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ASSESSMENT OF THE ANTIBACTERIAL EFFECT OF NANO-ZINC OXIDE AND NANO-TITANIUM DIOXIDE AGAINST *E. COLI* (ATC 25922 TM) ON THE QUALITY AND SHELF LIFE OF CHICKEN KOFTA

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ABSTRACT

Metal nanoparticles have recently gained significant attention across various fields of nanotechnology. This study investigated the antibacterial effects of zinc oxide (ZnO) and titanium dioxide (TiO_2) nanoparticles on *E. coli* and their subsequent impact on the quality and shelf life of chicken kofta. The samples were inoculated with *E. coli* and treated with different concentrations of the two nanomaterials: 5 mM and 10 mM ZnO, 5 mM and 10 mM TiO_2, and a combination of 5 mM ZnO and 5 mM TiO_2. These samples were then stored at 4 °C for 18 days. The *E. coli* counts were assessed to evaluate how ZnO NPs and TiO2 NPs influenced the quality and shelf life of chicken kofta. The disc diffusion method confirmed that ZnO NPs (10 mM) exhibited the highest efficacy against *E. coli*. Additionally, the antibacterial properties of ZnO, TiO_2, and their combination were analyzed using transmission electron microscopy. Results indicated that ZnO NPs at a concentration of 10 mM significantly inhibited the growth and count of *E. coli* in the chicken kofta. The findings suggest that ZnO NPs (10 mM) could serve as effective antibacterial agents for enhancing food preservation and extending shelf life.

***Keywords*:** Chicken kofta, TEM, Titanium dioxide, Zinc oxide

INTRODUCTION

Poultry meat products are popular globally because of their high nutritional value, cost-effectiveness, and quick cooking times (Özlü *et al.*, 2023). The presence of nitrogenous substances and lipids creates an environment conducive to microbial growth (Odeyemi *et al.*, 2020). Pathogenic strains of *E. coli* pose significant risks, highlighting the need for effective antimicrobial agents to enhance the quality of chicken meat (Marcus *et al.*, 2017). Tracking sources of *E. coli* is crucial for managing foodborne illnesses (Liu *et al.*, 2020). Nanotechnology presents innovative solutions across various stages of the food supply chain to improve safety and storage time (Baltić *et al.*, 2013; Biswas *et al.*, 2022). Nanoparticles possess larger surface areas, compared to their bulk counterparts, enabling practical applications (Burmistrov *et al.*, 2022). Recently, inorganic materials such as TiO_2 and ZnO

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have gained significant research interest for their stability and effectiveness against foodborne pathogens under harsh processing conditions (Chong *et al.,* 2022; Pal and Bhunia, 2022). Metal oxide nanomaterials exhibit strong antimicrobial properties by interacting with bacterial membranes, disrupting protein structures, and inhibiting DNA multiplication (Kaur *et al.,* 2023). One notable application of nanoparticles in food as additives is to preserve color and prevent spoilage (Biswas *et al.,* 2022). The FDA has approved these nanoparticles for use in the food industry due to their biocidal properties and lack of adverse effects (Toker *et al.,* 2013). Zinc oxide nanoparticles (ZnONPs) are particularly significant in the food industry due to their antibacterial properties (Gudkov *et al.,* 2021). ZnO nanostructures are chemically stable and non-toxic (Sirelkhatim *et al.,* 2015). Studies have shown that ZnO-NPs and TiO_2-NPs are effective against *E. coli* (Abd El-Aziz *et al.,* 2020a). Titanium oxide production began in the early twentieth century as a non-toxic white dye for paints (Almarbd and Abbass, 2022), with annual production now exceeding four million tons. This compound is used in pharmaceuticals, cosmetics, and plastics, and as a safe food pigment approved by European authorities (Al Taee and Al Shabander, 2022; Moayeripour and Behzadi, 2023). The antimicrobial efficacy of ZnO is enhanced when its size is reduced to the nanoscale (da Silva *et al.,* 2019a). Many nanomaterials demonstrate potent antimicrobial activity, with ZnO-NPs explored as food preservatives (Swain *et al.,* 2021). Their strong antimicrobial properties have sparked interest in using them directly in foods to reduce microbial contamination and extend shelf life (Meng *et al.,* 2014). While previous research mainly focused on nanoparticles in meat packaging or coatings, studies by EFSA (2008) and Avella *et al.* (2005) indicated minimal or no migration of nanoparticles from packaging materials. Additionally, Abd El-Aziz *et al.* (2020a) suggested that applying ZnO-NPs and TiO_2-NPs directly to minced meat effectively inhibits *E. coli* growth while prolonging shelf life. Consequently, this study intends to assess the antibacterial properties of ZnO and TiO_2 nanoparticles, both separately and in combination, when mixed directly with chicken kofta. It will also evaluate the in vitro antibacterial capabilities of these nanoparticles against *E. coli.*

MATERIALS AND METHODS

Collection and preparation of samples

Four kilograms of fresh, boneless chicken breast fillets were sourced from poultry abattoirs in El Monofia province. These samples were securely transported to the lab and stored in boxes with cooling packs at a temperature of 4±1ºC until needed for analysis. Chicken kofta was prepared, according to Sharada *et al.* (2023). The samples underwent sterilization using UVA light (320–400 nm) for 15 minutes on each side, as outlined by Wang *et al.* (2023) and Morsy *et al.* (2018).

