Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Antibacterial Effects of Zinc Oxide Nanoparticle and Ginger Oil Nanoemulsion in Fish Fillet Keeping Quality

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Abstract

IN THE current study, the antibacterial effect of zinc oxide nanoparticles (ZnO NPs) and ginger oil nanoemulsion (GNE) were determined by their application on experimentally inoculated fish fillet with nearly 4 $\log_{10}$ CFU/g *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) in separately treated groups and were kept in a refrigeration condition (4±1°C). For investigation of their antibacterial potential, ZnO NPs and GNE were used in concentrations of 12 mM and 5.0%, respectively either alone or in combination with each other. Characterizations results of the used nanomaterials revealed that the size of ZnO NPs and GNE were 20 and 17.94 nm, respectively. Regarding with their antibacterial effect, different investigated treatments inhibited significant antibacterial effect against *S. aureus* and *E. coli* through inhibition of their multiplication significantly in comparison with control group. Results revealed that the combination treated groups (ZnO NPs + GNE) showed higher reduction % than the other treated groups that strongly proved the synergistic effect of their combination against foodborne bacteria. In addition, extended organoleptic acceptability and shelf-life, consequently, of the treated fish fillet samples were recorded in comparing with the control untreated groups. Moreover, over longer time exposure, treated groups with GNE showed longer organoleptic acceptability criteria than that treated with ZnO NPs. Furthermore, *S. aureus* showed higher resistance potential against the used nanomaterials than *E. coli*. Through the recorded results, ZnO NPs and GNE showed potent antibacterial effects against *S. aureus* and *E. coli* fish borne bacteria; therefore, it is strongly recommended to be used as food additive; whereas, environmental safety and residue potential still need more investigations.

Keywords: Foodborne pathogens, Food safety, Nanomaterials.

Introduction

In the quest to enhance the preservation and quality of perishable food products, the application of innovative technologies has become increasingly vital. Among these advancements, the use of nanoemulsion has emerged as a promising method for improving food preservation. Nanoemulsion, which consist of finely dispersed droplets of one liquid within another, offer unique advantages in delivering bioactive compounds with enhanced efficacy. One such bioactive compound is ginger, renowned for its antimicrobial and antioxidant properties [1].

Fish, being highly perishable, is susceptible to rapid spoilage caused by microbial activity and oxidative deterioration. Traditional preservation techniques, while effective to some extent, often face limitations in maintaining the sensory and nutritional quality of fish products. This has prompted the exploration of alternative preservation methods that are both effective and sustainable [2].

Ginger, derived from the rhizome of *Zingiber officinale*, has been used traditionally for its health benefits, including its ability to inhibit microbial growth and combat oxidative stress. The incorporation of ginger into nanoemulsion form could revolutionize fish preservation by leveraging these properties more effectively than conventional methods [3].

Ginger nanoemulsion, with its enhanced bioavailability and stability of active compounds,

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(Received 22 September 2024, accepted 17 November 2024)
DOI: 10.21608/EJVS.2024.323044.2383

presents a novel approach to addressing these challenges. By potentially extending the shelf life of fish through improved antimicrobial and antioxidant effects, ginger nanoemulsion could offer a significant improvement over current preservation techniques. The encapsulation of ginger essential oil in nanoemulsion not only improves its stability and solubility but also enhances its antimicrobial activity against fish borne pathogens like *E. coli* and *S. aureus* [4].

On the same line, zinc oxide nanoparticles (ZnO NPs) are another effective tool in food preservation. These nanoparticles exhibit strong antimicrobial properties, making them ideal for reducing the growth of bacteria such as *L. monocytogenes, E. coli* and *S. aureus* on fish meat referring to the high surface area and charge density of ZnO NPs that enable them to interact effectively with bacterial cell membranes, leading to significant reductions in microbial populations [5].

Combining ginger essential oil nanoemulsion (GNE) with zinc oxide nanoparticles (ZnO NPs) could offer a synergistic approach to fish meat preservation. This dual strategy could potentially enhance the antimicrobial efficacy, improve the sensory characteristics, and extend the shelf life of fish products. By leveraging the strengths of both ginger oil nanoemulsion and ZnO NPs, the food industry can develop more effective and sustainable preservation methods, ensuring the quality and safety of fish meat for consumers [6].

