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Effectiveness of Zinc Oxide Nanoparticles, Red Cabbage and Beet Root in Reducing Bacterial and Fungal Growth in Refrigerated Beef Kofta

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ABSTRACT

This study determinate the antimicrobial effectiveness of zinc oxide nanoparticles (ZnO NPs), red cabbage, and beetroot in reducing bacterial and fungal growth in refrigerated beef kofta, a traditional meat product. Meat products are prone to microbial contamination and spoilage, even under refrigeration, making it crucial to develop effective and natural preservation strategies. ZnO NPs are known as strong antibacterial and antifungal properties, attributed to the generation of reactive oxygen species that disrupt microbial cell membranes and functions. Additionally, red cabbage and beetroot are rich in anthocyanins and betalains, natural compounds with potent antimicrobial and antioxidant activities. This study evaluated the effects of these agents, both individually and in combination, over a 21-day refrigerated storage period. Results showed that ZnO NPs, red cabbage, and beetroot significantly reduced the growth of various microorganisms. The ZnO NPs treatment alone was effective in lowering bacterial counts, including psychrophilic bacteria, *Staphylococcus aureus*, coliforms, and *Pseudomonas aeruginosa*. Notably, the combination treatments of ZnO NPs with 4% red cabbage (RC ZnONP4%) and 4% beetroot (BR ZnONP4%) demonstrated superior antimicrobial activity, achieving up to a 99% reduction in total bacterial counts throughout the storage period. These combinations also effectively controlled yeast and mold growth, maintaining low levels by day 21. Conclusion: The study suggests that combining ZnO NPs with red cabbage and beetroot can enhance meat preservation, extend shelf life, and provide a safer, more sustainable alternative.

Keywords: ZnO NPs, psychrophilic bacteria, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Kofta

INTRODUCTION

Ensuring the safety and quality of food products is a priority for the food industry, particularly when it comes to meat products like kofta, which are susceptible to microbial contamination and spoilage even under refrigerated conditions (Buncic and Sofos 2012). Traditional methods of preservation, such as refrigeration, while effective to some extent, do not completely prevent microbial growth. Therefore, the development of more effective antimicrobial strategies that are safe, natural, and sustainable is crucial for enhancing food safety and extending shelf life (Huang *et al.*, 2015).

Zinc oxide nanoparticles (ZnO NPs) have emerged as a promising solution in this regard due to their potent antimicrobial properties. ZnO NPs are known to exhibit strong antibacterial and antifungal activities against a wide range of pathogens, including those commonly found in meat products (Lallo da Silva *et al.*, 2019). The antimicrobial mechanism of ZnO NPs is primarily attributed to the generation of reactive oxygen species (ROS), which can damage microbial cell membranes, proteins, and DNA, leading to cell death (Sirelkhatim *et al.*, 2015). Additionally, ZnO NPs have the advantage of being generally recognized as safe (GRAS) for use in food applications, making them a suitable candidate for food preservation (Espitia *et al.*, 2012).

Alongside synthetic nanoparticles, the food industry is increasingly turning to natural plant extracts as alternative antimicrobial agents. Red cabbage and beetroot are two such plant sources that have been extensively studied for their health benefits and antimicrobial properties. Red cabbage contains anthocyanins, which are flavonoid compounds with antioxidant and antimicrobial activities, while beetroot is rich in betalains, which are natural pigments with similar properties (Clifford, 2000; Georgiev *et al.*, 2010). These compounds are effective against a variety of microorganisms and can also contribute to the preservation of food by preventing oxidation and spoilage (Kapadia *et al.*, 2011; Hidalgo *et al.*, 2018).

The potential of combining ZnO nanoparticles with natural extracts like red cabbage and beetroot in food preservation has been largely unexplored. The hypothesis is that the synergistic effects of these two agents could provide enhanced antimicrobial activity, thereby offering a more effective and natural solution for preserving meat products such as kofta. This research aims to investigate the effectiveness of zinc oxide nanoparticles, red cabbage, and beetroot, both individually and in combination, in reducing bacterial and fungal growth in refrigerated kofta. By doing so, it seeks to offer a novel approach to food preservation that aligns with consumer demand for natural and safe food products.

MATERIALS AND METHODS

Red Cabbage, Beet Roots and Pomegranate Peels

Red cabbage *(Brassica oleracea var. capitata)* and beet roots *(Beta vulgaris)* were purchased from the local

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market at Fayoum Governorate, Egypt. The vegetables were washed with distilled water, dried in vacuum oven at 40°C, and grinded into powder, and then stored in deep freezer at -18°C until use. Pomegranate, variety (Punica granatum L), was procured from the local market at El Fayoum Governorate, Egypt. The peels were separated from the pomegranate fruits, washed with tap water, subjected to drying in vacuum oven (electric heating constant temperature drying ovens) at 40°C, and powered. Pomegranate peel power was stored in sealed vials at room temperature until use.

Meat

Beef (Neck meat "douche"), and beef fat were purchased from the local market at Fayoum, Governorate, Egypt.

Other Ingredients

Flour, Salt (NaCl), Onion powder, garlic powder and Spices were obtained from the local market in Fayoum, Egypt.

Synthesis of Zinc-oxide Nanoparticles from Pomegranate Peel Extract (PPE)

Pomegranate peel extract (PPE) was prepared according to the method prescribed by Abdelmigid *et al.* (2021). In summary, 5.0 mL of the extract was combined with 95.0 mL of 0.01 M Zinc acetate dehydrate solution (Zn (C2H3O2)2.2H2O). The mixtures were continuously stirred for one hour at 70°C during the incubation period. After generation of the mixtures, the powdery precipitates were centrifuged for 30 min. at 3000 rpm. The precipitate was carefully washed with distilled water and the supernatant was decanted. Pellets were then moved to petri dishes and left overnight at 40°C to ensure thoroughly drying (Naseer *et al.*, 2020).

Preparation of beef kofta

According to Hosny and Nassif, (2020) precisely 3 kg of beef meat have been minced and then stored in sterile plastic bags. Samples of beef Kofta were made with 70% defatted meat, 12% fat, 9% wheat flour (It helps to hold the ingredients of the kofta together), 2.1% common salt, 1.2% onion, 1% garlic powder, and a variety of spices mixture. 1.2%, as described in Table 1.

