird Parker ager base (HIMEDIA, M043) after 48 hours of incubation at 37°C (FDA, 2001), total coliform count was done on violet red bile agar media (HIMEDIA, M049) in accordance with ISO 4832 (2006), followed by a 24-hour incubation period at 37 °C.

2.6. Physico-chemical evaluation

The pH was measured using the Digital Jenco 609 pH meter (ES 63-11/2006), and the TVB-N concentration was calculated using the procedure outlined in ES 63-10/2006 and reported as mg N/100 g of sample. According to ES 63-9/2006, the thiobarbituric acid (TBA) values were used to assess the samples' oxidative status.

2.7 Statistical analysis

The graph pad prism application (Version 8.0.2) for Windows was used to examine the data. The analysis of variance was performed on all data using one way ANOVA. Values were expressed as means and SD. Significant p-values were found at $p < 0.05$ at confidence level 95%.

3. RESULTS

3 Data in fig. (1) showed that the sensory scores exhibited a significant reduction in the score of odor, texture and overall acceptability as they decrease while storage period proceeded. When chicken meat spoiled, it develops rancid and putrid odor. The control group spoiled at 6th day of storage with score below 4 as its mean score was 3.5 ± 0.25 while the samples treated with ZnO 10 mM spoiled at 21th day of spoilage.

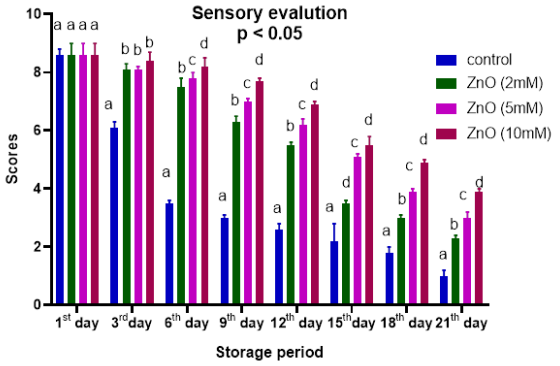

Fig (1). The effect of varying concentrations of ZnO nanoparticles on sensory attributes of chicken panne samples during chilling storage at 4°C

The results presented in Table 1 indicate that the average aerobic plate count (APC) at first day of storage were 4.11, 4.07, 4.05, and 4.01 for the untreated group and treated groups with ZnO nanoparticles at concentrations of 2 mM, 5 mM, and 10 mM, respectively (Abd El-Aziz et al., 2020). The APC of the control samples exhibited a gradual increase during the trail with around 2.5 log cfu/g. In contrast, the samples treated with ZnO nanoparticles demonstrated a significant inhibitory effect on APC, with statistically significant differences ($P < 0.05$) observed between the untreated and treated groups.

Table 1. The effect of varying concentrations of ZnO nanoparticles on the aerobic plate count (log10 cfu/ g) of chicken panne samples during chilling storage at 4°C.

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day	21st day
Control	4.11 ± 0.11^{a}	4.52± 0.16^{a}	4.93 ± 0.21^{a}	5.44 ± 0.11^{a}	5.9 ± 0.14^{a}	6.2 ± 0.45^{a}	6.8 ± 0.2^{a}	6.79 ± 0.11^{a}
ZnO (2mM)	4.07 ± 0.11^{a}	3.99 ± 0.2^{b}	3.9 ± 0.11^{b}	3.54 ± 0.11^{b}	3.11 ± 0.11^{b}	3.95 ± 0.3^{b}	4.20 ±0.12^{b}	4.88 ± 0.3^{b}
ZnO (5mM)	4.05 ± 0.11^{a}	3.80 ± 0.15^{c}	3.63 ± 0.12^{c}	3.38 ±0.16^{c}	3 ± 0.3^{c}	2.5 ± 0.28^{c}	3.1 ± 0.19^{c}	3.42 ± 0.19^{c}
ZnO (10mM)	4.01 ± 0.11^{a}	3.25 ± 0.25^{d}	3 ± 0.11^{d}	2.61 ± 0.2^{d}	2.04 ± 0.11^{d}	1.65 ± 0.15^{d}	1.54 ± 0.19^{d}	2.75 ± 0.19^{d}

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference ($P < 0.05$). EOS (2005) established that APC should not exceed 10^{6}/g.