Bacterial strain and nanoparticles

The bacterial strain used in this research was *E.coli* (ATCC® 25922TM), approximately 8 log CFU/ml. was provided by the Food Safety Reference Lab, Animal Health Research Institute (AHRI), Dokki, Egypt. Zinc oxide nanoparticles were prepared according to Wang *et al.* (2007), while titanium dioxide nanoparticles were synthesized following the method by Yin et al. (2001). Both types of nanomaterials were sourced from the National Research Centre in Dokki, Cairo, Egypt.

Assessment of In Vitro Antibacterial Activity

To test the antibacterial activity of ZnO (1, 2, 4, 6, 8, and 10 mM) and TiO_2 (1, 2, 4, 6, 8, and 10 mM) against *E. coli and* compared with the inhibition effect of Ciprofloxacin (5µg) and Amoxicillin (10µg). The nanoparticles were suspended in double-distilled water and mixed to make a homogeneous colloidal suspension. A precise amount of bacterial culture was put

into Mueller-Hinton agar plates. Sterile paper discs (Whatman No.1, 6 mm) were inserted on freshly inoculated plates, and 10 µL of each dilution was applied. The plates have been incubated for 24 hours at 37 degrees Celsius (Bauer *et al.*, 1966).

Electron Microscopy Observations

The biocidal effects of the nanoparticles on *E. coli* were evaluated using a transmission electron microscope. A JEOL JEM1400 TEM was utilized for this analysis at Cairo University Research Park (Yashroy, 1990).

Assessment of Antibacterial Activity in Chicken Kofta

Minced chicken fillet was combined with an additive and inoculated with approximately 8 log CFU/ml of *E. coli* to achieve a final concentration of around 5 log CFU/g. The mixture was thoroughly blended by hand to ensure even distribution, and allowed to rest for 30 minutes to promote adhesion between the *E. coli* and the minced chicken. Initial levels of *E. coli* were measured before introducing the nanomaterials. Control samples were treated with phosphate-buffered saline (PBS), while other groups received varying concentrations: 5 mM ZnO, 10 mM ZnO, 5 mM TiO2, 10 mM TiO2, and a combination of both ZnO and TiO_2 at 5 mM each. The nanomaterials were incorporated into the minced chicken fillet samples for an additional 30 seconds to ensure uniform mixing, before forming the chicken kofta under sterile conditions. The kofta samples were categorized into six groups (each weighing 350 g): Group 1 (PBS + *E. coli*), Group 2 (5 mM ZnO + *E. coli*), Group 3 (10 mM ZnO + *E. coli*), Group 4 (5 mM TiO_2 + *E. coli*), Group 5 (10 mM TiO_2 + *E. coli*), and Group 6 (5 mM ZnO + 5 mM TiO_2 + *E. coli*). Each sample was placed in a sterile polyethylene bag, appropriately labeled, and stored at a temperature of 4 ± 1 °C until spoilage occurred. *E. coli* counts and sensory evaluations were performed on days 1, 3, 6, 9, 12, 15, and 18. Each experiment was replicated three times to calculate average values.

Sensory Evaluation

The assessments were performed by nine trained panelists aged between 40 and 50 years, selected per ISO guidelines (2012). The panelists conducted descriptive sensory analyses on both treated and control samples to provide reliable comparative evaluations based on organoleptic criteria rated on a continuous hedonic scale (ISO, 2003). They scored attributes such as odor, color, and texture on a scale from 0 (very poor) to 10 (excellent), according to Cullere *et al.* (2018). Each panelist received disposable dishes containing three samples arranged in a triangle format for scoring.

***E. coli* Enumeration**

Using Eosin-methylene blue agar plates (HIMEDIA, M317) as described in FDA (2001). *E. coli* colonies were counted as log CFU/g of the sample.

Chemical Analysis

On days 1, 3, 6, 9, 12, 15, and 18 during storage, chemical analyses including pH measurement were performed for each treatment using a Digital Jenco 609 pH meter as per ES standards (63-11/2006).

