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Antibacterial efficacy of chemically and plant-synthesized zinc oxide nanocomposite against *Staphylococcus aureus* and *Escherichia coli* inoculated in Tilapia fillet

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ABSTRACT

Aims: Edible coatings developed from biodegradable materials such as starch and zinc oxide nanoparticles (ZnO-NPS) are efficient antimicrobials that could be used as a food additive to reduce the bacterial load on the food surface. Therefore, this study was aimed to examine the effect of chemical and green synthesized ZnO-NPS with different concentrations on the survival of *Escherichia coli* and *Staphylococcus aureus* in fish fillets during chilling storage at 4 ± 1 °C.
Methodology and results: ZnO-NPS were chemically prepared by mixing zinc acetate dihydrate with sodium hydroxide. *Lavandula officinalis* was used for the green synthesis of ZnO-NPS. The sterile biodegradable coating containing 2 and 5% of both chemically and green synthesized ZnO-NPS were made using starch, gelatin, xanthan gum and glycerol. Different bacterial cocktail strains of both *E. coli* and *S. aureus* were inoculated onto Tilapia fillet samples. The coating solution with different antimicrobials was aseptically spread in Tilapia fillets and examined periodically within two days intervals for the survival of *S. aureus* and *E. coli* during chilling at 4 ± 1 °C. Both chemically and plant-synthesized ZnO-NPS reduced the growth of both *S. aureus* and *E. coli* by about 3.7 $\log_{10}$ CFU/cm^2 of Tilapia fillet. The incorporation of *L. officinalis* increased the antibacterial activity of ZnO-NPS. *Staphylococcus aureus* was more sensitive than *E. coli* for both chemically and plant-synthesized ZnO-NPS. Moreover, zinc oxide biodegradable coating extended the shelf-life of chilled Tilapia fillets by about 4 days.
Conclusion, significance and impact of study: The results of the current study demonstrated the incorporation of *L. officinalis* into ZnO-NPS biodegradable coating which may be promising in reducing microbial growth on food surfaces.

Keywords: Fish fillets, food additive, Seafood, starch, zinc oxide nanoparticles

INTRODUCTION

Seafood is a significant source of human nutrition because they are rich in polyunsaturated fatty acids (PUFAs) and protein (Ozogul *et al*., 2006; Kykkidou *et al*., 2009). Despite their nutritional value, seafood are susceptible to oxidation due to their high content of polyunsaturated fatty acids, volatile nitrogen bases and higher final pH; therefore, they have a short shelf-life (Mexis *et al*., 2009).

Although there are several improved facilities and implementation of effective process control measures such as the Hazard Analysis Critical Control Point system by seafood companies, the number of seafood-related foodborne illnesses is still increasing (Dehghani et *al*., 2018).

Microbial growth on the product surface is the leading cause of spoilage of many kinds of seafood, with nearly 600 million people becoming ill after consuming contaminated food, resulting in 420,000 deaths every year. Due to such foodborne pathogenic microorganisms, the scientific community has focused research on materials for active and intelligent packaging (IP) specially designed to prevent the growth of microbes in foods and maintain their quality, safety and freshness (Nopwinyuwong *et al*., 2010; Hoseinnejad *et al*., 2018).

An antimicrobial agent is a chemical compound that could be incorporated into the coating material to prevent microbial growth. It could be divided into three major groups: chemical agents, natural spices and probiotics (Han, 2005).

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Edible coatings are useful substances produced mainly from edible biopolymers and food additives. Most biopolymers are natural substances, including proteins, polysaccharides and lipids (Gennadios *et al*., 1997). They can decrease the microbial growth in food products by reducing the diffusion rate of antimicrobial agents from coating materials into the food, so a high concentration of antimicrobial agents will be on the surface of the food product for a long time (Appendini and Hotchkiss, 2002).

Nanomaterials or nanostructure materials are particles with a nano-scale structure ranging from 1-100 nm (Chandra, 2016). This acquires them several properties that weren't present when the materials were in their original size (Bajpai *et al*., 2018). Union of nanostructure materials with other biomolecules, polymers and other nanostructure materials or existing in the aggregate form can result in larger particle size (>100 nm), leading to the formation of nanocomposite material (Koch *et al*., 2010).