Table 1. Composition of beef Kofta formulas

Ingredients	(%)
Meat	73.5
Fat	12
Flour	9
Salt (NaCl)	2.1
Onion powder	1.2
garlic powder	1
Spices	1.2

Beef kofta were prepared by mixing the ingredients (minced meat, flour, salt, onion, and garlic powder and spices mixture) in a blender to obtain meat dough. The meat dough was divided into two groups. The first group (250 g) was kept untreated (control), and the second group (treated group) was divided into 10 subgroups (250 gm. of each) and were mixed with $NaNo_2$ (150 ppm), Zinc oxide nanoparticle(10ppm), red cabbage powder 2.0 %, red cabbage powder 4.0 %, beet root powder 2.0 %, beet root powder 4.0 %, red cabbage powder 2.0 % + Zinc oxide nanoparticle, red cabbage powder 4 % + Zinc oxide nanoparticle, beet root powder 2 %+ Zinc oxide nanoparticle and beet root powder 4 %+ Zinc oxide nanoparticle. Meat dough was shaped in round balls manually to get raw meat kofta. Kofta samples was packed in polyethylene bag, labeled and stored at 4 °C for 21 days.

Microbiological examination

Kofta samples are sliced with sterilized scissors and forceps and carefully combined by a sterilized spoon. The mechanical shaker was used to homogenize aseptically 10 ml of prepared samples to 90 ml of sterile 0.1 ml percent peptone water for one minute. 1.0 ml of the previous homogenate was applied to 9 ml of sterilized diluents. The kofta samples prepared were subjected to the following inspections as followed:

Total viable bacteria count

Total viable bacteria count was assessed according to ISO (2003a) and the counts were expressed as (CFU/g). Duplicate plates of Standard Plate Count Agar were inoculated with 0.1 ml of the previously prepared decimal dilutions, evenly spreaded onto the surface of the plates and incubated at 30 °C for 72 hours.

Total count of *Staphylococcus aureus*

From the previously prepared decimal dilutions, 0.1 ml was transferred onto the dry surface of duplicate plates of Baird-Parker medium supplemented with egg yolk tellurite (OXOID 2010) and spread with sterile bent glass spreader until the surface of the medium appears dry. The plates were incubated at 37ºC for 24-48 hours. Plates which contain a maximum of 150 typical and/ or atypical colonies were choosing to calculate the Staphylococci count. Typical colonies (circular, smooth, convex, moist, grey to jet black, shiny greater than one mm in diameter with or without white margined surrounded by clear zone extending to the opaque medium) were counted and recorded.

Yeast and molds counts

From each of the previously prepared sterile dilutions, 1ml aliquots were delivered into duplicate sets of petri dishes, previously inoculated with 10ml of sterile Potato Dextrose agar medium after solidification, inoculated as well as control plates were incubated at an inverted position at 3 for 5 days. (OXOID 2010).

***Coliform* count**

From each of the previously prepared sterile dilutions, 1ml aliquots were delivered into duplicate sets of petri dishes, previously inoculated with 10ml of sterile MacConkey Agar After solidification, inoculated as well as control plates were incubated at an inverted position at 37° C for 48 hours (OXOID 2010).

Psychrophilic bacteria count

Plat Count Agar medium for psychrophilic bacteria, petri dishes were incubated in refrigerator at 4 ° C for 10 days Yousif and Makki (2020).

***Pseudomonas aeruginosa* count**

Pseudomonas aeruginosa count according to ISO, (2004) on Pseudomonas media at 25 °C for 48 hours after which all developed colonies (greenish yellow colonies) were enumerated. Typical Formula (g/l): Gelatin Peptone 16.0, Casein Hydrolysate 10.0, Potassium Sulfate, Anhydrous 10.0, Magnesium Chloride, Anhydrous1.4, Agar 15.0, Final pH 7.1 ± 0.2.

RESULTS AND DISCUSSION

Changes on the total count of psychrophilic bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days.

Psychrophilic bacteria are cold-loving microorganisms that can grow and multiply at refrigerated temperatures, which

can cause spoilage in refrigerated meat products. The total count of psychrophilic bacteria provides an indication of the microbial quality and potential spoilage of the meat over time (Kanekar *et al.*, 2022). In the Table 2 the bacterial counts were measured at zero time (initial), after 7 days, 14 days, and 21 days of refrigerated storage. The control sample had an initial bacterial count of 13×10^3 CFU/g (colony-forming units per gram). Bacterial Growth over Time: The bacterial count increased significantly over time, reaching 8×10^4 CFU/g by day 7. By days 14 and 21, the sample was marked as "S*", indicating spoilage, which suggests an extremely high bacterial count, beyond the threshold for safe consumption. The initial count was 13×10^3 CFU/g, similar to the control. Bacterial Growth: The bacterial count increased to 6×10^4 CFU/g by day 7 and further to 14×10^5 CFU/g by day 14, before slightly decreasing to 11×10^5 CFU/g by day 21. This treatment showed some control over bacterial growth compared to the control but still exhibited high counts indicating spoilage risk. ZnO NP (10 mg/kg) Treatment: The initial count was slightly lower at 12×10^3 CFU/g. Bacterial growth was slower compared to $NaNO_2$, increasing to 5×10^4 CFU/g by day 7, and then to 12×10^5 CFU/g by day 14, decreasing to 9×10^5 CFU/g by day 21. This treatment was more effective in controlling bacterial growth compared to $NaNO_2$. RC2%: Initial count was 14×10^3 CFU/g. Bacterial counts increased to 7×10^4 CFU/g by day 7, 16×10^5 CFU/g by day 14, and 12×10^5 CFU/g by day 21. This indicates moderate control over bacterial growth. On the other side RC4%: Initial count was 13×10^3 CFU/g. Bacterial counts were 6×10^4 CFU/g by day 7, 13×10^5 CFU/g by day 14, and 10×10^5 CFU/g by day 21, showing better control than RC2%. BR2% and BR4% Treatments: BR2%: Initial count was 13×10^3 CFU/g. Counts increased to 5×10^4 CFU/g by day 7, 11×10^5 CFU/g by day 14, and 8×10^5 CFU/g by day 21. This treatment provided good control over bacterial growth. BR4%: Initial count was 11×10^3 CFU/g. Bacterial counts were 4×10^4 CFU/g by day 7, 9×10^5 CFU/g by day 14, and 6×10^5 CFU/g by day 21, indicating effective bacterial growth inhibition, better than BR2%. Combination Treatments with ZnO NP: RC ZnO NP 2%: The initial count was 14×10^3 CFU/g. Counts increased to 7×10^4 CFU/g by day 7, 10×10^5 CFU/g by day 14, and 7×10^5 CFU/g by day 21, showing strong inhibition of bacterial growth. RC ZnONP4%: The initial count was 10×10^3 CFU/g, which increased to 5×10^4 CFU/g by day 7, 7×10^5 CFU/g by day 14, and decreased significantly to 11×10^4 CFU/g by day 21, demonstrating excellent control over bacterial growth. BR ZnO NP 2%: Initial count was 12×10^3 CFU/g. Counts were 6×10^4 CFU/g by day 7, 12×10^5 CFU/g by day 14, and significantly reduced to 5×10^5 CFU/g by day 21, indicating effective control over microbial growth. BR ZnONP4%: This sample had the lowest initial bacterial count of 9×10^3 CFU/g. Bacterial counts increased to 4×10^4 CFU/g by day 7, 6×10^5 CFU/g by day 14, and further reduced to 7×10^4 CFU/g by day 21, showing the best control of bacterial growth among all treatments. Nearly similar results were recorded by Shraouba, and Makkia, (2022).

