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Research article

Investigation of antimicrobial effects of zinc-based nanoparticles on food-borne pathogens

Sezen Ozcelik*[1]

[1] *Hakkari University, Faculty of Engineering, Department of Food Engineering, 30000, Hakkari, Türkiye*

Abstract

In this study, the antimicrobial effects of three different zinc-based nanoparticles, namely zinc oxide (ZnO), zinc chloride ($ZnCl_2$), and zinc ferrite ($ZnFe_2O_4$), on food-borne pathogen were investigated. ZnO and $ZnCl_2$ nanoparticles were obtained as commercially, but $ZnFe_2O_4$ nanoparticles were produced via sol-gel auto-combustion method. From the XRD results of $ZnFe_2O_4$ nanoparticle, it was found that all the peaks agreed with the literature. However, there was also small amount of the secondary phase peaks corresponding to the ferrite (Fe_2O_3) phases. Significant differences were observed between the inhibition effects of nanoparticles on bacteria in the disc diffusion method (p<0.005), except for the $ZnFe_2O_4$ nanoparticle, which has no effect on bacteria at the used dose. ZnO nanoparticle was observed to have the lowest inhibition zone on the Gram-negative bacterium *Campylobacter jejuni* of inhibition compared to other test bacteria. It was found that $ZnFe_2O_4$ had the highest value of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against Gram-negative bacteria.

Keywords: *Antibacterial activity; food-borne pathogen; nanoparticle; sol-gel; zinc oxide*

1. Introduction

Considered to be a new revolution in the 21st century, nanoscience and nanotechnology are seen at the forefront of modern and new studies (Sebastian and Gimenez, 2016; Al-Byati and Al-Duhaidahawi, 2023). Research and innovations in the field of nanotechnology contribute to many sectors such as medicine, health services, energy, defense industry, agriculture, food, textile, and cosmetics (Bhushan, 2010; Erkoc, 2012; Singh, 2017; Malik et al., 2023; Yalcin, 2022). It is thought that it is very important to produce functional foods with bionanotechnology and nanotechnology, which are advancing at a great pace. Nano-emulsion structures, nanoparticles, biopolymeric nano-composite materials, nanofibers, nanotubes, and nanosensors consisting of macromolecules in the structure of foods can also be used in applications on foods with different purposes. It has been reported that the substances in the structure of foods can have the desired properties at molecular levels. It is possible to develop different and new food products by using the mechanical and sensory properties of the food, and while the protection of the products and the extension of their shelf life are provided in the packaging with nano composites, the safety of the foods will be under control with the help of nanosensors (Tarhan et al., 2010; Ansari, 2023).

As an application area of nanotechnology science in the food industry, various topics such as detection of pathogens in foods, keeping food safety at a high level, development of antimicrobial packaging methods, controlled distribution with the transport of bioactive substances and production of functional foods can be listed (Robinson and Morrison, 2009; Siddiqui et al., 2023; Zhang et al., 2023).

Many different antimicrobial biomaterials are produced that are used in the prevention of diseases caused by infection, in the preservation of the long shelf life of the structures of medicines, food and cosmetics/dermo-cosmetic products, and in the prevention of biofilm formation in medical implants

* Corresponding author.
E-mail address: sezenozcelik@hakkari.edu.tr (S. Ozcelik).
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(Kanematsu and Barry, 2015).

In addition to being primary or secondary metabolite products of plants and animals or microorganisms, substances such as metal or nonmetal compounds, minerals, alloys, and mixtures may also have antibacterial properties. On the other hand, it has been possible to develop antibacterial carrier systems through chemically synthesized or purpose-built polymers, biopolymers, metal or metal oxide nanomaterials, carbon nanotubes, nanoemulsions and nanocrystalline structures (Bueno, 2015). It is known that the demand for high-quality and safe foods is increasing day by day. The presence of pathogens in food products is not desirable and is one of the leading causes of foodborne diseases worldwide (Mshelia et al., 2023). The presence of pathogens such as *Clostridium* spp., *Escherichia coli*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp. are the most important microbial hazards found in food products. Pathogenic bacteria such as *Clostridium* spp., *Yersinia* spp., *Salmonella* spp., *Escherichia coli* and *Staphylococcus aureus* are the causes of numerous diseases related to gastric and intestinal symptoms such as vomiting and diarrhea (Friedman et al., 2002; Demirci et al., 2008; Negrut et al., 2020). Concerns about food safety are observed due to the increase in food-borne diseases caused by pathogenic microorganisms.