The results demonstrated in Table 2 the average values of *S. aureus* count at the first day were 3.24, 3.20, 3.15, and 3.09 for the treated and treated groups by ZnO nanoparticles at concentrations of 2 mM, 5 mM, and 10 mM, respectively. Over the storage period of 21 days, the *S. aureus* count of the control samples exhibited a gradual increase, while the samples treated with ZnO nanoparticles demonstrated a significant inhibitory effect on *S. aureus*, with statistically significant differences ($P < 0.05$) observed between the

control and treated groups, as well as among the different concentrations of ZnO nanoparticles.

Table 2. The effect of varying concentrations of ZnO nanoparticles on the *S. aureus* count (log10 cfu/ g) of chicken panne samples during chilling storage at 4°C.

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day	21th day
Control	3.24 ± 0.6^{a}	4.08 ± 0.12^{a}	4.85 ± 0.14^{a}	5.51 ± 0.2^{a}	5.98 ± 0.11^{a}	6.61 ± 0.2^{a}	6.90 ± 0.23^{a}	7.32 ± 0.11^{a}
ZnO (2mM)	3.20 ± 0.14^{a}	3.10 ± 0.14^{b}	2.93 ± 0.2^{b}	2.72 ± 0.12^{b}	2.55 ± 0.11^{b}	3.21 ± 0.21^{b}	3.58 ± 0.17^{b}	3.99 ± 0.17^{b}
ZnO (5mM)	3.15 ± 0.11^{ab}	3.04 ± 0.2^{b}	2.61 ± 0.12^{c}	2.42 ± 0.23^{c}	2.03 ± 0.13^{b}	2.52 ± 0.22^{c}	3.02 ± 0.32^{c}	3.31 ± 0.32^{c}
ZnO (10Mm)	3.09 ± 0.12^{b}	2.85 ± 0.11^{c}	2.15 ± 0.21^{d}	1.99 ± 0.13^{d}	1.41 ± 0.44^{c}	ND*	ND*	ND*

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference (P < 0.05). EOS (2005) established that *S. aureus* count should not exceed 10^2/g.

Also, the data in Table 3 show that the mean coliform values at first day of the trail were 3.48, 3.41, 3.35, and 3.29 for the control group and the groups treated with ZnO nanoparticles at concentrations of 2 mM, 5 mM, and 10 mM, respectively. Throughout the 21-days of storage period, the coliform count in the control samples steadily increased. The samples treated with ZnO nanoparticles showed a considerable inhibitory impact, with statistically significant differences (P < 0.05) observed between the control and treated groups.

Table 3. The effect of varying concentrations of ZnO nanoparticles on the coliform count (log10 cfu/ g) of chicken panne samples during chilling storage at 4°C

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day	21th day
Control	3.48 ± 0.11^{a}	3.71 ± 0.13^{a}	3.85 ± 0.16^{a}	4.62 ± 0.3^{a}	4.61±0.4^{a}	5 ± 0.2^{a}	5.1 ± 0.15^{a}	5.4 ± 0.4^{a}
ZnO (2mM)	3.41 ± 0.21^{a}	3.20 ± 0.11^{b}	2.92 ± 0.12^{b}	2.47 ± 0.2^{b}	2.11 ± 0.19^{b}	2.38 ± 0.11^{b}	2.61 ± 0.01^{b}	3.11 ± 0.01^{b}
ZnO (5mM)	3.35 ± 0.15^{a}	3.15 ± 0.21^{b}	2.80 ± 0.18^{c}	2.14 ± 0.12^{c}	ND*	ND*	ND*	ND*
ZnO (10Mm)	3.29 ± 0.14^{a}	3.10 ± 0.1^{b}	2.60 ± 0.4^{d}	1.85 ± 0.15^{d}	ND*	ND*	ND*	ND*

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference (P ≤ 0.05).

As shown in Tables 4, 5 and 6, the mean pH, TBA and TVB-N values of chicken panne exhibited a gradual increase throughout the cold storage period. The rate of increase was most pronounced in the control group, followed by the groups treated with ZnO nanoparticles at concentrations of 2 mM, 5 mM, and 10 mM, respectively.

Table 4. The effect of varying concentrations of ZnO nanoparticles on sensory criteria (pH) of chicken panne samples during chilling storage at 4°C.