Table 2. Changes on the total count of psychrophilic bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 day
Control	13×10^3	8×10^4	S*	S*
$NaNo_2$(150 ppm)	13×10^3	6×10^4	14×10^5	11×10^5
ZnO NP(10 mg/kg)	12×10^3	5×10^4	12×10^5	9×10^5
RC2%	14×10^3	7×10^4	16×10^5	12×10^5
RC4%	13×10^3	6×10^4	13×10^5	10×10^5
BR2%	13×10^3	5×10^4	11×10^5	8×10^5
BR4%	11×10^3	4×10^4	9×10^5	6×10^5
RC ZnO NP2%	14×10^3	7×10^4	10×10^5	7×10^5
RC ZnO NP4%	10×10^3	5×10^4	7×10^5	11×10^4
BR ZnO NP2%	12×10^3	6×10^4	12×10^5	5×10^5
BR ZnO NP4%	9×10^3	4×10^4	6×10^5	7×10^4

S*: spoilage, $NaNo_2$ (150 ppm): sodium nitrite, ZnO NP: zinc oxide nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnO NP2%: red cabbage 2% + zinc oxide nanoparticle, RC ZnO NP4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnNP2%: beet root 2% + zinc oxide nanoparticle and BR ZnO NP4%: beet root 4% + zinc oxide nanoparticle.

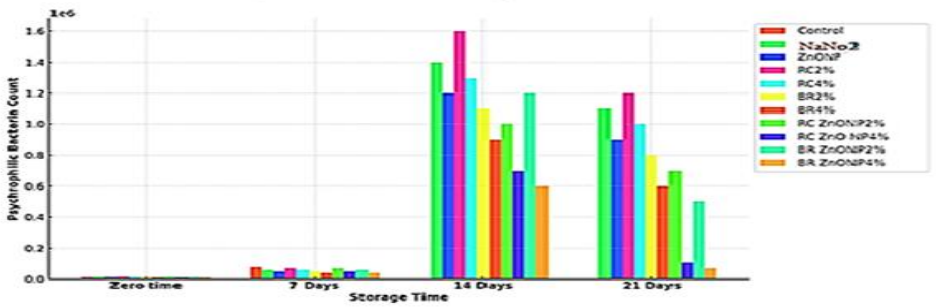

Fig. 1. Changes on the total count of psychrophilic bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days

Changes on the total counts of bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days.

The total bacterial count is an important indicator of the microbial quality and safety of meat products during storage. High bacterial counts indicate poor microbial quality and potential spoilage (Katiyo *et al.*, 2020). In the Table 3, the total bacterial counts for various beef kofta samples were measured at zero time (initial), after 7 days, 14 days, and 21 days of refrigerated storage. The control sample started with an initial bacterial count of 5.7×10^5 CFU/g (colony-forming units per gram). The bacterial count increased significantly to 9.22×10^6 CFU/g by day 7. By days 14 and 21, the sample reached spoilage levels, indicated as "S*," suggesting that bacterial counts were too high to measure accurately and exceeded safe consumption limits. In the other hand in $NaNO_2$ (150 ppm) Treatment: The initial count was 5.2×10^5 CFU/g. Bacterial Growth: The bacterial count increased to 8.34×10^6 CFU/g by day 7, then slightly decreased to 5.66×10^6 CFU/g by day 14, before drastically rising to 4.88×10^8 CFU/g by day 21. Although $NaNO_2$ initially slowed bacterial growth compared to the control, it was less effective at preventing a significant increase by the end of the storage period. ZnO NP (10 mg/kg) Treatment: Initial The ZnO NP treated sample had a lower initial count of 5.0×10^5 CFU/g. Bacterial Growth: The

count increased to 7.66×10^6 CFU/g by day 7 and 3.00×10^7 CFU/g by day 14, then significantly increased to 2.61×10^8 CFU/g by day 21. This treatment was more effective than $NaNO_2$ in the early stages but showed substantial bacterial growth by day 21. RC2%: The initial count was 5.6×10^5 CFU/g. Bacterial counts increased to 8.80×10^6 CFU/g by day 7 and 6.00×10^7 CFU/g by day 14, reaching 4.81×10^8 CFU/g by day 21. RC2% provided moderate control over bacterial growth. In RC4%: The initial count was 5.2×10^5 CFU/g. The count increased to 7.45×10^6 CFU/g by day 7 and 5.00×10^7 CFU/g by day 14, with a lower final count of 2.11×10^8 CFU/g by day 21, indicating better control than RC2%. In BR2%: The initial count was 5.6×10^5 CFU/g. Bacterial counts increased to 8.67×10^6 CFU/g by day 7, 6.34×10^7 CFU/g by day 14, and 3.65×10^8 CFU/g by day 21. BR2% showed moderate control over bacterial growth. While BR4%: The initial count was 5.3×10^5 CFU/g. Counts increased to 7.32×10^6 CFU/g by day 7, 6.27×10^7 CFU/g by day 14, and 4.60×10^8 CFU/g by day 21. This treatment exhibited moderate bacterial growth inhibition, similar to BR2%. Combination Treatments with ZnO NP: RC ZnONP2%: The initial count was 5.6×10^5 CFU/g. Bacterial counts were 7.20×10^6 CFU/g by day 7, 4.87×10^7 CFU/g by day 14, and 3.01×10^8 CFU/g by day 21. This treatment showed better bacterial control than RC2% and RC4%. RC ZnONP4%: The initial count was 5.4×10^5 CFU/g, which increased to 6.83×10^6 CFU/g by day 7, 3.56×10^7 CFU/g by day 14, and significantly lower to 6.97×10^7 CFU/g by day 21, demonstrating excellent control over bacterial growth. BR ZnONP2%: The initial count was 5.3×10^5 CFU/g. Counts were 7.00×10^6 CFU/g by day 7, 4.00×10^7 CFU/g by day 14, and 1.87×10^8 CFU/g by day 21, indicating effective control over microbial growth. BR ZnONP4%: The initial count was 5.0×10^5 CFU/g. Bacterial counts increased to 6.27×10^6 CFU/g by day 7, 3.25×10^7 CFU/g by day 14, and were the lowest at 5.80×10^7 CFU/g by day 21, showing the best control of bacterial growth among all treatments. Nearly similar results were recorded by Hosny and Amin (2020).