ZnO-NPS is reported as safe material that has wide antimicrobial activities. Using zinc in low doses (less than 100 mg/day) is relatively non-toxic to humans and animals (Salama *et al*., 2018), so it could be used in the food industry as an additive. Moreover, it is considered one of the most important metal oxide nanoparticles (Smijs and Pavel, 2011) due to its excellent physical and chemical properties (Ruszkiewicz *et al*., 2017). Nanoparticles (NPs) are produced by different chemical, physical and biological methods (El-Belely *et al*., 2021). Green synthesis of NPs could be done by using several biological methods involving plants, bacteria, fungi (actinomycetes) and or macroalgae (Salem and Fouda, 2021). Plant-mediated biological synthesis of nanoparticles has become important due to its simplicity, photocatalytic activity and eco-friendly (Elumalai *et al*., 2015; Moosa *et al*., 2015). Recently, several NPs have been synthesized by green nanotechnology approaches such as ZnO, Ag, Cu, CuO, Au, Se and others which can be used in different biological activities (Collenburg *et al*., 2017). Using plants in green synthesis of nanoparticles has significant advantages over other biological systems as the plants are safe to handle and available, and the nanoparticles synthesized by using plant extracts are more stable (Iravani, 2011). *Lavandula* leaf extract has great antimicrobial activity against both Gram-positive and Gram-negative bacteria such as *S. aureu*s and *E. coli* (Jianu *et al*., 2013).

Several approaches are used in ZnO-NPS syntheses, such as chemical precipitation (Rao *et al*., 2011), simple solution-based (Song *et al.*, 2005), sol-gel (Akbar *et al.*, 2004) and solvo-thermal methods (Liu *et al*., 2013); however, electrochemical and photochemical reduction techniques are more widely used (Wang *et al*., 2012). The chemical precipitation method of zinc oxide nanoparticles is one of the most important techniques which can be performed by using several precursors and different conditions of time, temperature and concentration of reactants (Sabir *et al*., 2014).

ZnO-NPS, prepared by chemical or green synthesis methods, has been examined against the survival of different microbes in fish products. However, the incorporation of *L. officinalis* is still not further investigated. Therefore, the main objective of the current study was to examine the effect of chemical and green synthesized ZnO-NPS with different concentrations on the survival of *E. coli* and *S. aureus* in fish fillets during chilling storage at 4 ± 1 °C.

MATERIALS AND METHODS

Materials

Corn starch and *L. officinalis* dried plants were obtained from the local market in Beni-Suef city. Glycerol, zinc acetate dihydrate, sodium hydroxide and gelatin were obtained from Sigma (St. Louis, MO. USA). Xanthan gum, Muller Hinton broth (MHB), Muller Hinton agar (MHA), Buffered Peptone water, Sorbitol MacCkonkey agar (SMAC), Baird Parker agar (BP) and egg yolk tellurite emulsion were obtained from Oxoid (Hampshire, UK).

Bacterial strains

Different bacterial cocktail strains of both *E. coli* (E.O114, E.O25 and E.O44) and *S. aureus* (S23, S26, S40) were obtained from the Microbiology Lab., Faculty of Veterinary Medicine, Beni-Suef University. The strains were confirmed by PCR at Animal Health Research Institute, Dokki, Giza, Egypt .The strains were transferred to Muller Hinton broth (MHB) medium and incubated at 37 °C for 18 h, loopfuls from stock culture were transferred to Muller Hinton agar (MHA) medium and incubated at 37 °C for 18 h, then stored in Muller Hinton agar (MHA) slopes at 4 °C after incubation at 37 °C for 18 h for further use throughout the study.

Preparation of plant extract

The aqueous extract of *L. officinalis* was prepared according to the method described by Yedurkar *et al*. (2016) with slight modifications. Briefly, dried leaves of *L. officinalis* were finely chopped using a food chopper (Moulinex La Moulinette Chopper, France) into small pieces. Ten grams of the chopped plant were heated with 100 mL of distilled water at 60 °C for about 20 min till the aqueous solution changed to light brown color. The extract was cooled to room temperature and filtered with Whatman No.1 filter paper. Then it was stored in a refrigerator at 4 ± 1 °C to be used for further experiments during the study.

Synthesis of zinc oxide nanoparticles (ZnO-NPS)

Chemical synthesis of ZnO-NPS was carried out according to Osman and Mustafa (2015) with some modifications. Fifty mL of (0.1 M) of zinc acetate dihydrate ($Zn(CH_3COO)_2.2H_2O$) was mixed with fifty mL of (4%) sodium hydroxide (NaOH) that was dissolved separately in deionized water. Zinc acetate dihydrate was added drop by drop to NaOH solution with continuous stirring at room temperature; the solution was filtered and then dried

