Egypt. J. Vet. Sci., pp. 1-8

Egyptian Journal of Veterinary Sciences

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

Antibacterial Effect of Some Herbal-based Extract Nanoemulsions Against Some Foodborne Bacteria

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Abstract

NANOEMULSIONS have emerged as a promising strategy for enhancing the preservation of meat products, leveraging their antimicrobial and antioxidant properties. Therefore, the current study was planned to evaluate the antibacterial effect of ginger (GNE), thyme (TNE) and lemongrass (LNE) oil nanoemulsions (NEs) against *Escherichia coli* and *Staphylococcus aureus* strains of meat borne isolates by the concentrations of 1.0 and 2.0% for each. For that purpose, fresh minced meat samples were inoculated with microorganisms and NEs and were kept in refrigerator for investigating the bactericidal effect of the used additives which was recorded as reduction in the bacterial counts. Results revealed that the different investigated treatments inhibited *E. coli* and *S. aureus* multiplication significantly in comparison with control group. Results revealed a direct correlation between the additive's concentration, time of interaction and the bacterial concentration; where, higher concentration (2.0%) resulted in greater reductions. Results revealed reduction between 1.6-2.8 logs for *E. coli* and 2.1-3.3 logs for *S. aureus* revealing higher sensitivity of *S. aureus* to the used additives than *E. coli*; where the maximum reduction was recorded in the LNE 2.0% treated group (68.3 and 76.7%, respectively). Furthermore, all of the used NEs had a preservative potential appeared through extension of the overall sensory acceptability of the treated samples; so, application of essential oil-based nanoemulsion of such traditional herbs is highly recommended for meat preservation in refrigerated storage.

Keywords: Food preservation, Minced meat, Nanoemulsion.

Introduction

Beef is a significant source of nutrition and plays an essential role in many diets around the world. Its nutrient density and high-quality protein make it a valuable food choice for various demographics[1].

Foodborne bacteria pose significant health hazards, leading to a range of illnesses that can vary from mild gastrointestinal discomfort to severe, life-threatening conditions. Foodborne illnesses affect millions globally each year, with the World Health Organization (WHO) estimating that unsafe food causes approximately 600 million cases and 420,000 deaths annually. Children under five years old are particularly vulnerable, accounting for nearly 30% of foodborne deaths[2].

The increasing prevalence of foodborne illnesses has prompted research into natural antimicrobial agents, particularly essential oils. Nanoemulsions, which enhance the solubility and bioactivity of these oils, have shown promise in combating foodborne pathogens [3].

Nanoemulsions consist of tiny droplets (typically less than 100 nm) that improve the bioavailability of essential oils. The reduction in droplet size increases the surface area, enhancing contact with bacterial cells and facilitating penetration through bacterial membranes. This mechanism allows for more effective disruption of bacterial cell integrity and metabolism compared to non-nanoemulsified oils[4].

Ginger (*Zingiber officinale*) has a rich history of use in food preservation, dating back thousands of years, particularly in Asian cultures. Historically,

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(Received 26 November 2024, accepted 22 December 2024)
DOI: 10.21608/EJVS.2024.339583.2517
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ginger was utilized not only for its flavor but also for its medicinal properties, serving as a remedy for ailments such as nausea and digestive issues. Its antibacterial qualities made it an effective preservative, helping to extend the shelf life of various foods. In ancient Rome, ginger was highly valued and traded extensively, often being incorporated into preserved forms for culinary use. By the medieval period, ginger was commonly found in Europe, where it was used in sweet confections like gingerbread, which were believed to have medicinal benefits. Studies have shown that ginger paste can effectively inhibit microbial growth in perishable foods such as fish, demonstrating its potential as a natural preservative that enhances food safety while maintaining quality. The active compounds in ginger, such as gingerol and shogaol, contribute to its antibacterial effects, making it a valuable ingredient in both historical and contemporary food preservation practices[5].

Ginger essential oil has been noted for its antimicrobial properties. When formulated into a nanoemulsion, its effectiveness against pathogens such as Salmonella and *E. coli* is significantly enhanced. Studies indicate that ginger nanoemulsion can achieve lower minimum inhibitory concentrations (MICs) compared to its non-nanoemulsified form, making it a potent natural preservative in food applications[6].

Thyme (*Thymus vulgaris*) has been historically significant in food preservation, particularly due to its potent antimicrobial and antioxidant properties. Ancient Egyptians utilized thyme for embalming, while the Greeks valued it for its aromatic qualities, often using it in baths and as incense in temples. Its preservation capabilities stem from bioactive compounds such as thymol and carvacrol, which effectively inhibit microbial growth and lipid oxidation, making thyme a popular choice for extending the shelf life of meat and seafood products. In traditional medicine, thyme was recognized not only for its culinary uses but also for its therapeutic effects, including its application as an antiseptic and antibiotic. The herb's rich composition of flavonoids and phenolic acids further enhances its role as a natural preservative, providing both flavor and health benefits while aligning with modern consumer preferences for natural food additives [7].

Thyme essential oil, particularly its active component thymol, has demonstrated strong antibacterial activity. Research has been showed that thyme nanoemulsion exhibits superior efficacy against foodborne pathogens like *S. aureus* and *L. monocytogenes*. The MIC values for thyme nanoemulsion are lower than those for pure thyme oil, indicating enhanced antibacterial properties due to the nanoencapsulation process. Furthermore, thyme nanoemulsion has shown significant antiadhesion effects, reducing bacterial adhesion by over 67% at sub-inhibitory concentrations[8].

