Effect of zinc oxide Nano particals treatment on the preservation of broiler chicken meat

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Abstract
Because to the biological makeup of fresh meat, it is a product that must be consumed quickly. Meat's shelf life and freshness are influenced by a variety of interconnected elements, including maintaining temperature, ambient oxygen (O2), internal enzymes, moisture, light, and—most importantly—microbes.In the current study, a total of (100) sample of broiler breast samples was collected from different market centers in Basra city to analyzed. Preparation of the zinc nanoparticles (ZNO-NPs) from Pomegranate peels extract to extend shelf life of chicken meat held under refrigerated storage at 4 ° C. This study evaluated the antibacterial effectiveness of synthetic zinc oxide nanoparticles made using pomegranate aqueous extract. This work studied the effects of zinc oxide nano-particles on meat preservation, broiler breast samples were treated with solutions containing different concentration 0.4, 0.6 and 0.8 of zinc oxide nanoparticle solution and were kept for 1, 3 and 6 days. Bacterial count, oragano leptic characteristics, physical characteristics and chemical properties were determined using standard methods.
Keywords: ZnO nanoparticles - broiler breast and Microbial Enumeration

1. Introduction

The main cause of the spoilage of chilled chicken flesh is microbial development (Rukchon, et al 2014). For the continuation and growth of microorganisms during processing, distribution, and storage, chicken meat is an excellent medium due to the availability of proteins, free amino acids, lipids, vitamins, mineral salts, and moisture. Bacterial metabolism leads to the production of metabolites, which change the physical, chemical, and sensory properties of chicken flesh (Dave & Ghaly, 2011).
Several techniques are applied to extend the shelf life of food products. However, risk of recontamination may occur. One of the main problems facing the food business is microbial contamination of foods, which has consequences for public health due to foodborne diseases and waste of ruined goods. (Carbone et al, 2016). Nanotechnology has recently seen a considerable increase in the fields in which it is used, including the food sector. Nanotechnology has emerged using nanomaterials in order to improve growth and production in economic plants, as demonstrated by many applied studies(Hadi & Abass,2021; Yang, Fan, et al,2006).
As a result of their small mass and high surface-to-mass ratio, nanoparticles possess special properties. Nanoparticles have been created using a variety of metallic nanoparticles, including zincoxide. Nanotechnology increases the shelf life of different foods and decreases food waste due to microbial contamination (Pradhan et al, 2015).ZnO is used by the food industry as a source of zinc, a vital vitamin that is crucial for human and animal growth, development, and wellbeing (Shi et al, 2008; Saleh 2018).
ZnO has a wide range of uses, including those in the ceramics, glass, rubber, food, cosmetics, pharmaceutical, and commodity chemical sectors. As reported by Özgür et al, (2005).
Zinc oxide (ZnO) has strong bioactivity for a variety of microorganisms, is chemically stable, and is nearly nontoxic to humans (Sirelkhatim et al, 2015).

2. Materials and Methods

Sample collection and storage conditions

Raw chicken chest samples were collected from retailer market of Basrah province. They were kept in a sterile polythene bag sealed to prevent contamination. The samples were then delivered to the public health laboratory, College of Veterinary Medicine, University of Basrah within 1h of purchase under chilled conditions and stored at 4 °C. Samples were then analysed for 1, 3, and 6 days of storage.

Synthesizes Zno NPs

Preparations of aqueous pomegranate extract

The Preparation of aqueous pomegranate extracts was done using the method as described previously (Loo et al, 2012; Al-Hashimi et al,2018). Briefly, 1 kg of pomegranate was weighed, washed with distilled water, dried at room temperature, cut in small pieces, and grounded using blinder. Then, the pomegranate powder (10 gram) was weighed and mixed with 100 ml of distilled water at room temperature The solution was heated to boiling point, maintained at that temperature for five minutes, and allowed to cool for two hours. After cooling, the solution was centrifuged at 4000 rpm to separate the liquid from the solid particles. The liquid was then filtered again using mesh fabrics to get rid of any remaining particles, yielding pure liquid

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extracts..The extracts thus prepared were used immediately.