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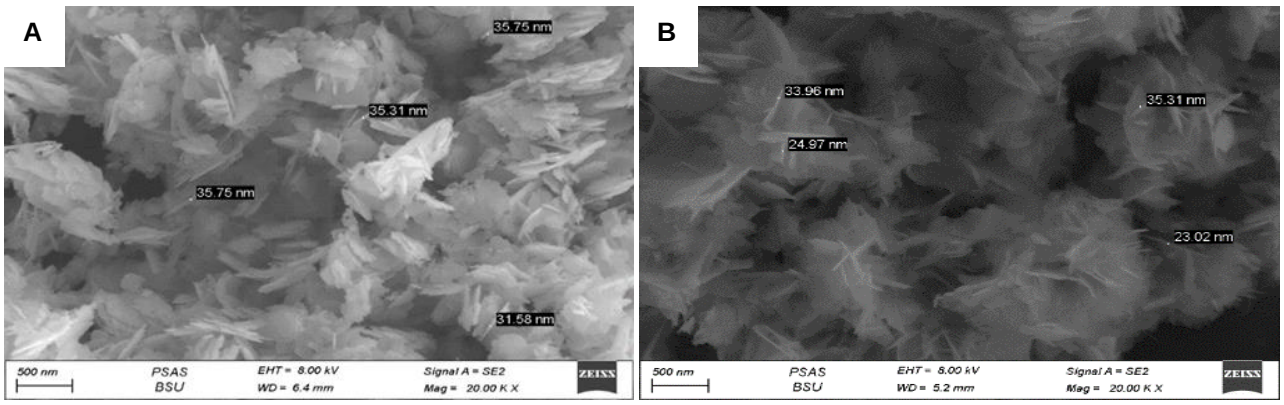

Figure 1: Characterization of ZnO-NPS by SEM imaging. (A) Chemically synthesized ZnO-NPS; (B) Biosynthesized ZnO-NPS.

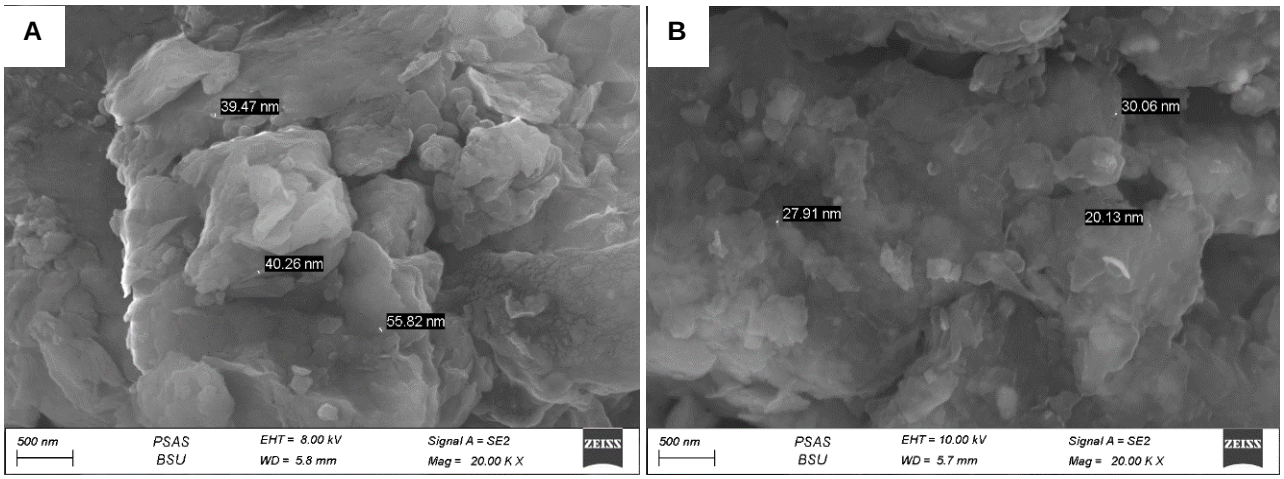

Figure 2: Characterization of ZnO biodegradable antimicrobial coating. (A) Chemically synthesized ZnO-biodegradable coating; (B) Biosynthesized ZnO-biodegradable coating.

in a hot air oven overnight (Heraeus, Germany) at 60 °C for 8 h.

For the green synthesis of ZnO-NPS, 50 mL of *L. officinalis* plant extract was added drop by drop to zinc acetate dihydrate and sodium hydroxide during stirring till white precipitation of nanoparticles appeared, then the solution was filtered and dried in a hot air oven at 60 °C for 8 h.

Characterization of ZnO-NPS using scanning electron microscope (SEM)

Scanning electron microscopy (Sigma, 500vp) was used to confirm the shape, size and distribution of zinc oxide nanoparticles (Figure 1A and 1B) as well as zinc oxide biodegradable coating (Figure 2A and 2B). These images demonstrated that ZnO-NPS had a rod shape, homogenous and heavy distribution with a particle size of about 20-40nm.