Statistical analysis

The study employed a completely randomized design with a 6×7 factorial arrangement, consisting of six treatments: 5 mM ZnO, 10 mM ZnO, 5 mM TiO_2, 10 mM TiO_2, a combination of 5 mM ZnO and TiO_2, and a control group. These treatments were assessed over seven sampling days (1^{st}, 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, and 18^{th}) under refrigerated conditions at 4±1°C. All collected data underwent analysis of variance (ANOVA) using the SPSS software (Version 22). Considerable differences were determined at $p \leq 0.05$ based on F-values (Duncan, 1955). Results are reported as means ± standard deviation.

RESULTS

The results revealed that ZnO with concentrations 1Mm and TiO_2 with concentrations 1 and 2 mM do not affect *E.coli* (Figure 1). The most effective nanoparticles against *E. coli* were ZnO 8 and 10 mM with inhibition zone diameters of 15.5 and 20.2 (mm), respectively, followed by TiO_2 with concentrations of 8 and 10 mM as the diameter of inhibition zones were 10.1 and 14.3 (mm), respectively. These nanoparticles were more effective than Ciprofloxacin (5µg) and Amoxicillin (10µg).

Figure 2 (A and B) showed under TME that ZnO and TiO_2 (NPs) sizes range from 30-40 nm. Smaller ZnO and TiO_2 (NPs) exhibit greater antibacterial activity compared to larger particles.

The TEM observations presented in Figure 3 (A, B, C and D) showed the morphological changes of *E.coli* cells, and the difference between control and treated strains by ZnO and TiO_2 with a diameter of 30-40nm, as well as their mixture. ZnO caused irregular, corrugated cell membranes, elongation of bacterial cells and ruptured bacterial cells. Also, the results indicated that TiO_2 nanoparticles led to the elongation of bacterial cells and thinning of the cell membrane. The mixture of ZnO and TiO_2 NPs was found to accumulate within the bacterial cells, leading to cellular destruction and disruption of the cytoplasmic structure.

The mean value of odor, texture, and the overall acceptability scores showed a significant difference ($P<0.05$) between the control and treated groups (Table 1). The control had a lesser score of 4.5 on the 6^{th} day of cold storage, then the samples spoiled on the 9^{th} day. Whereas the ZnO NPs with a concentration of 10 mM had a better score during cold storage, as they remained acceptable till the 15^{th} day of storage and then spoiled, followed by the mixture group, TiO_2 NPs (10 mM), ZnO NPs (5 mM) and TiO_2 NPs (5 mM) respectively. As storage time increased, all groups exhibited a significant ($P<0.05$) decline in sensory attributes, although this decrease was more pronounced in the control group.

Table (2) presents the antibacterial effects of ZnO and TiO_2 and their mixture on nanoparticles of *E. coli* inoculated in chicken kofta stored at 4 °C. In the control group, the average *E. coli* count increased from 5.26 to 7.62 (log cfu/g) during the storage period, which was significantly different from all treated samples. At a concentration of 10 mM, ZnO nanoparticles exhibited a notable antibacterial effect against *E. coli*, showing significant differences ($p < 0.05$) compared to TiO_2 at the same concentration. However, no significant difference ($p > 0.05$) was found between ZnO NPs at 10 mM and a combination of ZnO + TiO_2 at 5 mM.

Data in Table (3) presented that the pH levels of the treatments ranged from 5.81 to 8.82 on the first day of storage. Over time, all treated samples showed a significant increase in pH ($p < 0.05$), with the control sample experiencing a more substantial rise than the treated ones. The control group started with a pH of 5.82 and reached 7.55 by day 18 of refrigeration. Among treated samples, those with ZnO nanoparticles at a concentration of 10 mM exhibited the least increase in pH, followed by those containing a mixture of ZnO and TiO_2 at 5 mM. However, TiO_2 nanoparticles at 5 mM showed the highest increase in pH levels.