In addition, problems arising from chemical preservatives and artificial antibacterials used to inactivate or prevent the growth of pathogenic microorganisms that cause food safety risk are also observed (Turner et al., 2007; Chowdhury et al., 2023). Therefore, the use of natural antibacterials is important to keep microorganisms under control (Tajkarimi et al., 2010; Li et al., 2022). Although the presence and concentration levels of metals in the ecosystem, whose concentrations in the biological system produce toxic effects after a certain level, have been investigated by many scientists, their interaction with nanoparticles, which are increasing day by day, and the toxic effects they cause are a very important subject of research today (Lazar et al., 2022; Morais et al., 2022). Although heavy metals and nanoparticles have very common uses, they can also be found as mixtures in nature. Therefore, it is thought that it is very important to investigate the negative aspects of the pollutants in mixtures on the ecosystem and the lives of living things.

Zinc (Zn), which is an essential trace element for the normal development and growth of living things, is an essential food source for all living organisms and is a heavy metal that has duties in many enzymatic activities (Palmgren et al., 2008; Asad et al., 2023). Zn is used in the automotive industry and galvanizing to make casting molds. Apart from this, $ZnCl_2$ is also known to be used in the textile industry, as an activator in the tire industry, as a disinfectant and in the construction of battery bodies. ZnO structures in nanostructure are one of the favorite research topics of the researchers due to their optical and electrical properties (Chen et al., 2016; Khan et al., 2016; Sutradhar and Saha, 2016; Santhoskumar et al., 2017). ZnO nanoparticles are known to inhibit the growth of different bacterial strains and exert a cytotoxic effect against many cancer cells (Hanley et al., 2008; Nair et al., 2009; Premanathan et al., 2011). ZnO nanoparticle is known to be used as an additive in sunscreens, fertilizer, dyes, toothpastes, food, and food packaging (Baker et al., 2014; Shetty et al., 2015; Zhang et al., 2015; Kuang et al., 2016). Among the spinel ferrite compounds, $ZnFe_2O_4$ is used in soft magnet applications at high frequency values due to its different structures consisting of inverse, normal and mixed spinel structures and their high electromagnetic performance, excellent chemical stability, mechanical hardness, medium saturation magnetism property and their physical properties have been studied extensively (Naseri et al., 2012; Heiba et al., 2022; Badiger et al., 2023; Garg et al., 2023; Joshi et al, 2023). Recently, much research on Zn-based nanoparticles have been performed in the various field (Hatami et al., 2023; Kavitha et al., 2023; Senturk et al., 2023; Yalcin et al., 2023). The aim of this study was to evaluate the antimicrobial activities of different zinc nanoparticles (ZnO, $ZnCl_2$ and $ZnFe_2O_4$) on Gram-negative and positive food-borne pathogens.

2. Materials and methods

2.1. Bacteria and nanomaterials used in the study

Standard bacterial cultures such as *Yersinia enterocolitica* (NCTC 11175), *Listeria monocytogenes* (ATCC 19112), *Campylobacter jejuni* (ATCC 33560), and *Staphylococcus aureus* (ATCC29213) were used as food-borne pathogenic bacteria in the study. In the study, zinc chloride ($ZnCl_2$, Sigma Aldrich 229997) and zinc oxide (ZnO, Sigma Aldrich 209998) nanoparticles were used as stocked up, while $ZnFe_2O_4$ nanoparticles were prepared by sol-gel auto-combustion method (Ergin et al., 2023).

2.2. Analysis of antimicrobial activity

2.2.1. Agar well diffusion method

The levels of the minimum inhibition zone of Zn nanoparticles on food-borne pathogens were evaluated by the well diffusion method (Hwanhlem et al., 2017) using Mueller-Hinton Agar (MHA, Merck 1.05437, Darmstadt, Germany). The food-pathogenic bacteria were standardized to a Mcfarland cell density of 0.5 Mcfarland (10^8 cfu/mL) after 24 h growth of bacteria at 37°C in Nutrient Broth (Merck 1.05443, Darmstadt, Germany). Each bacterial cell culture (100 µL) was infused with a petri dish containing 20 mL of Muller Hilton agar. Three wells of 6 mm were formed in the solid medium. Both wells were inoculated with a 50 µL Zn nanoparticle stock solution. The petri dishes were then incubated at 37°C for 24 hours. After incubation, the zones of inhibition formed around each well were measured in mm with the help of a caliper.