In vitro* determination of the antimicrobial activity of ZnO - biodegradable coating against *E. coli and S. aureus

Preparation of bacterial inocula

Loopful of *E. coli* and *S. aureus* colonies previously stored on MHA slopes were grown overnight in MHB medium at 37 °C (Naskar *et al*., 2020), followed by dilution of the cells to an optical density of 0.5 McFarland turbidity standard. The cell cultures were used within 30 min. After dilution to prepare samples for detection of antimicrobial activity of ZnO-NPS and estimation of antimicrobial activity of biodegradable coating, then different bacterial strains were cultivated on MHA medium and incubated overnight at 37 °C.

Development of ZnO - biodegradable coating

The sterile, biodegradable coating containing the antimicrobials was made by adding starch (10-12g),

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gelatin (1-3g), xanthan gum (0.05-0.3g) and glycerol (3-5 mL/L) to 100 mL sterile distilled water. Firstly, distilled water was heated to 90 °C on a hot plate stirrer, gelatin was added with stirring, followed by starch and then xanthan gum to stabilize the mixture with continuous stirring. When all components were completely dissolved, the pH was adjusted to the isoelectric point of gelatin (8.5) using 1 M NaOH and the mixture was autoclaved at 121 °C for 15 min and then cooled to 55 °C. Chemically and biosynthesized ZnO-NPS were added to the coating at concentrations of 2% and 5% (for each). Starch-based biopolymer mixtures were prepared without antimicrobials to be used as a control in subsequent experiments.

Determination of the antimicrobial properties of antimicrobial biodegradable coating (ABC) using disc diffusion assays (DDAs)

Agar plates were inoculated with different cocktails of *S. aureus* and *E. coli* (one plate for each bacterium). Then filter paper discs, about 6 mm in diameter, enclosing the previously prepared biodegradable coating containing biosynthesized and chemically synthesized ZnO-NPS with a concentration of 5% and 2% were placed on the agar surface. Starch coating without any antimicrobials was used as a control negative. The Petri dishes were incubated at 37 °C and the diameters of inhibition zones produced by chemically and biosynthesized ZnO-NPS with different concentrations were measured (Balouiri *et al*., 2016).

Challenge study

Inoculation of Tilapia fillets samples with E. coli and S. aureus

Tilapia fillet samples were cut into thin slices of 25 cm^2 surface area (5 cm × 5 cm). The slices were subjected to UV light treatment in a biological safety cabinet for 15 m /side to reduce the bacterial loads prior to inoculation (Cutter and Siragusa, 1994). Bacterial cocktails were inoculated onto the samples by aseptically spreading 1 mL of the 8 log_{10} CFU/mL bacterial cocktail on each slice side to achieve a final concentration of approximately 6.6 log_{10} CFU/cm^2. The inoculated side was left undisturbed for 20 min to allow for bacterial attachment before inoculation of the other side. Un-inoculated slices were used as negative controls. The coating solutions with different antimicrobials and the control one were aseptically spread in Tilapia fillet samples under aseptic conditions and then stored at 4 ± 1 °C until spoilage.

Pathogen enumeration

Tilapia fillet samples were evaluated for *E. coli and S. aureus* populations at days 0 (4 h), 2, 4, 6, 8 and 10 of refrigerated storage (Suo *et al*., 2017). The samples were analyzed in triplicate; at each time point, samples were removed from their coats and aseptically stomached (Stomacher® 400, Seward, UK) in a sterile stomacher bag for 2 min with 225 mL buffered peptone water (BPW), then serially diluted in BPW. *E. coli* and *S*. *aureus* were spread-plated onto SMAC agar and BP agar, respectively. The remaining colonies were counted manually and converted to log_{10} CFU/cm^2.

Overall acceptability of Tilapia fillets

Sensory analysis was performed by semi-trained panelists in the Faculty of Veterinary Medicine, Beni-Suef University, to evaluate the overall acceptability of Tilapia fillet samples through a scoring method of 1 to 5 for each attribute, and the overall acceptability was calculated as the sum of all attributes according to the method of Neumann *et al.* (1983). The panel members (seven) were selected, based on their performance during an initial evaluation trial, from students and workers of the Food Hygiene Department at the Faculty of Veterinary Medicine, Beni-Suef University.

Statistical analysis

The challenge study was carried out in triplicate. Bacterial counts were converted to log_{10} CFU/cm^2. Mean and standard deviation (SD) were calculated using Microsoft Excel (Trinetta *et al*., 2010). A one-way ANOVA test (Minitab 18 statistical software) was used for the estimation of significant differences and comparison among means at $p \leq 0.05$.