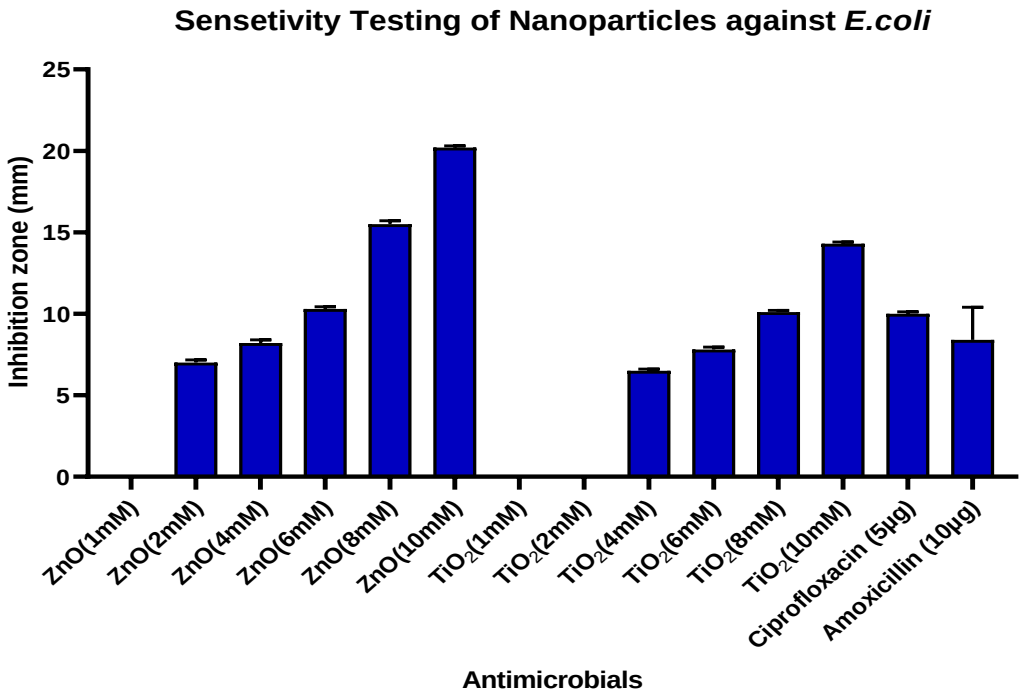

Figure (1) Antibacterial activity assessment of ZnO, TiO_2 and reference antibacterial agent against *E. coli* (ATCC 25922) using disc diffusion method

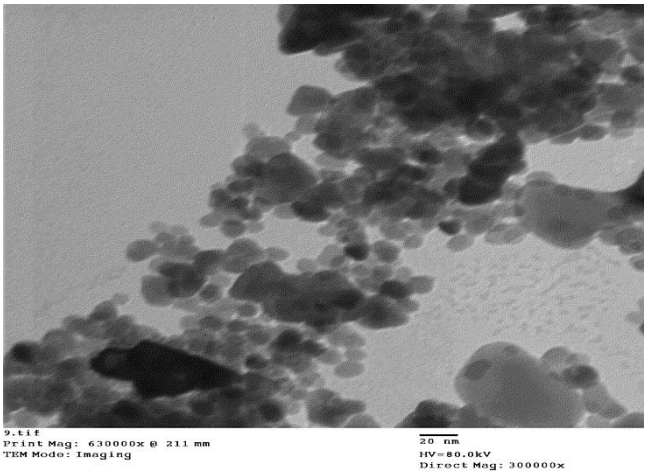

Figure (2A) The physical characteristics of zinc oxide nanoparticles observed under transmission electron microscopy (TEM) revealed white, spherical crystals with an approximate size of 25 ± 5 nm and a purity of 99.9%. The analysis was conducted at a magnification of 300,000 times.

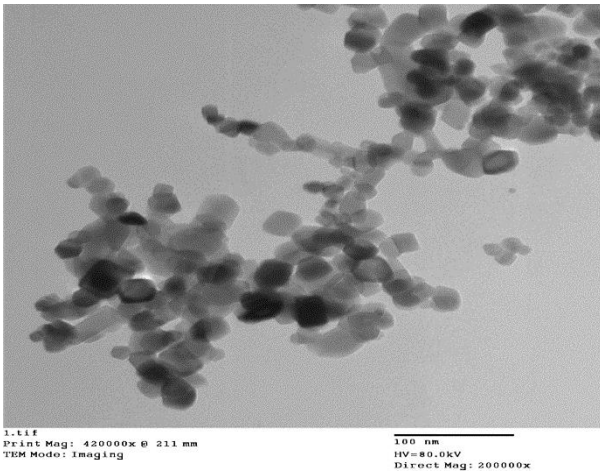

Figure (2B) The physical properties of titanium dioxide nanoparticles observed through transmission electron microscopy (TEM) indicated white, spherical crystals with an approximate size of 25 ± 5 nm. With purity of 99.9% with magnification power 200000 X.

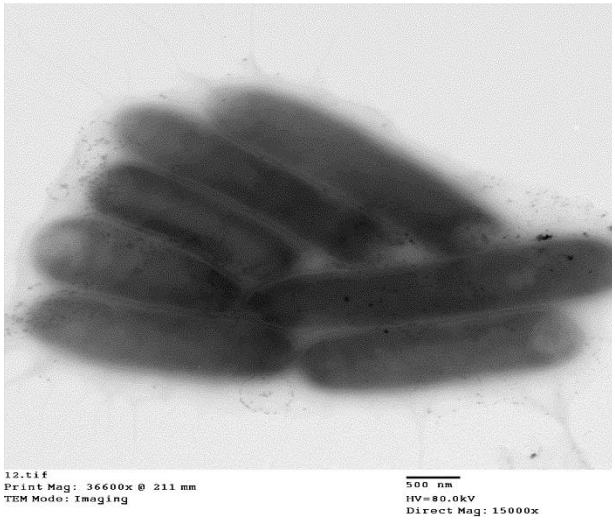

Figure (3A) Normal morphology of *E. coli* using transmission electron microscope showed straight rod shape bacterium showed intact cell membrane with magnification power 15000X.