Synthesis of zinc nanoparticles

6.58 g of zinc nitrate was melted in 300 mL of double-distilled water to create the 0.1 M zinc nitrate hexahydrate solution (Zn (NO3)26H2O). To achieve complex formation, ten milliliters of th The aqueous pomegranate extracts were gradually included into the mixture dropwise. e aqueous pomegranate extracts were gradually added dropwise into the solution while being stirred magnetically at 60 °C for around two hours. After stirring, the complex was collected and kept in the dark for 72 hours. Following 72 hours of observation of color changes from reddish brown to dark brown, the pellets were collected after being centrifuged at 10,000 rpm for 10 minutes. The separated pellets were kept in airtight vials for future research after being dehydrated in an oven at 80 C for 8 hours. As opposed to (Umar et al, 2018). noticed a shift in color from brown to dark brown.

3. Statistical Analysis

Two-way analysis of variance was used to statistically examine all the gathered data (ANOVA)using SPSS package (SPSS version22). Significant P values ($P < 0.05$) were applied to analyze the obtained results. Data were expressed as means ± standard deviation (SD).

4. Results

Microbial Enumeration

Total bacterial count

Investigations were conducted on the total bacterial count of raw chicken meat samples without or with ZnONPs treatment at different doses and times during refrigerated storage (Table 1). During the storage period, the mean total bacterial count for untreated beef samples (control group) steadily increased ($P \leq 0.05$).In contrast, a raw chicken meat sample treated with ZnONPs showed a significantly lower total bacterial count ($P \leq 0.05$)at various concentrations when stored in the refrigerator as compared to an untreated meat sample. At various stages of storage, the amount of microbiological multiplying in samples treated with ZnONPs was typically lower than that of the untreated meat samples (control group).

Table(1) : Microbial count of untreated (control) and zinc nanoparticle-treated chicken meats stored aerobically at 4°C

Storage time(days)	Untreated and zinc oxide Nanoparticle-Treated Group			
Aerobic mesophilic count	0%	0.4%	0.6%	0.8%
1	$^{A}2.5*10^{4a}$	$^{A}2.5*10^{3b}$	$^{A}2.5*10^{2c}$	$^{A}2.5*10^{d}$
3	$^{B}2.5*10^{5a}$	$^{B}2.5*10^{4b}$	$^{B}2.5*10^{3c}$	$^{B}2.5*10^{2d}$
6	$^{C}2.5*10^{6a}$	$^{C}2.5*10^{5b}$	$^{C}2.5*10^{4c}$	$^{C}2.5*10^{3d}$
Psychotropic count				
1	$^{A}1.5*10^{5a}$	$^{A}1.5*10^{4b}$	$^{A}1.5*10^{3c}$	$^{A}1.5*10^{2d}$
3	$^{B}2*10^{5a}$	$^{B}2*10^{4b}$	$^{B}2*10^{3c}$	$^{B}2*10^{2d}$
6	$^{C}2.5*10^{6a}$	$^{C}2.5*10^{5b}$	$^{C}2.5*10^{4c}$	$^{C}2.5*10^{3d}$
Coliform count				
1	$^{A}2.5*10^{5a}$	$^{A}2.5*10^{4b}$	$^{A}2.5*10^{3c}$	$^{A}2.5*10^{2d}$
3	$^{B}2.5*10^{6a}$	$^{B}2.5*10^{5b}$	$^{B}2.5*10^{4c}$	$^{B}2.5*10^{3d}$
6	$^{C}2.5*10^{7a}$	$^{C}2.5*10^{6b}$	$^{C}2.5*10^{5c}$	$^{C}2.5*10^{4d}$
Staphylococcal count				
1	$^{A}1.5*10^{4a}$	$^{A}1.5*10^{3b}$	$^{A}1.5*10^{2c}$	$^{A}1.5*10^{d}$
3	$^{B}2*10^{5a}$	$^{B}2*10^{4b}$	$^{B}2*10^{3c}$	$^{B}2*10^{2d}$
6	$^{C}2.5*10^{6a}$	$^{C}2.5*10^{5b}$	$^{C}2.5*10^{4c}$	$^{C}2.5*10^{3d}$

Results are expressed as mean colony forming units per 1 gram. Means with a different capital letter in the same column are significantly different ($P<0.05$). Means with a different small letter in the same row are significantly different ($P<0.05$).

