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# Effect of Zinc Oxide Nanoparticles Synthesis by( *Zingiber Officinale*) Ginger Extract in Some Microbiological Quality of Fresh Chicken Meat During Referginated Storage

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**Abstract**

This study, include the green synthesis of ZnO nanoparticles using ethanol extract of (*Zingiber officinale*) ginger was investigated in order to evaluate its antibacterial effect on fresh chicken meat. To Determining the microbiological quality of meat involves performing various plate counts indicators include, total bacterial count (TBC) and the total coliform counts TCC the UV-Visible Spectrophotometry and FTiR (Fourier Transform infrared spectroscopy) were used to characterize the GnZnO nanoparticles. A total of 180 samples of fresh chicken meat were subjected to (0.5, 1, and 1.5) mg/ml of GnZnONPs and bacteriologically examined was done . significant absorption of UV region at peak370nm verified the presence of ZnO NPs. The FT-IR spectra recorded the existence of biomolecules responsible for the reduction and capping of the green synthesized , Also observed result of a meat sample treated with GnZnONPs showed a significant ($p < 0.05$) reduction in (TBC) through 2, 5, and 7 days of storage at 4°C (1.5 mg). While the highest count recorded in control (untreated samples) reached 11.360.04 CFU.g-1 at the end of the experiment, the TCC also decreased significantly ($p< 0.05$) after 5,7 days of storage at $4^0$ C when the meat samples were treated with GnZnONPs compared to the control sample. The findings show that treating poultry meat with GnZNoNPs extends the shelf-life of the meat, and maintain its quality throughout the storage period.

**Keywards:** GnZnONPs , Ginger, Chicken Meat, Microbiological Quality

## تأثير تصنيع الجسيمات النانوية من أكسيد الزنك بواسطة مستخلص الزنجبيل (Zingiber officinale) في بعض الجودة الميكروبيولوجية للحوم الدجاج الطازجة أثناء التخزين بالتبريد

الخلاصة

هذه الدراسة تظمنت تحضير التخليق الأخضر لجسيمات ZnOالنانوية باستخدام مستخلص الإيثانول من الزنجبيل (Zingiber officinale)من أجل تقييم تأثيره المضاد للبكتيريا على لحم الدجاج الطازج. لتحديد الجودة الميكروبيولوجية للحوم جرى اختبار . العدد الإجمالي للبكتيريا ( TBC)وإجمالي عد بكتيريا القولون TCC , تم استخدام المقياس الطيفي المرئي للأشعة فوق البنفسجية والتحليل الطيفي بالأشعة تحت الحمراء لتوصيف الجسيمات النانوية GnZnONPs. تم جمع 180 عينة من لحوم الدجاج الطازج ومعاملتها لـ (0.5 ، 1 و 1.5ملغم / مل ) GnZnONps وتم فحصهامايكروبيالوجيا . أثبت الامتصاص الكبير لمنطقة الأشعة فوق البنفسجية عند الذروة 370 نانومتر عن وجود و ZnO NPs. سجلت أطياف FT-IRوجود جزيئات حيوية مسؤولة عن تقليل وتغطية الجزيئات الخضراء المُصنّعة ، كما أظهرت نتائج العد البكتيري لعينة اللحم المعالجة بـ GnZnONPsانخفاضًا كبيرًا ($p <0.05$)في إجمالي عدد البكتيريا (TBC)حتى 2 ، 5 و 7 أيام من التخزين عند 4 درجات مئوية (1.5 ملغم ). في حين أن أعلى عد بكتيري لوحظ في مجموعة السيطرة (العينات غير المعالجة) بلغ 11.360.04 $CFU.g^{-1}$في نهاية التجربة ، انخفض أيضًا إجمالي عد بكتريا القولون TCC بشكل ملحوظ ($P <0.05$)بعد 5،7 أيام من التخزين عند 4 $C^0$. عند معاملت عينات اللحوم باستخدام GnZnONPsمقارنة بعينةالسيطرة . تظهر النتائج أن معاملة لحوم الدواجن باستخدام GnZNoNPsيطيل العمر التخزيني للحم بتقليل الحمل البكتيري طوال فترة التخزين