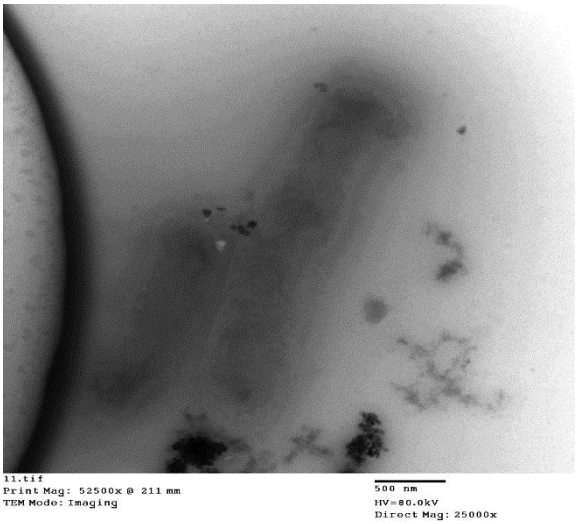

Figure (3B) The activity of ZnO NPs against *E. coli* assessed by transmission electron microscope. ZnO nanoparticles attached to *E. coli* showed irregular, corrugated cell membranes and elongation of bacterial cells and ruptured bacterial cells with magnification power 25000X.

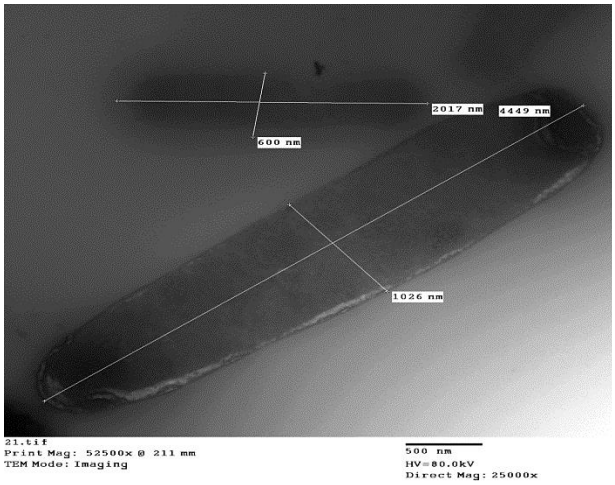

Figure (3C) The impact of TiO_2 nanoparticles on E. coli was examined using a transmission electron microscope. The results indicated that TiO2 nanoparticles led to the elongation of bacterial cells and thinning of the cell membrane.

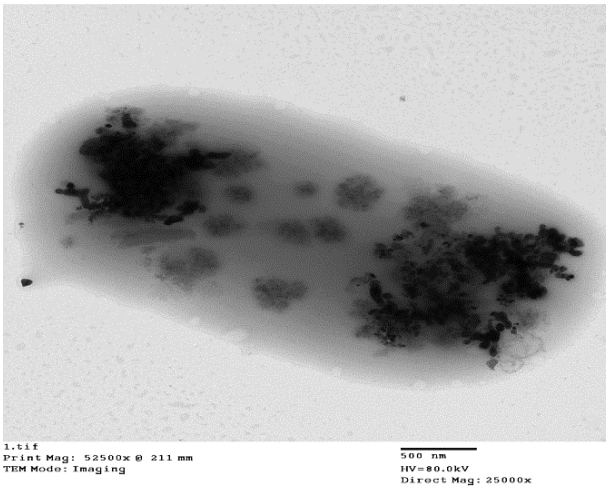

Figure (3D) The effectiveness of the nanoparticle mixture (ZnO + TiO_2) against *E. coli* was assessed using a transmission electron microscope. The nanoparticles were found to accumulate within the bacterial cells, leading to cellular destruction and disruption of the cytoplasmic structure.

Table 1: Effects of various ZnO and TiO_2 nanoparticle concentrations on the general acceptability of chicken kofta after 18 days of storage at 4 ˚C.