Organoleptic characteristics of untreated and zinc nanoparticle-treated samples

The organoleptic values showed a significant decrease($p<0.05$) in the value according to the time storage(1,3and6)in treated and non-treated groups with zinc oxide nanoparticles and also a significant difference in this value between non-treated and treated groups with zinc oxide nanoparticles and non-significant difference among doses of treated groups.

Effects of nanoparticle solution on physical traits of untreated and zinc nanoparticle-treated samples (Water-holding capacity (WHC), and PH level) were studied (Table3). The Water-holding capacity (WHCAs seen in a table (3), the percentage of chicken flesh samples (control) drastically dropped over time,The PH level showed asignificant in increase ($p<0.05$) according to the time storage(1,3,and 6) in treated and non-treated groups with zinc oxide nanoparticles and also asignificant decrease($p<0.05$) in PH level among non-treated and treated with0.4,0.6 and 0.6% with zinc oxide nanoparticles.

Table (2) : Organoleptic values of untreated (control group) and zinc oxide nanoparticle-treated chicken meats stored aerobically at 4°C.				
Storage time(days)	Untreated and Zinc Oxide Nanoparticle-Treated Group			
colour	0%	0.4%	0.6%	0.8%
1	$^{A}8.50 \pm 0.11^{a}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$
3	$^{B}5.50 \pm 0.11^{a}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 0.86^{b}$
6	$^{C}3.50 \pm 0.11^{a}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$
Tenderness				
1	$^{A}8.50 \pm 0.11^{a}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$
3	$^{B}5.55 \pm 0.13^{a}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 0.86^{b}$
6	$^{C}3.30 \pm 0.17^{a}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$
Juiciness				
1	$^{A}8.50 \pm 0.11^{a}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$
3	$^{B}5.55 \pm 0.13^{a}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 0.86^{b}$
6	$^{C}3.30 \pm 0.17^{a}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$
aroma				
1	$^{A}8.50 \pm 0.11^{a}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$	$^{A}8.75 \pm 0.57^{b}$
3	$^{B}5.50 \pm 0.11^{a}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 086^{b}$	$^{B}8.63 \pm 0.86^{b}$
6	$^{C}3.50 \pm 0.11^{a}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$	$^{C}8.50 \pm 0.11^{b}$

The results are expressed as mean± standard error. Means with a different capital letter in the similar column are considerably different (P<0.05). Means with a different small letter in the similar row are considerably different (P<0.05).

Physical characteristics of untreated and zinc nanoparticle-treated samples

Physical characteristics

Table (3): Physical values of untreated (control group) and zinc oxide nanoparticle-treated chicken meats stored aerobically at 4°C.				
Storage time(days)	Untreated and Zinc oxide Nanoparticle-Treated Group			
Water-holding capacity (WHC)	0%	0.4%	0.6%	0.8%
1	A0.34±0.01a	A0.36±0.004b	A0.38±0.01c	A0.40±0.01d
3	B0.33±0.01a	B0.35±0.01b	B0.37±0.003c	B0.39±0.01d
6	C0.35±0.01a	C0.37±0.003b	C0.39±0.01c	C0.41±0.01d
PH level				
1	A5.81±0.002a	A5.80±0.002b	A5.79±0.01c	A5.78±0.01d
3	B6.05±0.002a	B5.99±0.03b	B5.98±0.06c	B5.96±0.09d
6	C6.40±0.002a	C6.07±0.01b	C6.06±0.01c	C5.99±0.07d

Results are expressed as mean± standard error. Means with a different capital letter in the same column are considerably different (P<0.05). Means with a different small letter in the similar row are considerably different (P<0.05).

4.5. Chemical characteristics of untreated and zinc nanoparticle-treated samples

Impacts of the nanoparticle solution on chemical properties of untreated and zinc nanoparticle-treated samples (protein, fat, and ash) were studied in (Table4).

The meat samples' ash, lipid, and protein composition were significantly or not significantly impacted by the zinc nanoparticle concentration (P < 0.05).