Therefore, the current study was conducted to investigate the antibacterial activity of ginger nanoemulsion lonely and in combination with ZnO NPs in fish filet.

Material and Methods

Collection and preparation of samples

One kilogram and six-hundred grams of chilled bayad fish (*Bagrus bajad*) fillet were purchased from a local market in Benha city. Samples were divided into eight equal groups in the form of thin films, and were treated with ultraviolet light (wavelength 385 nm) for 30 min to eliminate background microflora before addition of the used nanomaterials or inoculation of the test strain[7], followed by soaking in bacterial suspension of *E. coli* and *S. aureus*, in a concentration of nearly 4 $\log_{10}$ CFU/g, separately up to 15 minutes before treatment of the inoculated samples with nanomaterials by soaking for further 15 minutes, after which the experiment zero time was recorded.

Preparation and characterization of ginger nanoemulsion

Ginger nanoemulsion was prepared in the unit of nanomaterials, Animal Health Research Institute (AHRI), with a concentration of 20%, which was prepared according to Pouton and Porter [8]. Nano-droplet size was determined in animal health research institute.

Preparation and characterization of zinc oxide nanoparticles

Zinc oxide nanoparticles were prepared in the unit of nanomaterials, Animal Health Research Institute (AHRI) according to Wang *et al.* [9]. From which, 12 mM was prepared by suspension of 976.6 g of ZnO NP powder in double-distilled water and constantly stirred until uniform colloidal suspension was formed. Zinc oxide nanoparticles were characterized using transmission electron microscopy (TEM) techniques (JEOL JEM 1400). TEM was conducted in Cairo University Research Park, Egypt.

Preparation of bacterial strain

Previously enriched field strain of *E. coli* and *S. aureus* were determined by serial dilution method, followed by plating on nutrient agar for counting of the original culture, from which, certain working culture count was adjusted by serial dilution technique on a sterile normal saline (0.9% NaCl) to reach nearly 4 $\log_{10}$ CFU/ml.

Experimental grouping

According to Noori *et al.* [10] and Maarouf *et al.* [11], 1600 grams of bayad fish fillet were equally divided into eight groups as follow:

G1: Control positive untreated fish fillet, inoculated with *S. aureus* without treatment

G2: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *S. aureus* + 12 mM ZnO NPs.

G3: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *S. aureus* + 5.0% ginger nanoemulsion (GNE).

G4: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *S. aureus* + 12 mM ZnO NPs + 5.0% ginger nanoemulsion (GNE).

G5: Control positive untreated fish fillet, inoculated with *E. coli* without treatment

G6: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *E. coli* + 12 mM ZnO NPs.

G7: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *E. coli* + 5.0% ginger nanoemulsion (GNE).

G8: 200 g fish fillet +~ 4 $\log_{10}$ CFU/g *E. coli* + 12 mM ZnO NPs + 5.0% ginger nanoemulsion (GNE).

Control and treated groups were kept all refrigerated at 4±0.5^{o}C. Sensory, microbiological and chemical examination at day zero (within 30 min. after treatment), and then periodically every 3 days of cold storage until organoleptic deterioration; which appeared after the 6th day of storage for control group.

The trial was repeated in triplicates.

The samples were examined for the following:

Sensory evaluation (color, odor, texture and overall) following Mörlein [12] in scores (1 to 5), where ≤1- represented the worst while 5- represented the excellent mark.

Bacteriological examinations

After preparation of serial dilutions according to ISO 6887-1 [13], Control and treated fish meat mince were examined for their *S. aureus* and *E. coli* counts every three days of refrigeration following the ISO standards "ISO 6888 [14] and ISO 16649-2 [15].

Reduction percent was calculated according the formula:

$$\text{Reduction rate (\%)} = \frac{A-B}{A} x100$$

A = Mean value of the bacterial count at zero time.

B = mean value of the bacterial count at the following days.