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day	21th day
Control	5.61 ± 0.21^{a}	6.17 ± 0.13^{a}	6.51 ± 0.01^{a}	6.65 ± 0.12^{a}	6.8 ± 0.09^{a}	7.25 ± 0.21^{a}	7.98 ± 0.2^{a}	8.05 ± 0.20^{a}
ZnO (2mM)	5.61 ± 0.21^{a}	5.92 ± 0.02^{b}	6.05 ± 0.02^{b}	6.35 ± 0.01^{b}	6.41 ± 0.08^{b}	6.81 ± 0.08^{b}	6.92 ± 0.09^{b}	7.41 ± 0.11^{b}
ZnO (5mM)	5.61 ± 0.21^{a}	5.85 ± 0.01^{b}	6.01±0.01^{b}	6.20 ± 0.01^{c}	6.31 ± 0.13^{c}	6.45 ± 0.06^{c}	6.7 ± 0.11^{c}	7.20 ± 0.21^{c}
ZnO (10Mm)	5.61 ± 0.21^{a}	5.70 ± 0.01^{c}	5.92 ± 0.01^{c}	6.02 ± 0.01^{d}	6.09 ± 0.10^{d}	6.22 ± 0.05^{d}	6.40 ± 0.06^{d}	6.88 ± 0.11^{d}

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference (P < 0.05).

Table 5. The effect of varying concentrations of ZnO nanoparticles on TBA (mg malondialhyde/kg) of chicken panne samples during chilling storage at 4°C.

Group	Zero day	3rd day	6thday	9th day	12th day	15th day	18th day	21th day
Control	0.21 ± 0.02^{a}	0.48 ± 0.01^{a}	0.98±0.01^{a}	1.12 ± 0.03^{a}	1.4 ± 0.05^{a}	1.61 ± 0.03^{a}	1.80±0.012^{a}	1.95± 0.01^{a}
ZnO(2mM)	0.21 ± 0.02^{a}	0.40 ± 0.01^{a}	0.54 ± 0.05^{b}	0.68 ± 0.02^{b}	0.79 ± 0.01^{b}	0.98 ± 0.01^{b}	1.11 ± 0.01^{b}	1.25 ± 0.02^{b}
ZnO (5mM)	0.21 ± 0.02^{a}	0.39 ± 0.01^{a}	0.42 ± 0.09^{bc}	0.46 ± 0.03^{c}	0.61± 0.06^{c}	0.85 ± 0.03 ^{c}	0.95 ± 0.02^{c}	1.10 ± 0.01^{c}
ZnO (10Mm)	0.21 ± 0.02^{a}	0.37±0.01^{a}	0.4 ± 0.002^{c}	0.42±0.006^{c}	0.59 ± 0.01^{c}	0.70 ± 0.02^{d}	0.81±0.01^{d}	0.97±0.01^{d}

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference (P < 0.05). EOS (2005) established that TBA count should not exceed 0.9 mg malondhyde/kg

Table 6. The effect of varying concentrations of ZnO nanoparticles on TVB-N (mg /100g) of chicken panne samples during chilling storage at 4°C.

Groups	Zero day	3rd day	6th day	9th day	12th day	15th day	18th day	21th day
Control	4.1 ± 0.1^{a}	12.11 ± 0.2^{a}	20.1 ± 0.1^{a}	22.3 ± 0.1^{a}	23.4 ± 0.2^{a}	26.5 ± 0.2^{a}	29.21 ± 0.4^{a}	30.24 ± 0.3^{a}
ZnO (2mM)	4.1 ± 0.1^{a}	7.2 ± 0.1^{b}	10.2 ± 0.1^{b}	13.3 ± 0.3^{b}	16.6 ± 0. 2^{b}	20.2 ± 0.13^{b}	22.6 ± 0.2^{b}	25.2 ± 0.1^{b}
ZnO (5mM)	4.1 ± 0.1^{a}	6.8 ± 0.2^{c}	9.6 ± 0.2^{cb}	12.1 ± 0. 1^{c}	15.2 ± 0.1^{c}	17.2 ± 0.11^{c}	20.5 ± 0.2^{c}	23.1 ± 0.1^{c}
ZnO (10Mm)	4.1 ± 0.1^{a}	5.6 ± 0.1^{d}	8.2 ± 0.1^{c}	10.1 ± 0.2^{d}	12.3 ± 0.1^{d}	15.15 ± 0.11^{d}	18.1 ± 0.2^{d}	20.2 ± 0.2^{d}

The data are presented as mean ± standard deviation. Means within the same column that have different superscript letters indicate a significant difference (P < 0.05). EOS (2005) established that TVB-N count should not exceed 20 mg /100g

4. DISCUSSION

The ability of ZnO nanoparticles to maintain sensory quality while extending shelf life is crucial for consumer acceptance and food safety. The sensory deterioration observed in the untreated group can be due to increase microbial loads and subsequent metabolic byproducts, which contribute to off-odors and undesirable textures (Burmistrov et al., 2022). In contrast, the treated samples exhibited significantly higher sensory scores throughout the storage period, indicating that ZnO nanoparticles effectively mitigate spoilage processes. The data indicated a significant decline in sensory characteristics as odor, texture as well as over-all acceptability of chicken panne as the storage period progressed. This decline is consistent with the typical spoilage characteristics of chicken meat, which develops rancid and putrid odors due to microbial activity and lipid oxidation (Alizadeh-Sani et al., 2020).