Table (4): Chemical contents of untreated (control) and zinc nanoparticle-treated chicken meats stored aerobically at 4°C				
Storage time(days)	Untreated and Zinc Oxide Nanoparticle-Treated Group			
Fat content	0%	0.4%	0.6%	0.8%
1	$^{A}4.42\pm0.004^{a}$	$^{A}3.51\pm0.004^{b}$	$^{A}3.50\pm0.005^{c}$	$^{A}3.49\pm0.005^{d}$
3	$^{B}3.08\pm0.003^{a}$	$^{B}2.65\pm0.004^{b}$	$^{B}2.64\pm0.005^{c}$	$^{B}2.63\pm0.006^{d}$
6	$^{C}2.59\pm0.002^{a}$	$^{C}1.77\pm0.003^{b}$	$^{C}1.76\pm0.004^{c}$	$^{C}1.75\pm0.004^{d}$
Protein content				
1	$^{A}29.36\pm0.004^{a}$	$^{A}29.00\pm0.002^{b}$	$^{A}28.9\pm0.01^{c}$	$^{A}28.8\pm0.02^{d}$
3	$^{B}29.1\pm0.004^{a}$	$^{B}28.9\pm0.01^{b}$	$^{B}28.8\pm0.02^{c}$	$^{B}28.7\pm0.02^{d}$
6	$^{C}28.1\pm0.02^{a}$	$^{C}28.8\pm0.02^{b}$	$^{C}28.7\pm0.02^{c}$	$^{C}28.6\pm0.01^{d}$
Ash content				
1	$^{A}8.5\pm0.02^{a}$	$^{A}8.6\pm0.05^{b}$	$^{A}8.7\pm0.04^{c}$	$^{A}8.8\pm0.03^{d}$
3	$^{B}8.2\pm0.02^{a}$	$^{B}8.3\pm0.02^{b}$	$^{B}8.4\pm0.06^{c}$	$^{B}8.5\pm0.05^{d}$
6	$^{C}8.1\pm0.02^{a}$	$^{C}8.2\pm0.03^{b}$	$^{C}8.3\pm0.02^{c}$	$^{C}8.4\pm0.05^{d}$

The results are expressed as mean± standard error. Means with a different capital letter in the same column are significantly different (P<0.05). Means with a different small letter in the similar row are considerably different (P<0.05).

5. Discussion

Total bacterial count

In general, moist, nutrient-rich, pH-neutral, warm, oxygen-rich environments are the ones where microorganisms reproduce the fastest (1) The nanoparticles are crucial in the suppression of microorganisms. Total bacterial counts (TBC) were much decreased in treated samples with ZnONPs

than in samples that weren't, and these findings were in line with those reported by Amjadi et al. (2019), who found that ZnO NPs had potent antibacterial properties against germs that cause food poisoning. It shows that ZnO NPs have high antibacterial action. It is hypothesized that the Zn+2 ions enter the microorganisms' cell walls, react with the chemicals inside the cells, and then alter the vitality of the cells (Tayel et al, 2011). It helped keep meat's quality high while it was being stored. It is well known that NPs have antibacterial activity against a variety of pathogenic and food-spoiled microorganisms with (Sagoo, Board, and Roller, 2002). Depending on the concentration, ZnO NPs could significantly reduce Staph.aureus count in broiler breast fillet samples. Our result is similar to those reported by (Etman, 2015; El-Fiky, 2014) who studied the effect of ZnO NPs on L. monocytogenes in ready to eat meat products and fresh minced meat, respectively. Finally, (Mirhosseini and Arjmand, 2014) proved the activity of ZnO NPs with acetic acid on Staph. aureus in mutton meat.

Organoleptic characteristics of untreated and zinc nanoparticle-treated samples

noted that meat samples from groups treated with NPs had good or favorable sensory scores because of the NPs' potential benefits against microbes, which can significantly lessen oxidative effects and increase shelf life (Gharibzahedi Mohammadnabi 2017). Total bacterial counts (Total bacterial counts TBC) in untreated samples of chicken meat (control) considerably increased ($P<0.05$) with advanced storage intervals, which had a detrimental impact on the stability of the features of tenderness, juiciness, color, and scent of control samples, whereas treated samples benefited from a decrease in a distinct microbe count, which may have an impact on panelists' assessments of the meat from treated groups and result in a favorable outcome.