## Introduction

Foodborne illnesses attributed to the consumption of poultry meat and its processed products are a significant public health problem around the world. It can be contaminated with a variety of microbes, including those capable of deteriorating the product during refrigerated conditions as well as some foodborne pathogens (1). Microorganisms that can contaminate poultry meat and its products are mainly transferred to humans through eating raw or undercooked chicken meat (2). Several studies have shown that chicken meat consumption is associated with the incidence of outbreaks of foodborne diseases (3). Chicken meat is the most preferred meat because it is an economical and available source of protein with a low fat content and low in cholesterol compared to other meats. , skin of raw chicken meat and cuts directly contact the air and equipment surface, so it is easily contaminated. (4) In order to reduce the economic burden of spoilage of raw poultry meat, methods for extending the shelf life and overall safety/quality of the meat are being researched. This is important because the initial bacterial load in the meat is the primary limitation on shelf life and overall safety/quality of the meat (5). When raw meat is kept refrigerated, it can spoil in two ways: through oxidative rancidity and through microbial growth. (6). Antimicrobial preservatives are ingredients that are used to extend the shelf life of meat by reducing the growth of microorganisms during slaughter, transportation, processing, and storage. Nanoparticles are employed in a variety of ways. Metal oxide nanoparticles are mainly used to reduce food pathogens and prevent food contamination. Nanoparticles having a large surface area and the ability to change their size can be employed in a variety of applications. In the food industry, nanoparticles help to enhance cleanliness, prolong product shelf life, and avoid food-borne illnesses and chemical contamination (7). Food processing and preservation ( nanocomposites, nanocoatings, nanosensors, edible coating NPs, etc.) and food packaging (nanocoatings, nanosensors, nanocomposites, etc.) are examples of NP applications in the food industry ( 8 ). Plant extract-based green synthesis of NPs has several advantages: it is non-pathogenic, cost-effective, creates a large number of metabolites, and is environmentally friendly. Ginger (Zingiber officinale) underground rhizomes are the medicinally useful part (9 ). All Ginger's major active ingredients, such as zingerone, gingerol, zingiberene, and shogaols, are known to possess antioxidant activities Antimicrobial and antioxidant nanoparticles could be a significant development in the development of antimicrobial therapeutic agents for the prevention and reduction of food pathogenic microorganisms and deterioration ( 10). The goal of this research is to develop an alternative to synthesize and characterize zinc oxide nanoparticles (ZnONPs) using ginger extract and evaluate the antibacterial effects.to extend its shelf life of the fresh chicken meat

## Material and methods

1. Green -synthesis of zinc oxide nanoparticles (ZnONPs)

Green -synthesis of ZnO nanoparticles was preferred by using ethanolic (Zingiber officinale) ginger root extracts 70% as studies by (11). Deionized water was used to prepare 50 mL of 0.01 M Zinc acetate dehydrate (Sigma Aldrich, Germany ) . In a magnetic stirrer, 500 ml of extract solution was slowly added by continuous stirring. 1.0 M sodium hydroxide( Fluka , Germany) was used to maintain pH at 12 , the mixture was stirred for 2 hours until a white precipitate formed, after which it was centrifuged for 10 minutes at 10,000rpm. Pellets were washed in deionized water and dried overnight in a hot air oven at 200°C. The resulting white powder was carefully collected and sent to be characterized. UV spectrum (Schimadzu 1601 spectrophotometer) in 250–900 nm range and

Fourier-transform infrared (FTIR) Shemadzu (Germany) spectrum, to performed the synthesized NPs. By adding a specific quantity of the organic solvent dimethyl sulfoxide (DMSO) to the solution a measured volume of ZnO nanoparticles (0.5,1, and 1.5 g/mL), ZnO nanoparticles were prepared., by allowed the mixture to sonicate for a few minutes

2. Collection and preparation of of meat samples:

A total of 180 fresh meat samples (500 g ) were collected from a local abattoir in Al Diwaniyah city and transported in ice boxes directly to the vetrenary college / public health laboratory. GZno-NPs powder dissolved in solution at the following concentrations (0.5mg/ml, 1mg/ml, and 1.5mg/ml) in deionized water the fresh meat samples divided in Four groups in accordance with ( Elsaid et al., 2019) First, the control group with out treatment. Meat sample dipping in nanomaterial . for I hr at room temperature (25 °C). then drained for 2 minutes before being packaged in polyethylene bags, labeled, and stored at 4 °C in order to evaluate microbiological count TBC Total Bacterial Counts And Total Coliforms Counts (TCC ) during 0, 2, 5, and 7 days of refrigerated storage.