Statistical Analysis

The obtained data was statistically treated by one-way ANOVA using SPSS software for Windows (Version 16). Duncan's post hoc analysis was used to analyze the data, with a p-value of 0.05 being regarded statistically significant

Results

Characterization of the used nanomaterials

Zinc Oxide NPs Characterized by white colored powder of 20 ± 5 nm of 99.9% Purity. It appeared spherical shaped under TEM. In addition, it formed a stable colloid in mixture of ethanol and chloroform and water. In addition, GNE droplet size was determined by Microtrac® size analyzer, results revealed that the droplet size was 17.94 nm.

Impact of the used additives on the sensory quality of the treated fish fillet

The sensory scores of all of the treated fish fillet samples showed a significant enhancement in the sensory quality appeared as an elongation in the physical acceptability longer than those were recorded for the control group; which showed spoilage characteristics after the 6th day of storage; whereas the combined treatment groups (G4 and G8) showed higher acceptability scores up to the 18th day of storage (Fig. 1 and 2).

Antibacterial effect of the used nanomaterials

In the current study, the antibacterial effect of ZnO NPs and ginger nanoemulsion (GNE) on experimentally inoculated *S. aureus* and *E. coli* in fish fillet during chilling condition at 4^{o}C±0.5^{o}C; where, *S. aureus* mean count at the zero day recorded 4.3 $\log_{10}$ CFU/g. Fig. (3) and Table (1), also, showed that the different investigated treatments inhibited *S. aureus* multiplication significantly in comparison with control group which showed spoilage signs in the 9th day of chilling storage (5.6 $\log_{10}$ CFU/g). Results revealed that the combination treated group (G4) showed higher reduction % than the other treated groups; where, the mean count of *S. aureus* was 1.1 $\log_{10}$ CFU/g in the 12th day of storage; while, the treated groups with ZnO and GNE alone showed re-raising of the bacterial count after the 9th day of the experiment. In addition, G2 and G3 showed spoilage signs at the 15th and 12th day of storage, respectively. Regarding with the reduction percent in relation to the staphylococcal count, the highest reduction % that was recorded for G2, G3 and G4 were 53.5, 48.8 and 74.4%; where the highest mean counts reduction were 2.3, 2.1 and 3.2 logs, respectively. Over longer time of exposure, treated groups with ZnO NPs showed longer organoleptic acceptability criteria than that treated with GNE.

In addition, Fig. (4) and Table (2), also, showed that the different investigated treatments inhibited *E. coli* multiplication significantly in comparison with control group which showed spoilage signs after the 6th day of chilling storage (6.0 $\log_{10}$ CFU/g). Results revealed that the combination treated group (G8) showed higher reduction % than the other treated groups; where, the mean count of *E. coli* was 1.0 $\log_{10}$ CFU/g in the 15th day of storage; while, the treated groups with ZnO and GNE alone showed re-raising of bacterial count after the 12th day of the experiment, however, they still with no apparently spoilage signs. Regarding with the reduction percent in relation to the *E. coli* count, the highest reduction % that was recorded for G6, G7 and G8 were 67.4, 56.5 and 78.3%; where the highest mean counts reduction were 2.5, 3.6 and 4.6 logs, respectively. Over longer time exposure, treated groups with ZnO NPs showed higher organoleptic acceptability scores than that was treated with GNE.

Results also showed higher sensitivity of *E. coli* to the used additives than *S. aureus*.

Discussion

Fish meat and food microbiology intersect in several important ways, especially concerning food safety, preservation, and quality because of that the fish meat can be a medium for various bacteria such as *E. coli* and *S. aureus*, which may possess a health hazard threats.

Microbial activity is a major factor in the spoilage of fish. Bacteria break down proteins and fats in fish, leading to off-flavors, odors, and changes in texture. This process can be slowed by refrigeration, freezing, or using preservatives, but it's crucial to monitor and manage microbial growth to maintain fish quality [16].

In the last decade, application of metal NPs as food preservative was extensively studied and discussed, especially for ZnO NPs. Unfortunately, numerous studies, also, recorded that, with chronic exposure to ZnO NPs, cellular toxicity may occur because of its potential to generate reactive oxygen species (ROS) that may lead to oxidative stress, as well as its ability to accumulate and interact with biological systems. Therefore, combination of ZnO NPs with natural herbal extract, such as ginger essential oil (GEO), have been studied [17, 18].