2.2.2. Minimum inhibitory (MIC) and bactericidal concentration (MBC) of nanoparticles

In the study, minimum inhibitory concentration (MIC) was determined according to microdilution method suggested by Clinical and Laboratory Standards Institute (CLSI, 2006) Test microorganisms incubated at 37°C for 24 hours were standardized to 0.5 MacFarland cell density. Mueller-Hinton Broth (MHB, Oxoid, CM0405) was used as the medium. Nanoparticle stock solution was prepared as 50 mg/ml and diluted up to 0.19 mg/ml in sterile tubes. Only tubes containing stock solution or pure culture were evaluated as control group. Other tubes to which MHB was added contained microorganisms and diluted stock solutions used in the study. The tubes used in the study were prepared repeatedly and incubated at 37°C for 24 hours. The bacterial growths to be observed in the tubes were compared with the control group tubes and the tubes with the lowest inhibition in bacterial

proliferation were recorded as the MIC value. In line with the MIC results, Mueller Hinton Agar surface was grafted from tubes without bacterial growth and petri dishes were incubated at 37°C for 24 hours and MBC values were recorded.

2.2.3. Production of zinc ferrite ($ZnFe_2O_4$)

Sol-gel automatic burning method was used in the production of nanostructured zinc ferrite powders. Zinc and iron nitrate compounds, which are used as zinc and iron sources, were obtained from Alfa Aeser company. Zinc nitrate (1:2) and iron nitrate [$Fe(NO_3)_3.9H_2O$] weighed according to the stoichiometric ratio were mixed in 50 ml of pure water at room temperature for about 1 hour until completely dissolved in the magnetic stirrer. Citric acid supplied from Sigma Aldrich company with 99% purity was added to the homogeneous solution as fuel, again according to the stoichiometric ratio, and mixed in pure water in the magnetic stirrer for 30 minutes. After the solution preparation process, the pH of the solution was adjusted to 7 with the help of ammonia. The prepared solution was evaporated and combusted at about 350°C under atmospheric conditions. The nano-ferrite powders obtained in dry form were ground and homogenized for 20 minutes with the help of agat.

2.2.4. Statistical analysis

Statistical analyses were performed using SPSS 180 (SPSS Inc., Chicago, IL.USA). The ANOVA test was used to indicate significant differences, defined as $p<0.005$.

3. Results and discussion

Since the properties of ZnO and $ZnCl_2$ nanoparticles were previously made by the manufacturer (Sigma-Aldrich) and the results were presented, no structural analysis studies have been carried out on these samples. However, prior to investigating the effect of $ZnFe_2O_4$ nanoparticles produced by us, their structural analysis was also examined. Fig. 1. shows the XRD patterns of $ZnFe_2O_4$ nanoparticles. As can be seen from the Fig. 1, the XRD patterns was labelled according to the JCPDS Card No.: 82-1042 and all the peaks agreed with the literature (Sarala et al., 2020; Lakra et al., 2023). However, there was also small amount of the secondary phase peaks corresponding to the Fe_2O_3 phases in the structure. Fig. 2. shows the size and structure of $ZnFe_2O_4$ nanoparticles produced in this study using Transmission Electron Microscopy (TEM). From the TEM images, it is seen that many nanoparticles are agglomerated and spherical in shape. This agglomeration is due to the magnetic nature of the nanoparticles. In addition, particle sizes are distributed on average between 5 nm and 40 nm. This may be due to the inhomogeneous heat dissipation that occurs during the production of nanoparticles. Results obtained were consistent with literature findings (Abbasian and Afarani, 2019).

Table 1 shows the inhibition diameter zone of Zn-based nanoparticles on food-borne Gram-negative and positive pathogenic bacteria. Statistically significant differences were observed on inhibition zones of nanoparticles on pathogenic bacteria ($p<0.05$). $ZnFe_2O_4$ had no antibacterial effect on food-borne pathogenic bacteria at the administered dose. However, among the nanoparticles tested, the $ZnCl_2$ nanoparticle was found to have the highest inhibition zone on bacteria ($p<0.05$). The $ZnCl_2$ particle exhibited the highest zone of inhibition on