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
Control	9.25 ± 0.08 [a]	5.9 ± 0.4 [a]	4.5 ± 0.2[e]	Spoiled	Spoiled	Spoiled	Spoiled
ZnO NPs (5 mM)	9.25 ± 0.28 [a]	8.75 ± 0.11[b]	7.1 ± 0.12 [c]	6.5 ± 0.2 [c]	5.4 ± 0.11 [c]	Spoiled	Spoiled
ZnO NPs (10 mM)	9.25 ± 0.08 [a]	8.9 ± 0.12 [c]	7.5 ± 0.11 [b]	7.2 ± 0.13 [d]	6 ± 0.2 [e]	5 ± 0.3 [f]	Spoiled
TiO_2 NPs (5 mM)	9.25 ± 0.08 [a]	8.61 ± 0.2 [d]	6.9 ± 0.13 [c]	6.11 ± 0.16 [e]	5 ± 0.3 [cf]	Spoiled	Spoiled
TiO_2 NPs (10 mM)	9.25 ± 0.08 [a]	8.7 ± 0.13 [bc]	7.1 ± 0.2 [c]	6.6 ± 0.3 [c]	5.5 ± 0.11 [c]	4.5 ± 0.11 [e]	Spoiled
ZnO + TiO_2 NPs (5 mM)	9.25 ± 0.08 [a]	8.85 ± 0.11 [c]	7.45 ± 0.11 [b]	7.13 ± 0.11 [d]	5.9 ± 0.3 [e]	4.8 ± 0.12 [f]	spoiled

The data are presented as mean ± standard error. Means within the same column having different superscript letters indicate a significant difference ($P \leq 0.05$).

Table 2. Effects of various ZnO and TiO2 nanoparticle concentrations against *E. coli* counts in chicken kofta inoculated with *E. coli* (~ 5 log CFU/g) during storage at 4 ºC for 18 days

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
Control	5.26 ± 0.12 [a]	5.71 ± 0.16 [a]	6.11 ± 0.14 [a]	6.53 ± 0.32 [a]	6.97 ± 0.3 [a]	7.2 ± 0.44 [a]	7.62 ± 0.34 [a]
ZnO NPs (5mM)	5.21 ± 0.11 [a]	4.92 ± 0.13 [b]	4.1 ± 0.1 [c]	3.9 ± 0.11 [b]	3.4 ± 0.2 [cd]	3.01 ± 0.19 [b]	4.5 ± 0.2 [b]
ZnO NPs (10 mM)	5.1 ± 0.13 [a]	4.35 ± 0.2 [c]	3.98 ± 0.11 [d]	3.78 ± 0.15[d]	3.2 ± 0.12 [d]	2.9 ± 0.2 [c]	3.01 ± 0.11 [b]
TiO2 NPs (5mM)	5.25 ± 0.12 [a]	4.96 ± 0.21 [b]	4.21 ± 0.1 [b]	4.09 ± 0.15 [b]	3.86 ± 0.13 [b]	3.44 ± 0.16 [b]	4.1 ± 0.21 [b]
TiO_2 NPs (10mM)	5.23 ± 0.11 [a]	4.71 ± 0.1 [b,c]	4.15 ± 0.2 [e]	4.02 ± 0.13 [b]	3.61 ± 0.17 [c]	3.15 ± 0.14 [d]	3.82 ± 0.10 [c]
ZnO+TiO_2 NPs (5mM)	5.23 ± 0.11 [a]	4.39 ± 0.2 [c]	4.02 ± 0.14 [d]	3.82 ± 0.11 [d]	3.29 ± 0.13 [d]	2.99 ± 0.2 [c]	3.15 ± 0.15 [b]

The data are presented as mean ± standard deviation. Means within the same column having different superscript letters indicate a significant difference ($P \leq 0.05$).

Table 3. Effects of various ZnO and TiO2 nanoparticle concentrations on pH in chicken kofta inoculated during storage at 4 ˚C for 18 days

Groups	1st day	3rd day	6th day	9th day	12th day	15th day	18th day
Control	5.82 ± 0.09 [a]	6.29 ± 0.11 [a]	6.44 ± 0.10 [a]	6.72 ± 0.07 [a]	6.99 ± 0.03 [a]	7.14 ± 0.04 [a]	7.55 ± 0.06 [a]
ZnO NPs (5mM)	5.81 ± 0.05 [a]	5.95 ± 0.06 [b]	6.20 ± 0.11 [c]	6.32 ± 0.04 [b]	6.40 ± 0.05 [cd]	6.59 ± 0.09 [b]	6.77 ± 0.04 [b]
ZnO NPs (10 mM)	5.81 ± 0.04 [a]	5.89 ± 0.03 [c]	6.08 ± 0.08[d]	6.19 ± 0.10[d]	6.28 ± 0.02 [d]	6.38 ± 0.02 [c]	6.62 ± 0.02 [b]
TiO2 NPs (5mM)	5.82 ± 0.06 [a]	5.98 ± 0.02 [b]	6.15 ± 0.09 [b]	6.36 ± 0.05 [b]	6.42 ± 0.04 [b]	6.66 ± 0.07 [b]	6.87 ± 0.04 [b]
TiO_2 NPs (10mM)	5.82 ± 0.10 [a]	5.92 ± 0.03 [b,c]	6.18 ± 0.02 [e]	6.28 ± 0.03 [b]	6.32± 0.07 [c]	6.45 ± 0.06 [d]	6.79 ± 0.09 [c]
ZnO + TiO_2 NPs (5mM)	5.81 ± 0.09 [a]	5.90 ± 0.02 [c]	6.13 ± 0.04 [d]	6.21 ± 0.09 [d]	6.30 ± 0.03 [d]	6.41 ± 0.05 [c]	6.67 ± 0.11 [b]

The data are presented as mean ± standard error. Means within the same column having different superscript letters indicate a significant difference ($P \leq 0.05$).