Table 3. Changes on the total count of bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 days
Control	5.7×10^5	9.22×10^6	S*	S*
$NaNo_2$ (150 ppm)	5.2×10^5	8.34×10^6	5.66×10^6	4.88×10^8
ZnO NP(10mg/kg)	5.0×10^5	7.66×10^6	3.00×10^7	2.61×10^8
RC2%	5.6×10^5	8.80×10^6	6.00×10^7	4.81×10^8
RC4%	5.2×10^5	7.45×10^6	5.00×10^7	2.11×10^8
BR2%	5.6×10^5	8.67×10^6	6.34×10^7	3.65×10^8
BR4%	5.3×10^5	7.32×10^6	6.27×10^7	4.60×10^8
RC ZnONP2%	5.6×10^5	7.20×10^6	4.87×10^7	3.01×10^8
RC Zn ONP4%	5.4×10^5	6.83×10^6	3.56×10^7	6.97×10^7
BR ZnONP2%	5.3×10^5	7.00×10^6	4.00×10^7	1.87×10^8
BR ZnONP4%	5.0×10^5	6.27×10^6	3.25×10^7	5.80×10^7

S*: spoilage. $NaNo_2$ (150 ppm): sodium nitrite, ZnO NP: zinc oxide nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnO NP2%: red cabbage 2% + zinc oxide nanoparticle, RC ZnO NP4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnO NP2%: beet root 2% + zinc oxide nanoparticle and BR ZnO NP4%: beet root 4% + zinc oxide nanoparticle

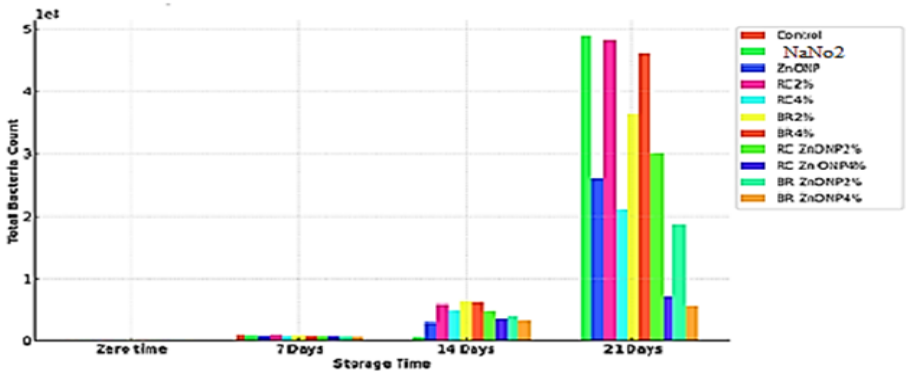

Fig.2. Changes on the total count of bacteria (CFU/g) on kofta samples during refrigerated storage for 21 days

Changes on *total count of Staphylococcus aureus* (CFU/g) on kofta samples during refrigerated storage for 21 days

Staphylococcus aureus is a pathogenic bacterium that can cause foodborne illnesses. Monitoring its presence in meat products during storage is crucial to ensure food safety (Abebe et al., 2020). the Table (4) showed that total count of Staphylococcus aureus in various beef kofta samples at refrigerated storage periods. The results illustrated that control sample had an initial Staphylococcus aureus count of 11.5×10^2 CFU/g (colony-forming units per gram). The count increased to 16×10^2 CFU/g by day 7. By days 14 and 21, the sample was marked as "S*," indicating spoilage and a high bacterial count, beyond safe consumption levels. $NaNO_2$ (150 ppm) Treatment: The initial count was 7.5×10^2 CFU/g. The count decreased slightly over the storage period, from 6×10^2 CFU/g by day 7 to 5×10^2 CFU/g by day 14, and then increased slightly to 7×10^2 CFU/g by day 21. This suggests that $NaNO_2$ had a moderate effect in controlling Staphylococcus aureus growth, maintaining the counts relatively stable. ZnO NP treatment sample had an initial count of 8×10^2 CFU/g. The count decreased steadily over time, from 5×10^2 CFU/g by day 7 to 3×10^2 CFU/g by day 14, and further to 1.5×10^2 CFU/g by day 21. This treatment was very effective in reducing Staphylococcus aureus counts, showing the best control among the treatments. RC2%: The initial count was 7×10^2 CFU/g. The bacterial count increased to 13×10^2 CFU/g by day 7 but then decreased to 5×10^2 CFU/g by day 14 and reached 0 CFU/g by day 21, indicating complete inhibition of Staphylococcus aureus by the end of the storage period. In addition to RC4%: The initial count was 8.5×10^2 CFU/g. Counts decreased to 4.5×10^2 CFU/g by day 7, increased to 9×10^2 CFU/g by day 14, and then decreased to 3.5×10^2 CFU/g by day 21. This treatment showed variable control over Staphylococcus aureus but was effective overall.

BR2% and BR4% Treatments: BR2%: The initial count was 9×10^2 CFU/g. The count increased to 14×10^2 CFU/g by day 7 and decreased to 10×10^2 CFU/g by day 14, then increased slightly to 11×10^2 CFU/g by day 21. This treatment showed moderate control over Staphylococcus aureus growth. BR4%: The initial count was 7×10^2 CFU/g. Counts increased to 13×10^2 CFU/g by day 7, decreased to 7×10^2 CFU/g by day 14, and increased again to 9×10^2 CFU/g by day 21. This treatment provided moderate control but with some fluctuations in bacterial counts. Combination Treatments with ZnO NP: RC ZnONP2%: The initial count was 9×10^2 CFU/g. Bacterial counts decreased to 5.5×10^2 CFU/g by day 7, 3×10^2 CFU/g by day 14, but increased again to 8×10^2 CFU/g by day 21. This treatment showed moderate control over bacterial growth. RC ZnONP4%: The initial count was 6.5×10^2 CFU/g. The bacterial count increased to 8×10^2 CFU/g by day 7, decreased to 6×10^2 CFU/g by day 14, and further decreased to 3.5×10^2 CFU/g by day 21, demonstrating good control over Staphylococcus aureus. BR ZnONP2%: The initial count was 8×10^2 CFU/g. Counts decreased to 5×10^2 CFU/g by day 7, increased to 10×10^2 CFU/g by day 14, and decreased again to 7×10^2 CFU/g by day 21, indicating moderate bacterial control. BR ZnONP4%: The initial count was 8×10^2 CFU/g. The count decreased to 6.5×10^2 CFU/g by day 7, further decreased to 4×10^2 CFU/g by day 14, and continued to decrease to 2.5×10^2 CFU/g by day 21, indicating effective inhibition of Staphylococcus aureus. Samples are not in accordance with EOS (2005) that does, not allow the presence of S. aureus kofta, and the limit (10^2 CUF/g)