The use of ZnO NPs and ginger extracts in antimicrobial applications has gained significant attention in recent years due to their potential in enhancing food safety and extending shelf life. The green synthesis method, which involves using plant extracts like ginger, has been particularly favored for its eco-friendly and cost-effective approach [19].

Referring to the present obtained results, significant enhancement in the organoleptic quality of the treated fish fillet samples was observed; that confirm the well-known antioxidant and antibacterial effects of the used additives (Figs 1 and 2); where, the ZnO NPs-GNE combination treated groups showed higher sensory scores that may be attributed to the synergistic effect of the powerful antibacterial effect of ZnO NPs and the antioxidant effect of ginger components. The recorded results came in agreement with that were recorded by Noori *et al.* [10] and Maarouf *et al.* [11] **.**

The mechanism of action of ZnO NPs against bacterial pathogens such as *E. coli* and *S. aureus* involves the release of Zn^{2+} ions that are toxic to bacterial cells, interfering with cellular processes and causing cell death [19]; ZnO NPs can generate Reactive Oxygen Species, which damage cellular components such as DNA, proteins, and lipids, leading to bacterial cell death [20]**;** beside that, ZnO NPs can cause physical damage to the bacterial cell membrane, leading to leakage of cellular contents and ultimately cell death [19]. Moreover, ginger extracts contain compounds like flavonoids and polyphenols, which have inherent antimicrobial properties that can enhance the antibacterial activity of ZnO NPs [21].

Because of its known stability and antimicrobial\antioxidant activity, ginger essential oil nanoemulsion have been extensively studied for their potential in various applications, including food preservation, pharmaceuticals, and agricultural uses. This nanoemulsion can be used as preservatives in food systems to extend shelf life. GEO has been shown to inhibit the growth of several bacterial strains, including *S. aureus*, *E. coli* and others, which may be attributed to its ability for disrupting the bacterial cell membrane, leading to the leakage of cellular contents and the inhibition of various virulence factors such as exopolysaccharides (EPS) production; in addition, it can inhibit the expression of genes linked to bacterial energy metabolism, tricarboxylic acid cycle, cell membrane-related proteins, and DNA metabolism [22].

For example, ginger essential oil (GEO) nanoemulsion has been incorporated into edible coatings to enhance the safety and quality of meat fillets in reference to Noori *et al.* [10] who developed nanoemulsion-based edible coatings containing ginger essential oil and demonstrated their antimicrobial and antioxidant efficiency, which improved the safety and quality attributes of chicken breast fillets.

In the current study, significant reductions in *E. coli* and *S. aureus* was recorded using ZnO NPs and ginger nanoemulsion alone, which was maximized by their combination with each other. The present results came in agreement with the recorded results by Izgis *et al.* [19] and Raj and Jayaylakshmy [20] who recorded that the combined ZnO NPs with ginger extracts exhibit significant antimicrobial activity against *E. coli* and *S. aureus*. For instance, a study using the disk diffusion method found that combined ZnO NPs with 2% ginger extract formed higher inhibition zones against these bacteria. in addition, over longer time of exposure, treated groups with GNE, alone or in combination with ZnO NPs, showed longer organoleptic acceptability criteria than that treated with ZnO NPs which may be attributed to the bioactive flavonoid contents of GEO that enhance its antioxidant activity.

While specific studies on the use of ZnO NPs and ginger nanoemulsion in fish meat are limited, the general principle of using these nanoparticles to extend shelf life can be inferred. For example, a study on minced beef found that ZnO NPs significantly reduced *E. coli* growth, suggesting a potential application in extending the shelf life of fish meat as well [18, 23].

Although different studies proved the significant antimicrobial effect of ZnO NPs and ginger nanoemulsion, variation in the extent of their antimicrobial effect may be attributed to the way of application such as the use of these nanoparticles as soaking, direct addition or incorporation in polyvinyl alcohol composite gels, which have been shown to be effective in maintaining the antimicrobial properties the NPs.

Maximization of ZnO NPs effectiveness, in combination with ginger nanoemulsion, can also be attributed to that the specific surface area of ZnO NPs combined with ginger extract has been found to be significantly higher than those synthesized

without the extract, indicating enhanced antimicrobial activity [24].