RESULTS AND DISCUSSION

Antimicrobial activity of biosynthesized and chemically synthesized ZnO biodegradable coating against *S. aureus and E. coli*

The antimicrobial activity of biosynthesized and chemically synthesized ZnO biodegradable coating was tested through the Agar disk diffusion method (Table 1). Both biosynthesized and chemically synthesized ZnO edible coating have an antimicrobial effect against different strains of *S. aureus* and *E. coli*, which could be detected through the size of the inhibition zone on MHA medium. ZnO-NPS, 2% concentration, was the lowest one able to cause inhibition against different pathogenic strains of *S. aureus and E. coli*. Moreover, ZnO-NPS, either prepared by chemical or green synthesis methods, was more effective against *S. aureus* (Gram-positive bacteria) than *E. coli* (Gram-negative bacteria). These results are consistent with those of Hajipour *et al*. (2012) and Basri *et al*. (2020), who reported that the cell wall of Gram-positive bacteria is simpler in its structure than Gram-negative bacteria. Gram-negative bacteria are surrounded by additional outer membranes composed of lipopolysaccharides which provide an extra layer of protection and resistance toward the antimicrobial agents, forming a very thick wall that decreases the penetration power of ZNO-NPS. In this context, Adams *et al*. (2006) and Reddy *et al*. (2007) concluded that *S. aureus* is sensitive to ZnO-NPS due to the high affinity between

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Table 1: Diameter of Inhibition zone of ZnO-biodegradable antimicrobial coating (ZnO-BAC) against *S. aureus and E. coli.*

Treatment	Biosynthesized ZnO-BC		Chemical ZnO-BC	
	S. aureus (mm)	*E. coli* (mm)	*S. aureus* (mm)	*E. coli* (mm)
ZnO-BAC 2%	8.6	2.6	7.3	2
ZnO-BAC 5%	9.6	5.3	8.3	4
ZnO-BAC 10%	10.5	6.3	9.6	6

ZnO-NPS and the bacterial cell. Furthermore, Jones *et al*. (2008) and Azam *et al*. (2012) indicated that ZnO nanoparticles showed the best antibacterial effects on *S. aureus* compared to other metal oxide nanoparticles.

On the contrary, Yamamoto (2001) and Applerot *et al.* (2009) concluded that *E.coli* has strong susceptibility to ZnO-NPS than *S. aureus* because *S. aureus* has strong antioxidant components such as carotenoid pigments, which act as great oxidizing agents in the interior of *S. aureus* in addition to the presence of potent detoxification agents such as antioxidant enzymes, particularly catalase which give resistance to *S. aureus* to several antimicrobial agents. Moreover, Brayner *et al*. (2006) found that ZnO-NPS had a bacteriostatic effect against *E. coli* due to the accumulation of ZnO-NPS in the bacterial cell membrane and cytoplasm leading to disorganization of *E. coli* membranes causing inhibition of their growth and multiplication.

In this study, the biosynthesized ZnO-NPS showed stronger antimicrobial activity against *S. aurues and E. coli* than the chemically synthesized one, which is regarded to the synergistic effect of ZnO-NPS and *L. officinalis* aqueous extract, which has a bactericidal effect against several microorganisms such as *S. aureus and E. coli*. These results are in good agreement with Jianu *et al*. (2013). However, Dostalova *et al*. (2014) reported that lavender aqueous extract hasn't a significant antimicrobial effect against several microorganisms, including *S. aureus* and *E. coli*.

Effect of biosynthesized and chemically synthesized biodegradable coating on the survival of *S. aureus* and *E. coli* in chilled Tilapia fillet samples stored at 4 ± 1 °C

The biodegradable antimicrobial coating containing different concentrations (2% and 5%) of biosynthesized or chemically synthesized ZnO-NPS, as well as control one, were used for coating Tilapia fillet samples inoculated with *S. aureus and E. coli* cocktails at a concentration of 6.6 $\log_{10}$ CFU/cm^2 then chilled stored at 4 ± 1 °C up to 10 days. The remaining counts of the inoculated *S. aureus* and *E. coli* cocktails are presented in Tables 2 and 3.

Both control coated and uncoated Tilapia fillet samples showed a continuous increase in *S. aureus* counts (Table 2), which progressed from 6.21 to 7.65 $\log_{10}$ CFU/cm^2 and 6.11 to 7.66 $\log_{10}$ CFU/cm^2, respectively at the end of the storage period (day 10). Similarly, the control coated and uncoated Tilapia fillet samples showed a continuous increase in *E. coli* counts (Table 3), which progressed from 6.38 to 7.65 $\log_{10}$ CFU/cm^2 and from 6.41 to 7.69 $\log_{10}$ CFU/cm^2, respectively at the end of the storage period (day 10).

The increment in *S. aureus and E. coli* counts in both coated and non-coated samples indicates that starch-based film did not have any antimicrobial activity. These results are in good agreement with research findings of Yoksan and Chirachanchai (2010), who found that starch-based films didn't have antimicrobial activity in comparison with chitosan film.