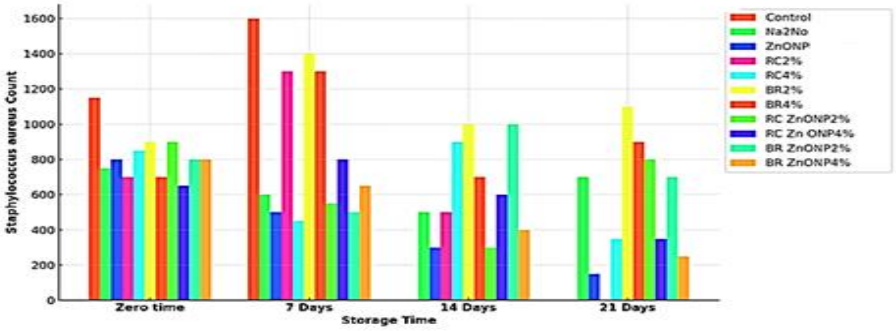

Fig. 3. Changes on total count of *Staphylococcus aureus* (CFU/g) on kofta samples during refrigerated storage for 21 days

Table 4. Changes on *total count of Staphylococcus aureus* (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 days
Control	11.5×10^2	16×10^2	S*	S*
$NaNo_2$ (150 ppm)	7.5×10^2	6×10^2	5×10^2	7×10^2
ZnO NP(10 mg/kg)	8×10^2	5×10^2	3×10^2	1.5×10^2
RC2%	7×10^2	13×10^2	5×10^2	0
RC4%	8.5×10^2	4.5×10^2	9×10^2	3.5×10^2
BR2%	9×10^2	14×10^2	10×10^2	11×10^2
BR4%	7×10^2	13×10^2	7×10^2	9×10^2
RC ZnONP2%	9×10^2	5.5×10^2	3×10^2	8×10^2
RC Zn ONP4%	6.5×10^2	8×10^2	6×10^2	3.5×10^2
BR ZnO NP2%	8×10^2	5×10^2	10×10^2	7×10^2
BR ZnO NP4%	8×10^2	6.5×10^2	4×10^2	2.5×10^2

S*: spoilage, $NaNo_2$ (150 ppm): sodium nitrite, ZnO NP: zinc oxide nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnO NP2%: red cabbage 2% + zinc oxide nanoparticle, RC ZnO NP 4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnNP2%: beet root 2% + zinc oxide nanoparticle and BR ZnO NP4%: beet root 4% + zinc oxide nanoparticle.

Changes on *Coliform group* counts (CFU/g) on kofta samples during refrigerated storage for 21 days

Coliform bacteria are a group of microorganisms commonly found in the environment, including in soil, water, and on plants (Some *et al.*, 2021). Their presence in food, especially meat products, indicates potential contamination and poor hygiene practices during processing and storage. Monitoring coliform counts helps assess the microbiological quality of food products. (Birhanu *et al.*, 2017). The control sample started with a coliform count of 5×10^2 CFU/g (colony-forming units per gram). Coliform Growth over Time: from table 5 the count decreased to 2×10^2 CFU/g by day 7. By days 14 and 21, the sample was marked with "S*", indicating spoilage. This suggests that the coliform levels reached unsafe limits, indicating potential contamination and spoilage. Throughout the 21-day storage period, samples treated with $NaNO_2$ consistently showed zero coliform counts at all-time points. This indicates that $NaNO_2$ was highly effective in preventing coliform contamination and maintaining the microbiological quality of the meat. Similar to NaNO2, the ZnO NP treated samples had zero coliform counts at all measured time points, from zero time through 21 days. This demonstrates that ZnO NP was equally effective in preventing the growth of coliform bacteria during refrigerated storage. RC2%: The initial coliform count was 6×10^2 CFU/g. The count decreased to 2×10^2 CFU/g by day 7 and dropped to zero by day 14, remaining at zero through day 21. This indicates that the RC2% treatment was effective in reducing coliform bacteria over time, eventually eliminating them. In addition to RC4%: The initial coliform count was 7×10^2 CFU/g. The count decreased to 5×10^2 CFU/g by day 7, further decreased to 8×10^1 CFU/g by day 14, and reached zero (0 CFU/g) by day 21. This showed that RC4% was effective in reducing coliform counts, although at a slower rate compared to RC2%. BR2%: The initial coliform count was 4×10^2 CFU/g. The count decreased to 2×10^2 CFU/g by day 7, dropped further to 8×10 CFU/g by day 14, and reached zero by day 21. This indicates good control over coliform bacteria, eliminating them by the end of the storage period. While BR4%: The initial coliform count was 3×10^2 CFU/g. The count decreased to zero (0 CFU/g) by day 7, increased slightly to 2×10 CFU/g by day 14, and returned to zero by day 21.

Table 5. Changes on *Coliform group* counts (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 days
Control	5×10^2	2×10^2	S*	S*
$NaNo_2$ (150 ppm)	0	0	0	0
ZnO NP(10 mg/kg)	0	0	0	0
RC2%	6×10^2	2×10^2	0	0
RC4%	7×10^2	5×10^2	8×10	0
BR2%	4×10^2	2×10^2	8×10	0
BR4%	3×10^2	0	2×10	0
RC ZnONP2%	2×10^2	0	25×10	0
RC ZnO NP4%	2×10^2	2×10^2	21×10	0
BR ZnONP2%	3×10^2	3×10^2	0	0
BR ZnONP4%	2×10^2	0	4×10	0

S*: spoilage., $NaNo_2$ (150 $_{ppm}$): sodium nitrite, ZnONP: zinc oxide nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnO NP2%: red cabbage 2% + zinc oxide nanoparticle, RC ZnO NP4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnO NP2%: beet root 2% + zinc oxide nanoparticle and BR ZnO NP4%: beet root 4% + zinc oxide nanoparticle.