It, also, worth noted that the higher sensitivity of *E. coli*, Gram-negative bacteria, to the applied additives than *S. aureus*, Gram-positive bacteria, may be attributed to the presence of peptidoglycan layer with thicker phospholipid cell wall in *S. aureus* that made it more resistant to the antibacterial compounds [25].

In summary, while nanotechnology holds promise for enhancing food preservation through improved packaging and nutrient delivery systems, its limitations related to health risks, regulatory challenges, environmental impact, and technical hurdles must be carefully addressed to ensure safe implementation.

Conclusion

ZnO NPs in combined with ginger extracts have shown promising results in antimicrobial applications, particularly against *E. coli* and *S. aureus*. The mechanism of action involves the release of Zn^{2+} ions and bacterial cellular membrane damage, which are enhanced by the antimicrobial compounds present in ginger extracts. While direct studies on fish meat are limited, the principles established in other food matrices suggest that ZnO NPs and ginger nanoemulsion could be effective in extending the shelf life of fish meat by inhibiting bacterial growth.

Acknowledgments

Authors want to present great appreciation for the all staff members of Food Hygiene Department, Faculty of Veterinary Medicine, University of Sadat City, Egypt; and Animal Health Research Institute, Agriculture Research Center for their valuable help and guidance.

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

TABLE 1. Reduction (%) of *S. aureus* counts in the treated groups

Day	G2	G3	G4
3rd day	25.6	18.6	30.2
6th day	30.2	34.9	48.8
9th day	53.5	48.8	65.1
12th day	41.9	34.9	74.4
15th day	30.2	25.6	58.1
18th day	Spoiled	Spoiled	41.9

TABLE 2. Reduction (%) of *E. coli* counts in the treated groups

Day	G6	G7	G8
3rd day	30.4	23.9	34.8
6th day	39.1	34.8	45.7
9th day	56.5	45.6	60.9
12th day	67.4	56.5	73.9
15th day	60.9	54.3	78.3
18th day	56.5	50.0	71.7

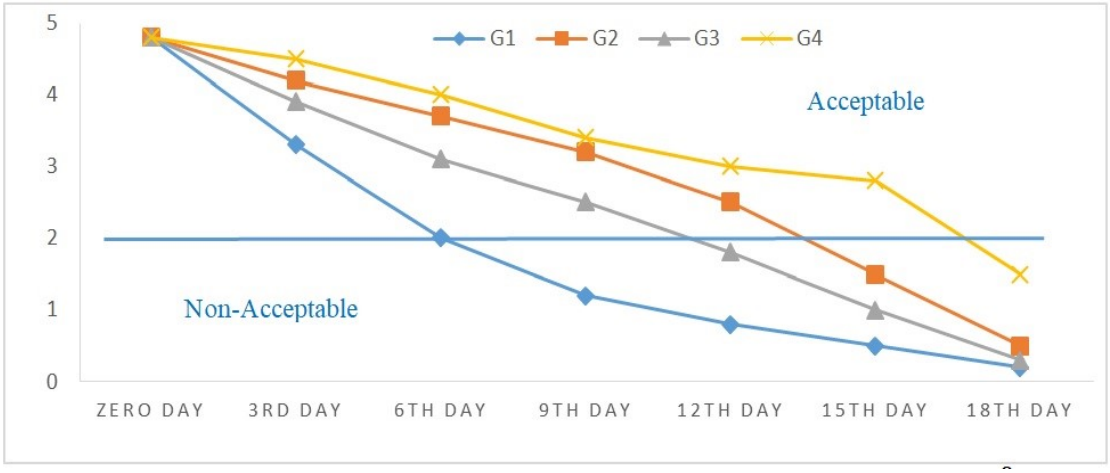

Fig. 1. Sensory profile of the examined fish groups inoculated with *S. aureus* in cold storage (4±0.5^{O}C).
According to this figure, if the final quality score is 2, the sample's quality is marginally acceptable. If this score is less than 2, the sample is unacceptable. While, less than 1, is spoiled apparently