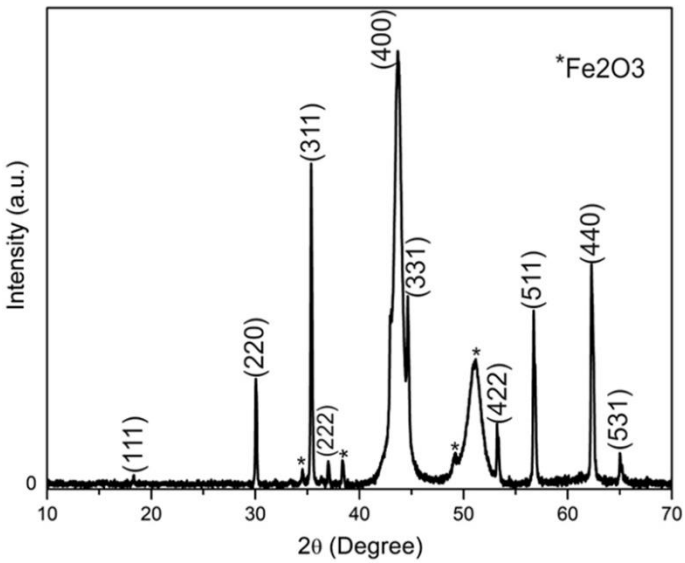

Fig. 1. XRD patterns of $ZnFe_2O_4$ nanoparticles.

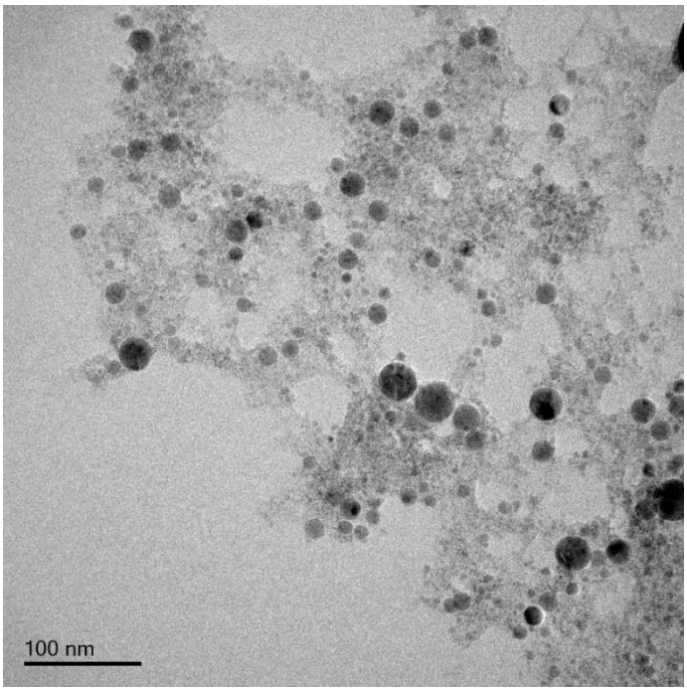

Fig. 2. TEM image of $ZnFe_2O_4$ nanoparticles.

Gram-positive *Listeria monocytogenes* (50 mm) and Gram-negative *Yersinia enterocolitica* (48.75 mm), while it showed statistically similar zone of inhibition (48 mm) on the other test bacteria. ZnO nanoparticle showed a lower zone of inhibition (12.25 mm) on *Campylobacter jejuni* compared to other test bacteria. *Staphylococcus aureus*, *Listeria monocytogenes*, and *Yersinia enterocolitica* had a statistically similar (19.25-19.50 mm) zone of inhibition against ZnO nanoparticles.

In the study, the minimum inhibitory (MIC) and minimum bactericidal concentration (MBC) values of the test microorganisms against nanoparticles are given in Table 2. The MIC value of ZnO nanoparticle on bacteria ranged from 6.25 mg/mL (*Yersinia enterocolitica*) to 25 mg/ml (*Listeria monocytogenes*). ZnO nanoparticle exhibited a similar bacteriostatic effect (12.5 mg/mL) against *Staphylococcus aureus and* Campylobacter *jejuni bacteria*. Gunay et al. (2021) determined the MIC values of ZnO nanoparticles synthesized with *Nosturtium officinale* extract against *Aeromonas hydro-*

Table 1
Minimum inhibition zones of zinc nanoparticles against foodborne Gram-positive and negative pathogenic bacteria.