DISCUSSION

Nanoparticles, especially ZnO and TiO_2 have garnered significant attention for their antibacterial properties, particularly against *Escherichia coli* (*E. coli*) (Babayevska *et al.,* 2022). Nanoparticles can be quantified by measuring the zones of inhibition they produce in disc diffusion tests. Similar results were recorded by (Albukhaty *et al.,* 2020; Chunchegowda *et al.,* 2021; Ahmad *et al.,* 2022). Also, ZnO NP's antibacterial efficacy is considerably controlled by their size. Many investigations have found a clear link between the size of particles and antibacterial action, particularly against various bacterial strains, including *Escherichia coli (*Babayevska *et al.,* 2022). This is primarily due to their increased surface area, which enhances interaction with bacterial membranes and facilitates penetration into cells (Abd El-Aziz *et al.,* 2020a).

Recent research has demonstrated the growing interest in various nanoparticles, particularly ZnO and TiO_2, due to their antibacterial characteristics (Jafar *et al.,* 2017; Ali *et al.,* 2017; Subhapriya and Gomathipriya, 2018). Notably, the antibacterial activity of ZnO nanoparticles is affected by both size and concentration.

Smaller ZnO particles tend to exhibit enhanced antibacterial activity, while an increase in concentration and surface area correlates with a stronger antimicrobial effect (Abebe, 2020). This relationship underscores the importance of optimizing particle characteristics to maximize their potential as antimicrobial agents.

The TEM observations showed some cells with irregular cell wall, perforated, swollen, enlarged and also undergo thinning of cell wall (Abebe *et al.* 2020; Yoo *et al.* 2021). Researchers have identified three primary mechanisms through which the antibacterial effects of ZnO nanoparticles (ZnO-NPs) and titanium dioxide nanoparticles (TiO_2-NPs) operate, with reactive oxygen species (ROS) production playing a crucial role. This process involves the generation of free radicals resulting from electron transitions between the valence and conduction bands due to appropriate excitation (da Silva *et al.,* 2019b). In the case of metal nanoparticles, cations and anions react with oxygen and water, leading to the formation of highly reactive ROS, including hydrogen peroxide (H_2O_2). This chemical is very toxic to bacterial cells because it disrupts internal structures, resulting in cell death (Priyadarshi *et al.,* 2020). Also, can interact with biological membranes, causing damage via lipid peroxidation (Rauf *et al.,* 2019). Thi *et al.* (2020) found a bactericidal activity against *E. coli* due to ROS generation even in the absence of particular excitation. Furthermore, Vijayaraghvan (2017) and Wang *et al.* (2018) found that oxygen shortages on the outermost layer of zinc oxide contribute to ROS formation. The electrostatic interactions between nanoparticles and bacterial cell walls facilitate their contact, allowing the nanoparticles to penetrate and disturb normal cellular organization and function (da Silva *et al.,* 2019b).

Moreover, *E. coli* are considered a significant source of morbidity and mortality in developing countries, often transmitted through contaminated food (Bintsis, 2017). Currently, nanotechnology is gaining attention as a cutting-edge field within materials science, offering a wide range of innovative applications. These applications span various sectors, including modern fabric production, food processing, agriculture, and advanced medical techniques (De and Goswami, 2022; Astashev *et al.,* 2022). Similar observations of using NPs were obtained by El Asuoty *et al.* (2023) and Abd El-Aziz *et al.* (2020a), who reported that the treated groups exhibited significant enhancements in sensory attributes compared to the control group in meat products, largely attributed to the antimicrobial effects of ZnO.