This showed that BR4% was effective in eliminating coliform bacteria early in the storage period and maintaining that control. Combination Treatments with ZnO NP: RC ZnONP2%: The initial coliform count was 2×10^2 CFU/g. The count dropped to zero by day 7, briefly increased to 25×10 CFU/g by day 14, and then returned to zero by day 21. This indicates some fluctuation in control but effective overall elimination of coliforms by the end of storage. RC ZnONP4%: The initial coliform count was 2×10^2 CFU/g. Counts remained stable at 2×10^2 CFU/g by day 7, then decreased to 21×10 CFU/g by day 14, and reached zero by day 21, showing good control of coliform growth. BR ZnONP2%: The initial coliform count was 3×10^2 CFU/g. The count remained at 3×10^2 CFU/g by day 7 and then decreased to zero by day 14 and stayed at zero through day 21. This demonstrates effective elimination of coliforms. BR ZnONP4%: The initial coliform count was 2×10^2 CFU/g. The count decreased to zero by day 7, increased slightly to 4×10 CFU/g by day 14, and returned to zero by day 21, indicating good control over coliform bacteria. These positive results exceeded permissible limit recommended by E.O.S. (2005) as negative Coliform bacteria.

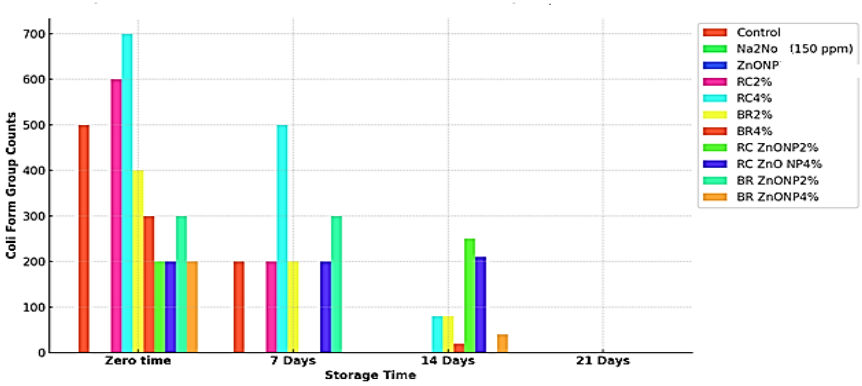

Fig.4. Changes on Coliform group (CFU/g) counts on kofta samples during refrigerated storage for 21 days

Changes in *Pseudomonas aeruginosa* (CFU/g) on kofta samples during refrigerated storage for 21 days

In table 6, the levels of *Pseudomonas aeruginosa* in beef kofta samples stored under refrigeration over a period of 21 days across different treatment groups: Control, NaNO2 (150 ppm), ZnO NP (10 mg/kg), RC2% (Red Cabbage 2%), RC4% (Red Cabbage 4%), BR2% (Beet Root 2%), BR4% (Beet Root 4%), RC ZnONP2% (Red Cabbage 2% + Zinc oxide Nanoparticle), RC ZnONP4% (Red Cabbage 4% + Zinc oxide Nanoparticle), BR ZnONP2% (Beet Root 2% + Zinc oxide Nanoparticle), and BR ZnONP4% (Beet Root 4% + Zinc oxide Nanoparticle). The control samples had initial *Pseudomonas aeruginosa* levels of 3.2×10^2 CFU/g. Changes Over Time: Levels increased to 5.65×10^2 CFU/g by day 7, and spoilage occurred by days 14 and 21, indicated by 'S*'. NaNO2 (150 ppm) The initial level was 3.2×10^2 CFU/g. Changes Over Time: The levels increased to 4.5×10^2 CFU/g by day 7, 6.8×10^2 CFU/g by day 14, and 7.6×10^2 CFU/g by day 21, showing a steady increase over time. ZnO NP (10 mg/kg) The initial level was 3×10^2 CFU/g. Levels increased to 4.2×10^2 CFU/g by day 7, 5.7×10^2 CFU/g by day 14, and 7.3×10^2 CFU/g by day 21, indicating a gradual rise throughout the storage period. RC2% (Red Cabbage 2%) The initial level was 3.2×10^2 CFU/g. Levels rose to 4.2×10^2 CFU/g by day 7, increased to 6.5×10^2 CFU/g by day 14, and reached 8×10^2 CFU/g by day 21. RC4% (Red Cabbage 4%) The initial level was 3.12×10^2 CFU/g. Changes Over Time: Levels increased to 4.2×10^2 CFU/g by day 7, 6×10^2 CFU/g by day 14, and 7.2×10^2 CFU/g by day 21, showing a steady rise. BR2% (Beet Root 2%) The initial level was 3.3×10^2 CFU/g. Changes Over Time: Levels increased to 4.2×10^2 CFU/g by day 7, rose to 6×10^2 CFU/g by day 14, and peaked at 8.8×10^2 CFU/g by day 21, indicating a significant rise in bacterial levels. BR4% (Beet Root 4%) The initial level was 3×10^2 CFU/g. Changes Over Time: Levels rose to 4.5×10^2 CFU/g by day 7, increased to 6×10^2 CFU/g by day 14, and further to 8.6×10^2 CFU/g by day 21. RC ZnONP2% (Red Cabbage 2% + ZnO NP) The initial level was 3×10^2 CFU/g. Changes Over Time: Levels increased to 3.9×10^2 CFU/g by day 7, rose to 5.8×10^2 CFU/g by day 14, and 6.8×10^2 CFU/g by day 21, indicating a moderated increase. RC ZnONP4% (Red Cabbage 4% + ZnO NP) Group: Initial Levels: The initial level was 3×10^2 CFU/g. Changes Over Time: Levels increased to 3.9×10^2 CFU/g by day 7, rose to 5.5×10^2 CFU/g by day 14, and remained at 6.8×10^2 CFU/g by day 21, showing controlled bacterial growth. BR ZnONP2% (Beet Root 2% + ZnO NP) The initial level was 3×10^2 CFU/g. Changes Over Time: Levels increased to 4×10^2 CFU/g by day 7, rose to 6.5×10^2 CFU/g by day 14, and reached 7×10^2 CFU/g by day 21, indicating moderate bacterial growth. BR ZnONP4% (Beet Root 4% + ZnO NP) The initial level was 3×10^2 CFU/g. Changes Over Time: Levels increased to 4×10^2 CFU/g by day 7, rose to 6.5×10^2 CFU/g by day 14, and reached 7.3×10^2 CFU/g by day 21.