Samples coated with biosynthesized and chemically synthesized ZnO biodegradable coating at different concentrations (2% and 5%) showed a significant difference in the counts of *S. aureus* after 4 h of chilling and all over the storage period. The remaining *S. aureus* counts were 5.25, 5.01 and 5.28, 5.09 $\log_{10}$ CFU/cm^2 after 4 h of storage, while at the end of the storage period, the final counts were 3.83, 3.71 and 3.87, 3.82 $\log_{10}$ CFU/cm^2, respectively (Table 2). The log reduction in *S. aureus* cocktail counts in the case of biosynthesized and chemically synthesized ZnO biodegradable coating with different concentrations (2% and 5%) at the end of the storage period was 3.82, 3.94 and 3.78, 3.83 $\log_{10}$ CFU/cm^2, respectively. Similarly, samples coated with biosynthesized and chemically synthesized ZnO biodegradable coating with different concentrations showed significant differences ($p \leq 0.05$) in the counts of *E. coli* after 4 h of chilling and all over the storage period. The remaining counts at 4 h of storage were 5.39, 5.33 and 5.40, 5.36 $\log_{10}$ CFU/cm^2, respectively, while the final counts reached 3.90, 3.78 and 3.95, 3.86 $\log_{10}$ CFU/cm^2, respectively. The log reduction in *E. coli* cocktail counts for both 2% and 5% biosynthesized and chemically synthesized ZnO biodegradable coating at the end of the storage period was 3.75, 3.87 and 3.7, 3.79 $\log_{10}$ CFU/cm^2, respectively.

In our study, starch-based biodegradable antimicrobial coating incorporated with biosynthesized and chemically synthesized ZnO-NPS achieved approximately 3.7 log reduction in both pathogenic *S. aureus* and *E. coli* cocktails; this indicates that ZnO biodegradable coating has a significant antimicrobial effect. Similar results were reported by Ma *et al*. (2016), who concluded that ZnO-starch nanocomposite coating has an efficient antimicrobial activity against *E. coli* and *S. aureus*. Moreover, Raigond *et al*. (2019) recorded the antimicrobial activity of potato starch-based nanocomposite films incorporated with clove oil against *S. aureus*.

It is important to point out that the log reduction of *S. aureus* and *E. coli* counts in Tilapia fillet coated with 2 and 5% biosynthesized and chemically synthesized ZnO-NPS

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Table 2: Remaining counts of *S. aureus* in artificially inoculated Tilapia fillet samples, coated and stored at 4 ± 1 °C up to 10 days.

Coating solutions	Populations (log_{10} CFU/cm^2) during storage at 4 °C					
	Zero day (4 h)	Day 2	Day 4	Day 6	Day 8	Day 10
C1	6.11 ± 0.06^{a}	6.31 ± 0.07^{a}	6.90 ± 0.30^{a}	7.65 ± 0.02^{a}	7.65 ± 0.02^{a}	7.66 ± 0.02^{a}
C2	6.21 ± 0.03^{a}	6.21 ± 0.03^{a}	6.82 ± 0.06^{a}	7.54 ± 0.03^{a}	7.65 ± 0.02^{a}	7.65 ± 0.02^{a}
BAC/B-ZnO NPS.2%	5.25 ± 0.03^{b}	5.18 ± 0.04^{bc}	5.11 ± 0.06^{b}	4.49 ± 0.02^{b}	3.83 ± 0.01^{b}	3.83 ± 0.01^{b}
BAC/B-ZnO NPS.5%	5.01 ± 0.06^{c}	4.95 ± 0.06^{bc}	4.87 ± 0.09^{b}	4.32 ± 0.04^{c}	3.71 ± 0.03^{c}	3.71 ± 0.03^{c}
BAC/Ch-ZnO NPS.2%	5.28 ± 0.03^{b}	5.24 ± 0.04^{b}	5.17 ± 0.05^{b}	4.52 ± 0.02^{b}	3.87 ± 0.01^{b}	3.87 ± 0.01^{b}
BAC/Ch-ZnO NPS.5%	5.09 ± 0.06^{bc}	5.03 ± 0.08^{b}	4.96 ± 0.07^{b}	4.46 ± 0.03^{b}	3.82 ± 0.01^{b}	3.82 ± 0.02^{b}

C1: Control positive noncoated sample; C2: Control positive starch coated sample; BAC/B-ZnO NPS: Biodegradable antimicrobial coating incorporated with biosynthesized zinc oxide nanoparticles; BAC/Ch-ZnO NPS: Biodegradable antimicrobial coating incorporated with chemically synthesized zinc oxide nanoparticles. Results are expressed as mean value ± SE; average values within the same column followed by different superscripts are significantly different at $p \leq 0.05$.