The usage of ZnO NPs at a concentration of 10 mM was very efficient, prolonging the shelf life of chicken panne till the 21th day of chilled storage while, the untreated control samples spoiled by the 6th day. This finding corroborates previous research demonstrating that ZnO nanoparticles possess significant antimicrobial properties, which help inhibit spoilage organisms and prolong the shelf life of meat (Morsy et al., 2018; Ahamad Khan et al., 2023).

The findings highlight the effect of ZnO NPs on the aerobic plate count (APC), *S. aureus* count as well as coliform count in chicken panne during 21 days of chilled storage. These data indicate that control group experienced a steady increase in microbial load throughout the storage period, which is consistent with expectations for untreated meat products due to natural spoilage processes (Papadochristopoulos et al., 2021). In contrast, the samples fortified with ZnONPs exhibited a significant decrease in APC, *S. aureus* count and coliform count demonstrating the antimicrobial properties of these nanoparticles (da Silva et al., 2019; Abd El-Aziz et al., 2020). The most notable decline in APC was presented in the group treated by 10 mM ZnO nanoparticles, indicating that higher concentrations of ZnO are more effective in controlling aerobic bacteria. These findings nearly similar to previous research that has documented the potent antibacterial activity of ZnONPs on foodborne pathogens *S. aureus, E. coli* and *B. cereus* (Alekish etal., 2018; Awasthi etal., 2020; Ye etal., 2022). The mechanism behind the effectiveness may entail a number of mechanisms, including the formation of reactive oxygen compounds that disrupt bacterial cell membranes and metabolic processes (da Silva etal., 2019; Mendes et al., 2022).

The results presented in tables 4, 5, and 6 demonstrate the significant effects of ZnO NPs on the pH, TBA and TVB-N

of chicken panne during 21 days chilled storage period. The gradual increase in mean pH values, from an initial 5.61 to 8.05 in the control group, indicates a shift towards more alkaline conditions as spoilage progresses. This increase was less pronounced in the groups treated with ZnO nanoparticles, where final pH values were recorded at 7.41, 7.20, and 6.88 for the 2 mM, 5 mM, and 10 mM treatments, respectively. Such results suggested that ZnO nanoparticles may help maintain a more stable pH environment, which is crucial for preserving meat quality (Alizadeh-Sani et al., 2020). In addition to pH changes, the TBA values also increased throughout the storage period, indicating lipid oxidation. The control group's TBA value rose from an initial 0.21 mg malonodialdehyde/kg to 1.95 mg malonodialdehyde/kg by the end of storage. Conversely, the groups treated with ZnO nanoparticles showed significantly lower TBA values (1.25 mg malonodialdehyde/kg for 2 mM, 1.10 mg malonodialdehyde/kg for 5 mM, and 0.97 mg malonodialdehyde/kg for 10 mM), suggesting that ZnO nanoparticles effectively mitigate lipid oxidation processes. This antioxidant effect is crucial as lipid oxidation can lead to off-flavors and rancidity in meat products (Burmistrov et al., 2022). Moreover, the TVB-N values reflected a similar trend, with significant increases observed in the control group compared to treated groups. The control group's TVB-N value escalated from 4.1 mg /100 to 30.24 mg /100g, while treated groups recorded values of 25.2 mg /100g for 2 mM, 23.1 mg /100g for 5 mM, and 20.2 mg/100g for 10 mM ZnO nanoparticles by 21days of chilled storage. These findings suggested that ZnO nanoparticles not only inhibit microbial growth only but also reduce the production of nitrogenous compounds associated with spoilage. These findings are consistent with other studies that have demonstrated the efficacy of ZnO nanoparticles as antimicrobial agents in food preservation (Morsy et al., 2018).

5. CONCLUSIONS

The incorporation of ZnO nanoparticles especially at concentration of 10mM into chicken panne not only prolongs shelf life but also preserves sensory quality by reducing spoilage-related changes as a viable strategy for enhancing meat product safety and quality during chilled storage.

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