Physical characteristics of untreated and zinc nanoparticle-treated samples

Water-holding capacity (WHC) .

The WHC measured the volume % of the meat that the moisture occupied (Dijkstra, 2004). Meat's protein makeup and pH had an impact on its ability to store water. After slaughter, meat with the highest water retention capacity will result from a high pH. (Gorge, 2000). Chicken meat samples treated with ZnONPs were able to prevent the cellular membrane from rupturing. Although samples treated with ZnONPs demonstrated a higher percentage of WHC than samples control chicken meat as a result of the ability to shield the cellular membrane from rupturing, inhibits proteolysis, prevents water from escaping, and keeps attached to the protein as a result of the nanoparticles' effect inhibition effect against proteolytic bacteria as shown in a table(3), the water-holding capacity (WHC) percentage of chicken flesh samples (control) considerably dropped over.According to the data indicated in (Nauman, 2018), the ability of nanoparticles to lower oxidation states as well as microbial inhibition may have contributed to the reduction of moisture loss during the study. This demonstrated an improvement over control in the preservation of moisture, reduction of loss during cooking, and maintenance of a water holding capacity.compared with control.

The pH value of chicken meat

in table (3), The pH level affects the color, juiciness, and tenderness of the meat, as well as other qualitative characteristics (Pires et al, 2018; Barbut, 2009). Chickens' typical postmortem pH values range from 6.2 to 6.5, whereas their typical final pH standards are approximately 5.8. (Berri et al, 2005). (Van Laack and colleagues, 2000) The treated samples' pH increased from a value of 5.81 to a maximum of 6.40 for control meat, While the pH of untreated samples was frequently higher, Because of this, panelists gave the controller meat a lower rating.

Chemical characteristics of untreated and zinc nanoparticle-treated samples.

In table (4), The zinc oxide nanoparticle concentration increased while the amount of protein and fat dropped. Protein denaturation is defined as changes in protein structures brought on by chemical bond breakdown and subsequent interactions with other elements (Alizadeh& Nomikos, 2009), The reduction in crude protein of the meat samples after treatment with solutions containing zinc oxide nanoparticles may be due to the progressive breakdown of the initial crude protein to more volatile compounds such total volatile bases (TVB), trimethyl amine (TMA), hydrogen sulfide, and ammonia. Additionally, it might be brought on by a decline in proteins that are water- and salt-soluble (Chomnawang et al, 2007) or to could be the result of autolytic degeneration brought on by the operations of bacteria and endogenous enzymes (Hultman andRustard, 2004). According to Olusanya (2011), lipids in the body have protective functions, and several crucial fatty acids generated from fats, such omega-3 fatty acids, are crucial for the healthy operation of various physiological systems (Obidoa et al, 2010). A rise in lipid oxidation is shown by the decrease in fat content. cellular organelles that have ruptured produce oxidative enzymes and pro-oxidants. may be the cause of the decrease in the fat content of the samples treated with zinc oxide nanoparticles (Boon sumrej et al, 2007). According to Akubugwo et al. (2007), Ash concentration is a gauge of the biota's mineral makeup. The As the quantity of zinc oxide nanoparticles rose, the samples' ash contents rose as well. Due to the An increase in ash levels could result from a concentration of soluble solids with relatively low melting points and decreases in other chemical elements (protein and fat).

6. Conclusions

The effectiveness of various zinc oxide nanoparticle concentrations against pathogenic bacterial strains was assessed.. High concentrations of nanoparticles successfully inhibited the growth of bacteria. In future, more research should be focused on the safety evaluation and antimicrobial activity of ZnO NPs,in vivo need to be undertaken pomegranate peel extract is ZnONPs are produced easily, quickly, and simply through biological synthesis using effective reducing and capping agents.The zinc oxide nanoparticles using pomegranate peel extract proved excellent antimicrobial activity. These zinc oxid nanoparticles may be used in food and pharmaceuticals industries.

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