Furthermore, research by Alizadeh-Sani *et al.* (2020) indicated that the application of TiO_2 could prolong the shelf life of beef by an additional 15 days. Sensory evaluation is crucial for gauging consumer perceptions regarding the palatability of meat products. Lipid oxidation can result in undesirable odors and flavors, while myoglobin oxidation may alter texture and color, leading to discoloration that affects consumer acceptance. Harmful byproducts from lipid oxidation, such as aldehydes, contribute to these sensory changes (Banerjee *et al.*, 2017). Conversely, *E. coli* counts in samples treated with nanoparticles decreased throughout storage, demonstrating the antibacterial properties of these nanoparticles as highlighted by Raj et al. (2021). At a concentration of 10 mM, ZnO nanoparticles exhibited a notable anti-bacterial effect against *E. coli*, showing significant differences ($p < 0.05$) compared to TiO_2 at the same concentration. However, no significant difference ($p > 0.05$) was found between ZnO NPs at 10 mM and a combination of ZnO + TiO_2 at 5 mM. Similar findings were reported by Marcous *et al.* (2017), who concluded that ZnO was more effective than TiO_2 against *E. coli* in minced meat. The FDA classifies ZnO nanoparticles as Generally Recognized as Safe (GRAS), making them suitable for use as preservatives due to their antibacterial properties (Souza *et al.*, 2020). Both TiO_2 and ZnO have garnered considerable research interest due to their stability and effectiveness against foodborne pathogens, with some products already available on the market (Chong *et al.*, 2022; Pal and Bhunia, 2022). As storage duration increased, all groups experienced a significant decline in sensory attributes ($P<0.05$), although this decrease was less pronounced in the control group compared to treated samples. The pH levels of the treatments ranged from 5.81 to 8.82 on the first day of storage. Over time, all treated samples showed a significant increase in pH ($p < 0.05$), with the control sample experiencing a more substantial rise than the treated ones. The control group started with a pH of 5.82 and reached 7.55 by day 18 of refrigeration. Among treated samples, those with ZnO nanoparticles at a concentration of 10 mM exhibited the smallest increase in pH, followed by those containing a mixture of ZnO and TiO_2 at 5 mM. However, TiO_2 nanoparticles at 5 mM showed the greatest increase in pH levels. This trend aligns with findings from Abd El-Aziz *et al.* (2020b) regarding how ZnO and TiO_2 nanoparticles influence pH and shelf life in meat products. The rise in pH can be attributed to bacterial activity-producing compounds, such as trimethylamine and ammonia, which have alkaline properties (Hassanzadeh *et al.*, 2018).

CONCLUSION

Zinc oxide and titanium dioxide nanoparticles demonstrate significant antibacterial properties against *E. coli* and contribute to extending the shelf life of chicken kofta, with their efficiency being significantly affected by size and concentration. Higher concentrations tend to produce larger inhibition zones, and their antibacterial mechanisms involve oxidative stress and ion release that disrupt bacterial integrity. This makes ZnO and TiO_2 nanoparticles promising candidates for applications in antimicrobial treatments and food safety measures.

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تأثير جسيمات أكسيد الزنك وأكسيد التيتانيوم النانومترية المضادة لبكتيريا الايشريشيا كولاى على جودة ومدة صلاحية كفتة الدجاج

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تهدف هذه الدراسة إلى دراسة التأثيرات المضادة للبكتيريا لجسيمات أكسيد الزنك وأكسيد التيتانيوم على الايشريشيا كولاى وتأثيرها على جودة ومدة صلاحية كفتة الدجاج. تم زرع عينات كفتة الدجاج بالايشريشيا كولاى ثم تم معالجتها بتركيزات مختلفة من المادتين بتركيزي ٥ ملي مول و١٠ ملي مول من أكسيد الزنك، و٥ ملي مول و١٠ ملي مول من أكسيد التيتانيوم، بالإضافة إلى مزيج من ٥ ملي مول من أكسيد الزنك و٥ ملي مول من أكسيد التيتانيوم .تم تخزين هذه العينات بعد ذلك عند درجة حرارة ٤°م لمدة ١٨ يومًا. تم تقييم الايشريشيا كولاى لمعرفة كيفية تأثير جسيمات أكسيد الزنك والتيتانيوم النانومترية على جودة ومدة صلاحية الكفتة الدجاج. أكدت النتائج على أن جسيمات الزنك النانومترية عند تركيز ١٠ ملي مول قد أوقفت بشكل كبير نمو بكتريا الايشريشيا كولاى المزروعة علي الوسط الغذائى في اختبار قياس منطقة تثبيط البكتريا. أيضا تم تحليل الخصائص المضادة للبكتيريا لجسيمات أكسيد الزنك وأكسيد التيتانيوم ومزيجهما باستخدام المجهر الإلكتروني النافذ وتبين أنها تسبب خلل في مكونات البكتريا وتغير شكلها. كما أظهرت النتائج أن تأثيرأستخدام جسيمات أكسيد الزنك ١٠ ملي مول الأكثر فعالية ضد الايشريشيا كولاى المحقونة في كفتة الدجاج وزيادة مدة صلاحيتها إلى ١٨ يوم. وبذلك خلصت نتائج الدراسة إلى أن جزيئات أكسيد الزنك وأكسيد التيتانيوم يمكن استخدامها كعوامل مضادة للبكتيريا ولتمديد مدة الصلاحية في حفظ الطعام.