Table 6. Changes on *Pseudomonas aeruginosa* (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 days
Control	3.2×10^2	5.65×10^2	S*	S*
$NaNo_2$ (150 ppm)	3.2×10^2	4.5×10^2	6.8×10^2	7.6×10^2
ZnO NP(10 mg/kg)	3×10^2	4.2×10^2	5.7×10^2	7.3×10^2
RC2%	3.2×10^2	4.2×10^2	6.5×10^2	8×10^2
RC4%	3.12×10^2	4.2×10^2	6×10^2	7.2×10^2
BR2%	3.3×10^2	4.2×10^2	6×10^2	8.8×10^2
BR4%	3×10^2	4.5×10^2	6×10^2	8.6×10^2
RC ZnONP2%	3×10^2	3.9×10^2	5.8×10^2	6.8×10^2
RC Zn ONP4%	3×10^2	3.9×10^2	5.5×10^2	6.8×10^2
BR ZnONP2%	3×10^2	4×10^2	6.5×10^2	7×10^2
BR ZnONP4%	3×10^2	4×10^2	6.5×10^2	7.3×10^2

S*: spoilage. $NaNo_2$ (150 ppm): sodium nitrite, ZnO NP: zinc oxide nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnO NP2%: red cabbage 2% + zinc oxide nanoparticle, RC ZnO NP4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnO NP2%: beet root 2% + zinc oxide nanoparticle and BR ZnO NP4%: beet root 4% + zinc oxide nanoparticle.

Changes on yeasts and molds (CFU/g) on kofta **samples during refrigerated storage for 21 days**

Yeasts and molds are types of fungi that can grow in refrigerated foods, potentially leading to spoilage and affecting food quality. Monitoring their presence in meat products during storage helps assess the risk of spoilage and contamination (Altunatmaz *et al.*, 2012). In Table 7, the counts of yeasts and molds in various beef kofta samples were measured at zero time (initial), after 7 days, 14 days, and 21 days of refrigerated storage. The control sample had no detectable yeasts and molds at zero time and after 7 days of storage. Later Storage Periods: By days 14 and 21, the sample was marked with "S*", indicating spoilage. This suggests that yeast and mold levels became too high to measure accurately, contributing to spoilage. The $NaNO_2$-treated samples had no detectable yeasts and molds at zero time and after 7 days. By day 14, the count increased to 2.2×10^2 CFU/g and further increased to 11×10 CFU/g by day 21.

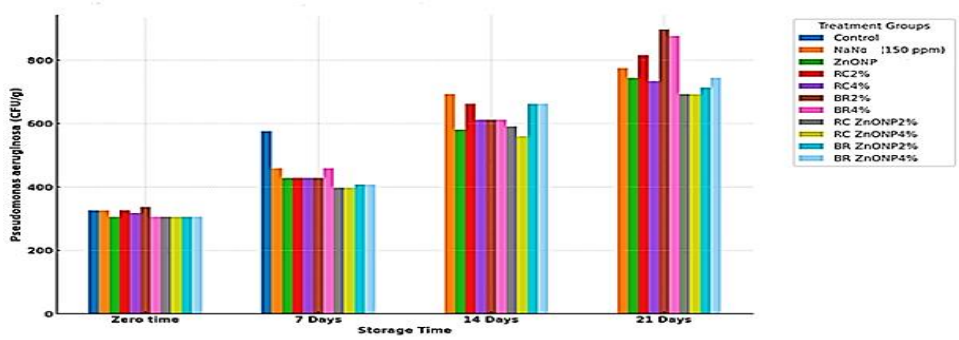

Fig. 5. Changes on *Pseudomonas aeruginosa* (CFU/g) on kofta samples during refrigerated storage for 21 days

Table 7. Changes on yeasts and molds (CFU/g) on kofta samples during refrigerated storage for 21 days

Kofta samples	Storage period			
	Zero time	7 Days	14 days	21 days
Control	0	0	S*	S*
$NaNo_2$ (150 ppm)	0	0	2.2×10^2	11×10
ZnO NP(10 mg/kg)	0	0	2.5×10^2	5×10
RC2%	0	5×10^2	7×10^2	16×10
RC4%	0	3×10^2	6.5×10^2	13×10
BR2%	0	4.5×10^2	7.5×10^2	6×10
BR4%	0	5×10^2	8.5×10^2	9×10
RC ZnONP2%	0	8×10^2	11×10^2	20×10
RC ZnO NP4%	0	6×10^2	8×10^2	12×10
BR ZnONP2%	0	7×10^2	9×10^2	18×10
BR ZnONP4%	0	3.5×10^2	7×10^2	6×10

S*: spoilage., $NaNo_2$ (150 ppm): sodium nitrite, ZnO NP: zinc nanoparticle, RC2%: red cabbage 2%, RC4%: red cabbage 4%, BR2%: beet root 2%, BR4%: beet root 4%, RC ZnNP2%: red cabbage 2% + zinc oxide nanoparticle, RC Zn NP4%: red cabbage 4% + zinc oxide nanoparticle, BR ZnNP2%: beet root 2% + zinc oxide nanoparticle and BR ZnNP4%: beet root 4% + zinc oxide nanoparticle.

This indicates that $NaNO_2$ was moderately effective in controlling yeast and mold growth but did not prevent it completely by the end of storage. The ZnO NP-treated samples also showed no yeasts and molds at zero time and after 7 days. However, by day 14, the count raised to 2.5×10^2 CFU/g and then decreased slightly to 5×10 CFU/g by day 21. This indicates that ZnO NP effectively controlled yeast and mold growth, better than $NaNO_2$ in the latter stages of storage.RC2% and RC4% Treatments: RC2%: The initial yeast and mold count was 0 CFU/g. By day 7, the count increased to 5×10^2 CFU/g, further increased to 7×10^2 CFU/g by day 14, and then decreased to 16×10 CFU/g by day 21. This showed moderate control over yeast and mold growth, with a decrease by the end of storage. RC4%: The initial count was 0 CFU/g. The count increased to 3×10^2 CFU/g by day 7, rose to 6.5×10^2 CFU/g by day 14, and decreased to 13×10 CFU/g by day 21. This indicates that RC4% provided similar control to RC2% over yeasts and molds, with a decrease towards the end of the storage period. BR2% and BR4% Treatments: BR2%: The initial count was 0 CFU/g. By day 7, the count increased to 4.5×10^2 CFU/g, further increased to 7.5×10^2 CFU/g by day 14, and then decreased to 6×10 CFU/g by day 21. This showed good control over yeast and mold growth, with significant reduction by the end of storage. BR4%: The initial count was 0 CFU/g. The count increased to 5×10^2 CFU/g by day 7, rose to 8.5×10^2 CFU/g by day 14, and decreased to 9×10 CFU/g by day 21. This indicates effective control, similar to BR2%, with some fluctuation but overall reduction towards the end of storage. Combination Treatments with ZnO NP: RC ZnONP2%: The initial count was 0. The count increased significantly to 8×10^2 CFU/g by day 7, rose to 11×10^2 CFU/g by day 14, and decreased to 20×10 CFU/g by day 21. This treatment showed some fluctuation in yeast and mold counts but eventually reduced them by the end of the storage period. RC ZnONP4%: The initial count was 0 CFU/g. Counts increased to 6×10^2 CFU/g by day 7, 8×10^2 CFU/g by day 14, and decreased to 12×10 CFU/g by day 21, indicating good control over yeast and mold growth. BR ZnO NP2%: The initial count was 0. Counts increased to 7×10^2 CFU/g by day 7, 9×10^2 CFU/g by day 14, and decreased to 18×10 CFU/g by day 21. This shows effective control over yeast and mold growth, similar to other