3.Preparation of samples for microbiological analysis :

The samples were prepared in accordance with FDA guidelines for bacteriological analysis (2001). homogenize 25 g of each sample with 225 mL of BPW ) Himedia / India ( solution . The total bacterial count was determined by preparing decimal serial dilutions (10-1 to 10-6) of each meat sample in sterile 0.1 % (wt/v) peptone water and then pouring onto plates of nutritional agar at 45°C, for 24 hr. each dilution was done in 3 triplicate. While total count of coliforms was estimated by serial dilutions (10-1 to 10-6) of each meat sample in sterile peptone water 0.1. Pour plated in violet red bile agar ( Himedia / India) for each dilution incubated for 48hr then Multiplication of the average number of bacterial colonies by reciprocal dilution (cfu/ml).

Statistical analysis:

The significance of the differences in mean values has to be determined. As a result of this research, two-way ANOVA was used to statistically evaluate the acquired findings using the SPSS program (SPSS 19.0, Chicago, IL, USA). The post-hoc Duncan's test was used. The data was provided as Mean ±Standard Error (SE) (13).

## Results and Discussion:-

1. Gn-ZnONPs Characterization :

a- Fourier-transform infrared Shemadzu (Germany) spectrum (FTIR): was used to investigated the functional groups of biomolecules in the ginger extract which are responsible for ZnO nanoparticle reduction and stabilization. Figure (1) The peaks at (3430) cm 1, (415) cm1, 1516 cm 1, 1384 cm 1, and 872 cm-1 correspond to N–H(Amine) or amide groups, Zn–O bond, and C=O stretch, indicating ketones. Respectively. (14) ( 15) When making Zn2 oxid nanoparticles, it is preferable to use natural stabilizers instead of artificial or chemical ones., Nanoparticlesize,shape, and morphology are significantly influenced by the phytochemicals in the plant extract. Consequently, the nanoparticles are extremely stable and have a narrow particle size distribution (16 ) .

b- UV-visible absorption spectrum : The synthesis of ZnO NPs was confirmed by( Schimadzu 1601 spectrophotometer) in 250–900 nm range a significant absorption band with a maximum wavelength at 370 nm was indicated (fig 2). This sharp peak confirm that the particles are in Nano sized, according to the spectrum which is identical to results has been reported for ZnO NPs (17).

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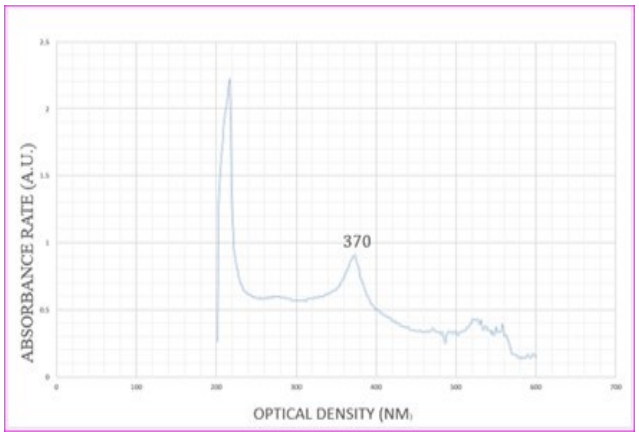


Figure (1): UV-Vis spectrophotometer of green synthesis GnZnO NPs showing characteristic peak (at 370 nm).

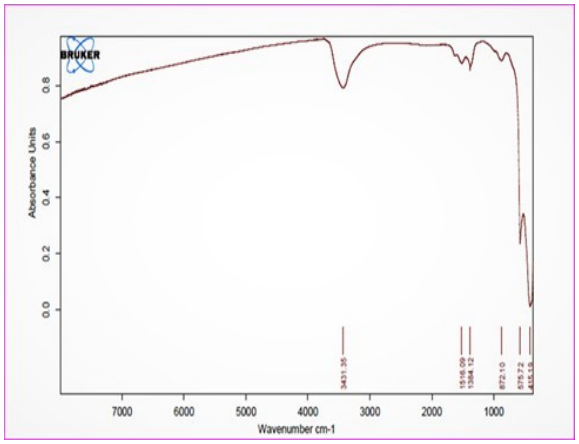


Figure (2 ): FTIR spectrum of ZnO-NPs, showing successful green synthesis of ZnO nanoparticles influenced by the phytochemicals in the plant extract.

c- Total bacterial count ( TBC)

The most important factors in meat shelf life according to past research, (18 ) estimations are measurements of microbial growth. Our Results of microbiological analysis showed in table(1) and figure (3 ) The initial mean of total bacterial count for ( control group) untreated meat samples was observed to be 3.95±0.02 CFU.g-1 these value was increased significantly ($P \leq 0.05$) during day of storage, the result showed significantly highest count recorded in control (untreated samples ) reaching 7.93±0.02 CFU.g-1 and 11.36±0.04 CFU.g-1 after storage for 5 day and 7 day respectively. While the sample treated with GnZnONPs shown a significant ($p \leq 0.05$).