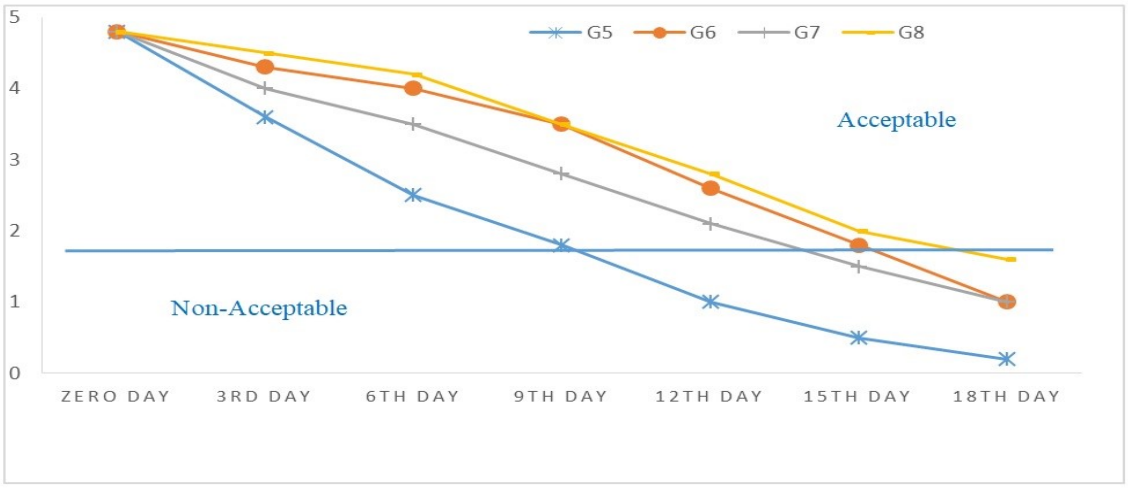

Fig. 2. Sensory profile of the examined fish groups inoculated with *E. coli* in cold storage (4±0.5^{O}C).
According to this figure, if the final quality score is 2, the sample's quality is marginally acceptable. If this score is less than 2, the sample is unacceptable. While, less than 1, is spoiled apparently

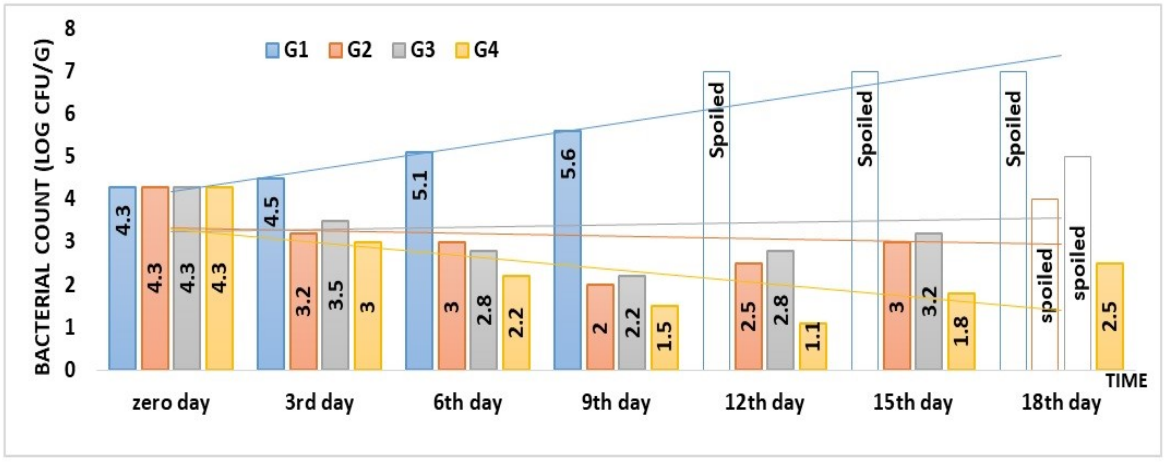

Fig. 3. Mean *S. aureus* counts (CFU/g) in the different groups of the experiment