Table 3: Remaining counts of *E. coli* in artificially inoculated Tilapia fillet samples, coated and stored at 4 ± 1 °C up to 10 days.

Coating solutions	Populations(log_{10} CFU/cm^2) during storage at 4 °C					
	Zero day (4 h)	Day 2	Day 4	Day 6	Day 8	Day 10
C1	6.41 ± 0.05^{a}	6.38 ± 0.02^{a}	7.00 ± 0.11^{a}	7.65 ± 0.03^{a}	7.67 ± 0.01^{a}	7.69 ± 0.02^{a}
C2	6.38 ± 0.04^{a}	6.21 ± 0.04^{a}	6.76 ± 0.14^{a}	7.63 ± 0.02^{a}	7.64 ± 0.03^{a}	7.65 ± 0.02^{a}
BAC/B-ZnO NPS.2%	5.39 ± 0.03^{b}	5.32 ± 0.04^{b}	5.23 ± 0.06^{b}	4.54 ± 0.02^{b}	3.90 ± 0.01^{b}	3.90 ± 0.02^{b}
BAC/B-ZnO NPS.5%	5.33 ± 0.04^{b}	5.27 ± 0.03^{b}	5.09 ± 0.05^{b}	4.38 ± 0.04^{c}	3.78 ± 0.03^{c}	3.78 ± 0.03^{c}
BAC/Ch-ZnO NPS.2%	5.40 ± 0.02^{b}	5.36 ± 0.04^{b}	5.32 ± 0.03^{b}	4.55 ± 0.03^{b}	3.95 ± 0.02^{b}	3.95 ± 0.02^{b}
BAC/Ch-ZnO NPS.5%	5.36 ± 0.03^{b}	5.31 ± 0.02^{b}	5.16 ± 0.06^{b}	4.49 ± 0.02^{bc}	3.85 ± 0.01^{c}	3.86 ± 0.01^{bc}

C1: Control positive noncoated sample; C2: Control positive starch coated sample; BAC/B-ZnO NPS: Biodegradable antimicrobial coating incorporated with biosynthesized zinc oxide nanoparticles; BAC/Ch-ZnO NPS: Biodegradable antimicrobial coating incorporated with chemical synthesized zinc oxide nanoparticles. Results are expressed as mean value ± SE; average values within the same column followed by different superscripts are significantly different at $p \leq 0.05$.

increased with increasing storage time which may be attributed to the accumulation of hydrogen peroxide on food surface (Zhang *et al*., 2008). Moreover, Sawai *et al*. (1996; 1998) found that ROS (reactive oxygen species) concentrations increased with the increase of ZnO-NPS, which are toxic to the bacterial cell membrane because they are a potent oxidizing agent.

Comparing the effect of biosynthesized and chemically synthesized ZnO-NPS on *S. aureus* and *E. coli* revealed that counts of *S. aureus* of the biosynthesized ZnO-NPS were significantly ($p \leq 0.05$) lower than chemically synthesized one all over the storage period except for day 4, while non-significant differences in *E. coli* inoculated samples were detected in days 6, 8 and 10. These significant differences are due to the synergistic effect of ZnO-NPS and *L. officinalis* aqueous extract, which has a bactericidal effect against several microorganisms such as *S. aureus and E. coli*. In this respect, Salari *et al*. (2017) concluded that *Lavandula* herbal extract had antioxidant and antimicrobial effect due to the reducing effect of flavonoid derivatives (like flavones and flavanone) and phenolic compounds (such as hydroxycinnamic acids) present in the plant extract, so when used in the biosynthesis of ZnO-NPS, it gives an antimicrobial and antioxidant effect.

Furthermore, the effect of biosynthesized ZnO-NPS versus chemical one had been assessed by many researchers. In this respect, Stan *et al*. (2016) demonstrated that ZnO-NPS biosynthesized from *Allium sativum* extract

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Table 4: Overall acceptability of treated Tilapia fillets samples during storage at 4 ± 1 °C.

Treatment	Zero-day	Day 2	Day 4	Day 6	Day 8
C1	20 ± 0^{a}	17 ± 0^{a}	14.4 ± 0.35^{a}	-	-
C2	19.5 ± 0.20^{a}	16.2 ± 0.47^{a}	13.5 ± 0.23^{a}	-	-
BAC/B-ZnO NPS.2%	19.8 ± 0.19^{b}	19.5 ± 0.10^{b}	17.5 ± 0.29^{b}	14.5 ± 0.06^{b}	12.1 ± 0.07^{b}
BAC/B-ZnO NPS.5%	19.8 ± 0.1^{b}	19.7 ± 0.19^{b}	18 ± 0.1^{b}	15.2 ± 0.12^{b}	13.3 ± 0.12^{b}
BAC/Ch-ZnO NPS.2%	19.7 ± 0.37^{b}	18.6 ± 0.69^{b}	16.3 ± 0.17^{b}	14.2 ± 0.24^{b}	11.3 ± 0.18^{b}
BAC/Ch-ZnO NPS.5%	19.6 ± 0.32^{b}	18.5 ± 0.8^{b}	16.5 ± 0.34^{b}	14.7 ± 0.22^{b}	12.5 ± 0.4^{b}