	ZnO	$ZnCl_2$	$ZnFe_2O_4$
Gram-positive bacteria			
Staphylococcus aureus	19.50±1.29*[a]	48.00±0.82[b]	-
Listeria monocytogenes	19.25±1.26[a]	50.00±0.00[a]	-
Gram-negative bacteria			
Campylobacter jejuni	12.25±1.00[b]	48.00±1.41[b]	-
Yersinia enterocolitica	20.13±1.03[a]	48.75±0.96[ab]	-

*Mean (n=3) ±Standard deviation. There is a significant difference ($p < 0.05$) between the groups in terms of different lettered (a-b) values in the same column.

Table 2
Minimum inhibitory (MIC) and bactericidal concentration (MBC) of zinc nanoparticles against foodborne Gram-positive and negative pathogenic bacteria.

	ZnO		$ZnCl_2$		$ZnFe_2O_4$	
	MIC	MBC	MIC	MBC	MIC	MBC
Gram-positive bacteria						
Staphylococcus aureus	12.5	25	12.5	12.5	12.5	25
Listeria monocytogenes	25	>50	25	25	25	>50
Gram-negative bacteria						
Campylobacter jejuni	12.5	50	6.25	12.5	25	50
Yersinia enterocolitica	6.25	>50	12.5	12.5	25	>50

phila and *Vibrio parahaemolyticus* strains as 15 and 7.5 mg/mL, respectively. In another study, it was determined that the MIC values of ZnO nanoparticles against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* were above 0.13 mg/mL (Erdogan et al., 2019). In this study, Campylobacter jejuni was one of the most sensitive bacteria to the $ZnCl_2$ nanoparticle (6.25 mg/mL), while Listeria monocytogenes was the bacterium with the highest MIC value (25 mg/mL) against the $ZnCl_2$ nanoparticle among the bacteria tested. $ZnFe_2O_4$ nanoparticle showed a MIC value of 25 mg/ml against test microorganisms other than S*taphylococcus aureus*. The bactericidal effect of the nanoparticles on the test organisms was generally 50 mg/ml or more for ZnO and $ZnFe_2O_4$, and 12.5 mg/ml for $ZnCl_2$.

On the other hand, studies with different nanoparticles and different nanoparticle production methods are also available in the literature. Eren and Baran (2019), synthesized silver nanoparticles (AgNP) by green synthesis method using the extract of *Pistacia vera lentiscus* plant and evaluated the antibacterial effect of the synthesized AgNPs in *Staphylococcus aureus* (ATCC 29213) *Escherichia coli* (ATCC 25922), and fungal strains *Candida albicans*. As a result, they stated that silver nanoparticles synthesized by the green synthesis method showed antimicrobial effects. They also reported that AgNPs from the leaves of the *Carica papaya* plant were >25 µg/mL of MIC and MBC against Gram-positive and Gram-negative bacteria in *Luria bertani* broth cultures. Moreover, the effects of the antimicrobial activities of biocompatible gold nanoparticles (AuNP) on food pathogens were evaluated by Baran et al. (2021). They reported that concentrations of 0.056 and 0.025 µg/mL were effective on gram-positive *Staphylococcus aureus* and *Bacillus subtilis*, whilst 0.50 and 0.25µg/mL concentrations were inhibitory effect on Gram-negative bacteria *Pseudemonas aeruginosa* and *Escherichia coli*, respectively.

4. Conclusion

As a result, statistically significant differences were observed on inhibition zones of nanoparticles on pathogenic bacteria ($p<0.05$). $ZnFe_2O_4$ had no antibacterial effect on food-borne pathogenic bacteria at the administered dose. However, the nanoparticle with the highest zone of inhibition on bacteria was identified as $ZnCl_2$.

On the Gram-negative bacterium *Campylobacter jejuni*, ZnO nanoparticle was observed to have the lowest zone of inhibition compared to other test bacteria, while other bacteria were determined to have a similar zone of inhibition against ZnO nanoparticle. It was determined that the minimum inhibitory (MIC) and bactericidal concentrations (MBC) values of zinc nanoparticles used in the study against foodborne Gram-positive bacteria were similar between the groups. The highest value of MIC and MBC against Gram-negative bacteria was found for $ZnFe_2O_4$ nanoparticle and has antibacterial effect. Apart from $ZnFe_2O_4$ nanoparticle, ZnO nanoparticle was determined to have an MBC value of <50 µg/mL against Gram-positive bacteria *Listeria monocytogenes* and Gram-negative bacteria *Yersinia enterocolitica*.

Conflict of interest: The author declares that she has no conflict of interests.

Informed consent: The author declares that this manuscript did not involve human or animal participants and informed consent was not collected.

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