treatments with ZnO NP. BR ZnONP4%: The initial count was 0 CFU/g. Counts increased to 3.5×10^2 CFU/g by day 7, 7×10^2 CFU/g by day 14, and decreased to 6×10^1 CFU/g by day 21. This indicates the best control over yeasts and molds among the combination treatments, with the lowest count by the end of the storage period.

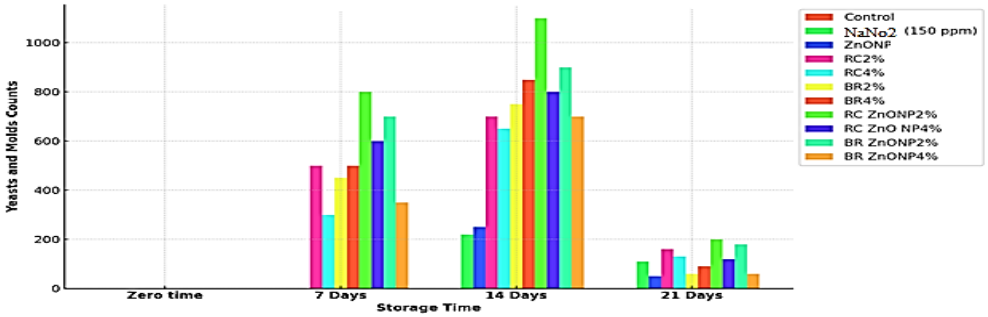

Fig. 6. Changes on yeasts and molds (CFU/g) on kofta samples during refrigerated storage for 21 days

CONCLUSION

This study explored the antimicrobial effectiveness of zinc oxide nanoparticles (ZnO NPs), with red cabbage, and beetroot in reducing bacterial and fungal growth in refrigerated meat kofta. The results of this study demonstrate that all tested treatments, particularly when combined, significantly reduced the growth of various microorganisms that contribute to spoilage in meat products. Individual Effectiveness of ZnO NPs: The ZnO NPs from pomegranate peel extracts alone proved to be highly effective in lowering bacterial counts of several pathogens, including psychrophilic bacteria, *Staphylococcus aureus, coliforms, and Pseudomonas aeruginosa*. The antimicrobial action of ZnO NPs is primarily due to their ability to generate reactive oxygen species (ROS), which damage microbial cell structures and lead to cell death. Both red cabbage and beetroot powder, rich in anthocyanins and betalains respectively, displayed significant antimicrobial and antioxidant properties. These natural compounds contributed to reducing microbial growth and maintaining food quality by preventing oxidation. Synergistic Effects of Combination Treatments: The combination treatments of ZnO NPs with red cabbage and beetroot powders (particularly at 4% concentration) provided superior antimicrobial activity compared to individual treatments. The combination of ZnO NPs with 4% red cabbage powder (RC ZnONP4%) and 4% beetroot powder (BR ZnONP4%) resulted in up to a 99% reduction in total bacterial counts, and significant control over yeast and mold growth during the 21day refrigerated storage period.

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فعالية جسيمات أكسيد الزنك النانوية والكرنب الأحمر و البنجر في تقليل النمو البكتيري والفطري في كفته اللحم البقري المحفوظة بالتبريد

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[٢] قسم الكيمياء، كلية العلوم، جامعة بني سويف، بني سويف ٦٢٥١١، مصر .

الملخص

تهدف هذه الدراسة الي دراسة الفعالية المضادة للميكروبات لجسيمات أكسيد الزنك النانوية (ZnO NPs) والكرنب الأحمر و البنجر في تقليل النمو البكتيري والفطري في كفتة اللحم المبردة، وهو منتج لحوم تقليدي. تتعرض منتجات اللحوم لخطر التلوث الميكروبي والفساد اثناء عملية التبريد، مما يجعل من الضروري تطوير طرق حفظ فعّالة وطبيعية. تُعرف جسيمات ZnO بخصائصها القوية المضادة للبكتيريا والفطريات، وذلك بفضل إنتاجها للأنواع النشطة من الأكسجين التي تعطل أغشية الخلايا الميكروبية ووظائفها. بالإضافة إلى ذلك، يحتوي كل من الكرنب الأحمر و البنجر على الأنثوسيانين والبيتالين، وهي مركبات طبيعية ذات أنشطة مضادة للميكروبات ومضادة للأكسدة قوية. تم تقييم تأثيرات هذه العوامل، سواء بشكل فردي أو مجتمعة، على مدار فترة تخزين مبردة مدتها ٢١ يومًا. أظهرت النتائج أن ZnO NPs والكرنب الأحمر و البنجر خفضت بشكل كبير نمو الميكروبات المختلفة. كانت معالجة ZnO NPsوحدها فعالة في تقليل أعداد البكتيريا، بما في ذلك البكتيريا المحبة للبرودة، و*Staphylococcus aureus*، والبكتيريا القولونية، و *Pseudomonas aeruginosa*. كما اظهرت عينات الكفته المعاملة ب ZnO NPs مع ٤% من الكرنب الأحمر (RC ZnONP4%) و٤% من البنجر (BR ZnONP4%) نشاطًا مضادًا للميكروبات متفوقًا، حيث حققت تخفيضًا يصل إلى ٩٩% في إجمالي الأعداد البكتيرية خلال فترة التخزين بالتبريد . كما تمكنت هذه التركيبات من التحكم الفعّال في نمو الخمائر والفطريات، مع الحفاظ على مستويات منخفضة حتى اليوم ٢١الخلاصة :تشير الدراسة إلى أن الجمع بين ZnO NPs والكرنب الأحمر والبنجر يمكن لن يطيل من فترة حفظ وصلاحية اللحوم المحفوظة تحت ظروف التبريد ، ويوفر بديلاً أكثر أمانًا واستدامة.

الكلمات الدالة :جسيمات ZnO النانوية، البكتيريا المحبة للبرودة، *Staphylococcus aureus*، البكتيريا القولونية، *Pseudomonas aeruginosa*، الكفتة.