Results are expressed as mean values ± SE. Average values within the same column followed by different superscripts are significantly different at $p \leq 0.05$.

inhibited the growth of *S. aureus*, *E. coli* and several types of Gram-positive and Gram-negative bacteria stronger than chemically synthesized ZnO-NPS. Moreover, Xu *et al*. (2021) concluded that ZnO-NPS synthesized by green extract showed a greater inhibitory effect against bacterial species than chemically synthesized ZnO-NPS However, Li *et al*. (2010) found that the ZnO-coated film inhibited both *E. coli* and *S. aureus* without significant observable difference. A similar result was reported by El-Waseif (2019) concluded that there was no significant difference between ZnO-NPS prepared by green synthesis or chemical precipitation methods.

The great reduction in *S. aureus* counts obtained in the current study compared to those of *E. coli* may be due to the best antibacterial effects of ZnO nanoparticles on *S. aureus* compared to other kinds of metal oxide nanoparticles (Jones *et al*., 2008; Azam *et al*., 2012).

The mechanism of ZnO-NPS antimicrobial activity may be attributed to the production of hydrogen peroxide in ZnO particles which penetrate the bacterial cell membrane causing injury, thus helping in the inhibition of growth and multiplication of *E. coli* and *S. aureus* (Yamamoto *et al*., 2004; Zhang *et al*., 2007; Li *et al*., 2009). The production of Zn ions as a result of ZnO decomposition may be another cause of its antibacterial activity (Yang and Xie, 2006; Tam *et al*., 2008).

Overall acceptability of treated Tilapia fillets samples during storage at 4 ± 1 °C

The overall acceptability of uncoated, chemically synthesized and biosynthesized ZnO biodegradable coated (2% and 5%) Tilapia fillet samples stored at 4 ± 1 °C is presented in Table 4. The highest score of overall acceptability was detected at day one of storage, for all samples, then a drop in the sensory features was found during the storage period. This reduction was significant in control-coated and non-coated samples compared with antimicrobial-coated samples. This ensures the fact that seafood have a short shelf-life due to their high content of free amino acids and volatile nitrogen bases and higher final pH. As a result, the control coated and non-coated samples spoiled earlier than the treated ones (Mexis *et al*., 2009). The control-coated and non-coated samples maintained good sensory characteristics until the fourth day of storage. On day 6 upwards, the control-coated and non-coated samples were not evaluated due to the appearance of signs of spoilage. Samples treated with 2 and 5% zinc oxide antimicrobial biodegradable coating still had good sensory characteristics till day 8 of chilled storage. This might allow 4 days extension of the shelf-life of samples coated with the antimicrobial biodegradable coating compared to the control samples. These results are in good agreement with Ahmed *et al*. (2022), who reported that starch-based film incorporated with Ag-NPS extends the shelf-life of chicken breast samples 4 days above the control one. Lee *et al*. (2016) prolonged the shelf-life of fatty tuna meat stored at 4 °C for 12 days by wrapping the product in a composite film (1% gelatin + 4% red pepper seed meal protein + oregano). Moreover, Thaker *et al*. (2017) reported that Indian salmon fillets coated with 10% gelatin solution, 10% gelatin + 1.5% chitosan + 30% lime juice, and 10% gelatin + 30% garlic extract + 1.5% chitosan had a shelf-life of 8, 16 and 16 days, respectively versus 4 days for the control. Similarly, the antimicrobial film based on agar/Konjac glucomannan loaded with 2% carvacrol, examined by Peng *et al*. (2022), showed antimicrobial material that inhibits *S. aureus* and extends the shelf-life of chicken breast samples.

CONCLUSION

Starch-based biodegradable coating incorporated with biosynthesized and chemically synthesized ZnO-NPS presented a significant reduction in the cocktails of *S. aureus* and *E. coli* strains experimentally inoculated in Tilapia fillet samples during chilling storage. The incorporation of *L. officinalis* increased the antibacterial activity of ZnO-NPS. *Staphylococcus aureus* was more sensitive than *E. coli* for chemically and plant-synthesized ZnO-NPS. The biodegradable antimicrobial coating showed 4 days extension in the shelf-life compared to the control coated and uncoated samples.

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