Table ( 1 ) Total bacterial count ( TBC) of raw chicken meat (log10 CFU.g-1 of sample) treated with different concentrations GnZnONPs during 7 days of refrigerated storage

| Period Group | Zero time | 2 days | 5 days | 7 days |
|---|---|---|---|---|
| Control | D3.95 ±0.02a | C5.45 ±0.03a | B7.93 ±0.02a | A11.36±0.04 a |
| T1 | D3.91 ±0.02a | C4.32 ±0.01b | B5.76 ±0.01b | A 7.28± 0.01b |
| T2 | D3.90 ±0.01a | C4.24 ±0.06c | B5.04 ±0.03c | A6.84 ±0.05c |
| T3 | D3.90 ±0.01a | C4.08 ±0.05d | B4.29 ±0.01d | A5.08 ±0.01d |
| LSD | 0.0627 | | | |

Means with a different small letter in the same column are significantly different (P< 0.05), Means with a different capital letter in the same row are significantly different (P< 0.05) Mean ±SE= Mean ±Standard Error control ( un treated ) , T1 = (0.5 mg/ml ZnO NPs) T2= 1 mg/ml ZnONPs) (T3=1.5 mg/ml ZnONPs)

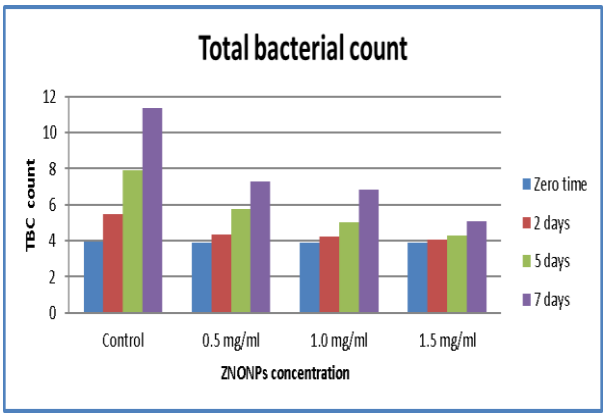


Figure (3 ): Total bacterial count of chicken meat samples treated with various GnZnONPs concentrations at zero, 2, 5, and 7 days of refrigerator storage

reduction on total bacterial count after 2, 5, and 7 days of storage at 4°C., it was observed the mean of TBC was the lowest recorded in sample treated with T3( 1.5 mg) 5.08±0.01 CFU.g-1 At 7 day 0f storage although there was decreases in TBC in sample treated with T1 7.28±0.01 CFU.g-1. And T2 6.84±0.05 CFU.g-1. In7 days of storage While The effect of storage periods on all groups was significant (P<0.05), the count increase gradually with the increase of storage periods, the highest count recorded in the last periods at7 days of storage in all groups, and the lowest count recorded in the first periods zero days of storage in all groups. However, there is a significant difference (P<0.05) between T1 ,T2 and T3 through the storage periods the highest significantly decrease (p<0.05) in sample treated with T3 observed result of the total bacterial counts after 7 days of refrigerated storage at 4 0C were affected significantly (p< 0.05) by treated the meat samples GnZnONPs compared with control . nanoparticles play important role in bacterial inhibition in samples treated with ZnONPs compared with samples without treated , these results agreed with mentioned by (19 ) that indicated during a study bio synthesis ZnO NPs exhibited strong antimicrobial activity against food-borne bacteria. also maintained the organoleptic features of chicken fillet during storage Also, agreement with mentioned by (20) that indicate by study extending the shelf-life of poultry breast meat packaged by nanocomposite films with ZnO NPs storage at 4◦C.