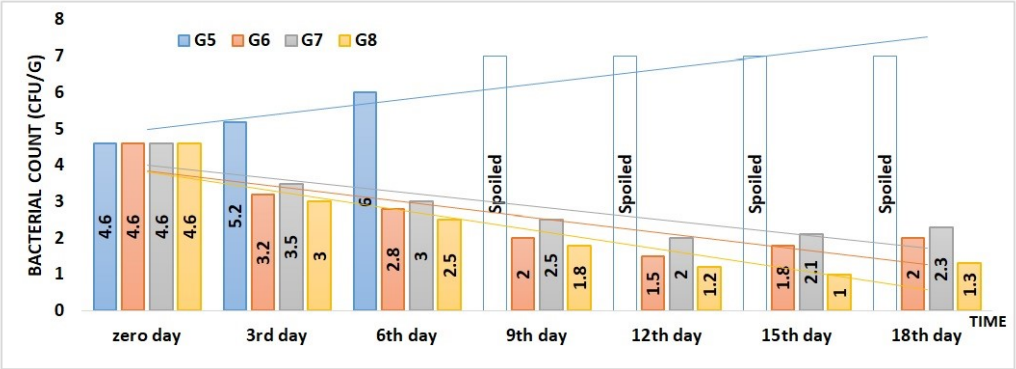

Fig. 4. Mean *E. coli* counts (CFU/g) in the different groups of the experiment

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التأثيرات المضادة للبكتيريا لجسيمات أكسيد الزنك النانوية ومستحلب زيت الزنجبيل النانوي في جودة حفظ شرائح السمك

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الملخص

في الدراسة الحالية، تم دراسة التأثير المضاد للبكتيريا لجسيمات أكسيد الزنك النانوية ومستحلب زيت الزنجبيل النانوي من خلال تطبيقهما على شرائح السمك الملقحة تجريبياً بما يقرب من 4لوج $_{10}$ خلية/جرام من المكورات العنقودية الذهبية والإشرشيا كولاي في مجموعات معالجة بشكل منفصل وتم الاحتفاظ بها في حالة تبريد عند درجة حرارة 4 ±0.5 درجة مئوية. للتحقيق في إمكاناتها المضادة للبكتيريا، تم استخدام جسيمات أكسيد الزنك النانوية ومستحلب زيت الزنجبيل النانوي بتركيزات 12 مليمول و5.0٪ على التوالي إما بمفردها أو بالاشتراك مع بعضها البعض. كشفت نتائج توصيف المواد النانوية المستخدمة أن حجم جسيمات أكسيد الزنك النانوية ومستحلب زيت الزنجبيل النانوي كان 20 و17.94 نانومتر على التوالي. فيما يتعلق بتأثيرها المضاد للبكتيريا، فإن المعالجات المختلفة قيد الدراسة قد أظهرت تأثيرًا مضادًا للبكتيريا بشكل كبير ضد بكتريا الاستافيلوكوكس اوريس والاشرشيا كولاي من خلال تثبيط تكاثرها بشكل كبير مقارنة بمجموعة التحكم. كشفت النتائج أن المجموعة المعالجة بخليط اوكسيد الزنك النانوية ومستحلب زيت الزنجبيل النانوي نسبة انخفاض في العد البكتيري أعلى من المجموعات المعالجة الأخرى التي أثبتت بقوة التأثير التآزري لتركيبتها ضد البكتيريا المنقولة بالغذاء. بالإضافة إلى ذلك، تم تسجيل معدلات قبول حسية ممتدة وعمر تخزين أطول لعينات شرائح السمك المعالجة بالمقارنة مع مجموعات التحكم غير المعالجة. علاوة على ذلك، على مدى فترة التعرض الأطول، أظهرت المجموعات المعالجة بمستحلب زيت الزنجبيل النانوي معايير قبول حسية أطول من تلك المعالجة بجسيمات اوكسيد الزنك النانوية. علاوة على ذلك، أظهرت بكتريا الاستافيلوكوكس اوريس إمكانات مقاومة أعلى ضد المواد النانوية المستخدمة من بكتريا الاشرشيا كولاي من خلال النتائج المسجلة؛ لذلك، يوصى بشدة باستخدام المواد المستخدمة كإضافات آمنة لمنتجات لحوم الاسماك؛ في حين أن السلامة البيئية وإمكانية المخلفات لا تزال بحاجة إلى مزيد من الدراسة.

الكلمات الدالة: ميكروبات التسمم الغذائي، سلامة الغذاء، المواد النانومترية.