d-Coliform Bacterial Count CBC :-

The inhibition effect of GnZnONPs in the Coliform Count of chicken meat samples was illustrated in table (2)and figure (4 ) which observed a Significant different in the mean of Coliform Count during storage days between the treated sample with GnZnONPs and untreated ( control ) the initial total coliform count for meat samples was observed in zero day to 3.59±0.02 ,3.67±0.02 ,3.65±0.02 ,3.62±0.03 CFU.g-1 for control and T1,T2,T3 These values significantly increased (P< 0.05) during storage, reaching 6.280.02 CFU.g-1, 4.860.01 CFU.g-1, 4.260.03 CFU.g-1, and 4.060.01 CFU.g-1 after 5 days and 9.360.04 CFU.g-1, 6.970.02 CFU.g-1, 5.580.03 CFU.g-1 after 7 days of storage for the above treatment. When meat samples were treated at 1.5 mg/ml with GnZnONPs 4.710.01 CFU.g-1, after 7 days of refrigerated storage the total coliform counts were affected significantly (p< 0.05) when compared to the control sample. Although the total coliform count in meat samples treated with 0.5 mg of record 3.950.07, 4.260.03, and 1 mg/ml of GnZnONPs decreased after 5,7 days of storage at 40C, the decrease was significant (p <0.05).Bacterial growth in control and treated samples increased exponentially from the first day of storage, and continued to increase steadily on day 7 of refrigerated storage. Microbial proliferation was generally slower in NP-treated samples than in controls than in the logarithmic phase, whereas NPs extended the lag phase in meat-treated samples. NPs have been shown to have antimicrobial activity against a variety of food spoilage and pathogenic microorganisms (21). During storage periods. Zn+2 ions are thought to affect cell viability (22) by penetrating microorganism cell walls, reacting with interior cell compounds, and thus affecting cell viability (23). According to several studies, antibacterial activity of ZnO-NPs was directly related to their concentration; therefore, larger surface area and higher concentration are responsible for ZnO-NPs antibacterial activity (24). Our findings were similar to those reported by (25), (26) and (27) when uses ZNONPs as antibacterial growth , FDA( Food and Drug Administration) - approved zinc compounds can be used in food production because they have been presumed safe for human consumption by the organization (28) . Further study in this area is needed in the future.

Table (2) Coliform Bacterial Count ( CBC CFU.g-1 ) of raw chicken meat treated with different concentrations GZnO-NPs during 7 days of refrigerated storage

| Period<br>Group | Zero time | 2 days | 5 days | 7 days |
|---|---|---|---|---|
| Control | D3.59± 0.02a | C5.23± 0.05a | B6.28± 0.02a | A9.36± 0.04a |
| T1 | D3.67± 0.02a | C4.15± 0.02b | B4.86± 0.01b | A6.97± 0.02b |
| T2 | D3.65± 0.02a | C3.95± 0.07c | B4.26± 0.03c | A5.58± 0.03c |
| T3 | D3.62± 0.03a | C3.85± 0.02d | B4.06± 0.01d | A4.71± 0.01d |
| LSD | 0.0667 | | | |

Means with a different small letter in the same column are significantly different (P< 0.05) Means with a different capital letter in the same row are significantly different (P< 0.05) Mean ±SE= Mean ±Standard Error control ( un treated ) , T1 = (0.5 mg/ml ZnO NPs) T2= 1 mg/ml ZnONPs) (T3=1.5 mg/ml ZnONPs)

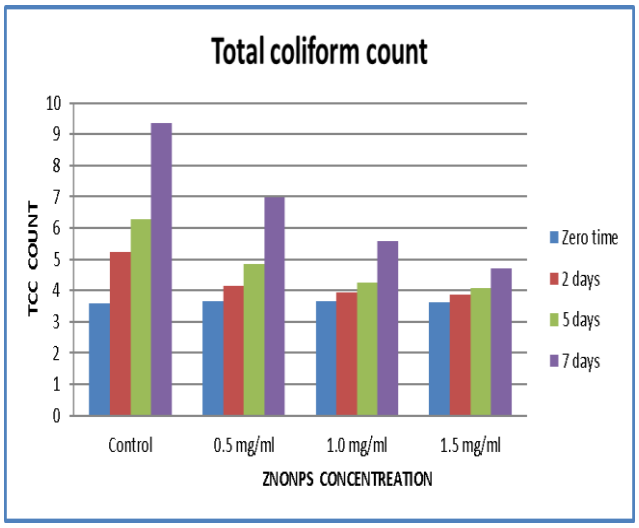


Figure ( 4 ): Total coliform count of chicken meat samples treated with various GZnONPs concentrations during zero, 2, 5, and 7 days of refrigerator storage.

## Conclusion

Nanotechnology is a novel application that has been deployed in the meat production . The antibacterial effect of green synthesis ZnO-NPs is concentration dependent . Bacterial count was considerably (P< 0.05) lower in samples treated with ZnO NPs than in controls. It demonstrates that ZnO NPs' has potent antibacterial action. The use of nanoparticles in the meat industry has effectively improved meat quality and safety. The US Food and Drug Administration has categorized zinc compounds as a generally recognized as safe (GRAS) substance, allowing them to be utilized in the food sector. More study should be conducted in this area in the future.

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