In addition, Lemongrass (*Cymbopogon citratus*) has a long history of use in food preservation, particularly in Southeast Asian cuisines, where it not only enhances flavor but also serves as a natural preservative due to its antimicrobial and antioxidant properties. Traditionally, lemongrass has been employed to inhibit the growth of bacteria and fungi in various food products, effectively extending their shelf life. The essential oil extracted from lemongrass, rich in compounds like citral, has been shown to possess significant antibacterial activity against common foodborne pathogens, making it an effective alternative to synthetic preservatives. In addition to its preservative qualities, lemongrass has been recognized for its medicinal uses, including its application in traditional medicine for digestive issues and respiratory conditions. Recent studies support its efficacy in preserving meat products and other perishables, demonstrating that formulations containing lemongrass can significantly reduce microbial counts while maintaining organoleptic qualities, thus highlighting its dual role as both a flavoring agent and a natural preservative in modern food preservation practices [9].

The use of ginger, thyme, and lemongrass nanoemulsions represents a promising approach to enhance the antibacterial properties of essential oils against foodborne bacteria. These formulations not only improve the efficacy of the natural compounds but also offer potential applications in food preservation and safety. Therefore, the current study aimed to investigate the antibacterial effects of the previously mentioned nanoemulsions against foodborne *S. aureus* and *E. coli* isolated from minced meat samples in Qalubiya governorate.

Material and Methods

Collection and preparation of samples

Two Kg and four-hundred and fifty grams of fresh beef were purchased from a high-quality butcher in Benha city. Meat was minced and prepared in Animal Health Research Institute – Benha lab. Samples were divided into fourteen 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[10].

Samples were followed by inoculating of bacterial suspension of *E. coli* and *S. aureus*, in a concentration of nearly 4 log10 CFU/g, separately; and were left up to 15 minutes before treatment of the inoculated samples with nanoemulsions (NEs) by direct addition for further 15 minutes, after which the experiment zero time was recorded.

Preparation and characterization of essential oil based nanoemulsion (NE)

Ginger, thyme and lemon grass nanoemulsions were prepared in the unit of nanomaterials, Animal Health Research Institute (AHRI), with a concentration of 20%, which was prepared according to Pouton and Porter[11]. Nano-droplet size was determined in animal health research institute.

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).

Experimental grouping [12]

2450 grams of minced beef were equally divided into six groups as follow:

G1: Control positive untreated minced beef, inoculated with *E. coli* without treatment

G2: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 1.0% ginger nanoemulsion (GNE).

G3: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 2.0% ginger nanoemulsion (GNE).

G4: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 1.0% thyme nanoemulsion (TNE).

G5: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 2.0% thyme nanoemulsion (TNE).

G6: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 1.0% lemon grass nanoemulsion (LNE).

G7: 175 g minced beef +~ 4 log10 CFU/g *E. coli* + 2.0% lemon grass nanoemulsion (LNE).

G8: Control positive untreated minced beef, inoculated with *S. aureus* without treatment

G9: 175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 1.0% ginger nanoemulsion (GNE).

G10: 175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 2.0% ginger nanoemulsion (GNE).

G11:175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 1.0% thyme nanoemulsion (TNE).

G12: 175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 2.0% thyme nanoemulsion (TNE).

G13: 175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 1.0% lemon grass nanoemulsion (LNE).

G14: 175 g minced beef +~ 4 log10 CFU/g *S. aureus* + 2.0% lemon grass nanoemulsion (LNE).

Bacteriological examinations

After preparation of serial dilutions according to 6887-1 [13], i.e. mixing of 25 g of mince samples with 225ml of sterile 0.1% peptone water by mean of stomacher; followed by tenth fold serial dilutions, Control and treated meat mince were examined for their *Staphylococcus aureus* and *Escherichia coli* counts every three days of refrigeration following the ISO standards [14 and 15].

Reduction percent was calculated according the formula:

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

Where B = mean bacterial count in the next examination days, A = mean bacterial count at the zero time.

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 ($P \leq 0.05$) being regarded statistically significant.

Results

Characterization of the used nanometrials

Nanoemulsions droplet size was determined by Microtrac® size analyzer, results revealed that the droplet size was 17.94, 139.5 and 49.3 nm for ginger, thyme and lemon grass NEs, respectively.

Antibacterial effect of the used nanoemulsions

In the current study, the antibacterial effect of GNE, TNE and LNE on *S. aureus* and *E. coli* experimentally inoculated, in minced beef during chilling condition at $4^{o}C\pm1^{o}C$ was recorded; where, mean counts at the zero day were 4.1 and 4.3 $\log_{10}$ CFU/g for *E. coli* and *S. aureus*, respectively.

Fig. (1) and Table (1) showed that the different investigated treatments inhibited *E. coli* multiplication significantly in comparison with control group which showed spoilage signs after the 6^{th} day of chilling storage (5.8 $\log_{10}$ CFU/g). Results revealed a direct correlation between the additive's concentration, time of interaction and the bacterial concentration; where, higher concentration revealed higher reduction %. Results revealed reduction between 1.6-2.8 logs; where the maximum reduction was recorded in the LNE 2.0% treated group (68.3%).

Fig. (2) and Table (2) showed that the different investigated treatments inhibited *S. aureus* multiplication significantly in comparison with control group which showed spoilage signs after the 6^{th} day of chilling storage (6.0 $\log_{10}$ CFU/g). Results revealed a direct correlation between the additive's concentration, time of interaction and the bacterial counts; where, higher concentration revealed higher reduction %.

Results revealed reduction between 2.1-3.3 logs; where the maximum reduction was recorded in the LNE 2.0% treated group (76.7%) at the 18^{th} day of storage. Referring to the recorded results, antibacterial effect of the used nanoemulsions (NEs) appeared to be dose dependent; where, higher additive concentration had higher reduction effects on the examined foodborne bacteria.

LNE showed the most potent inhibitory effect against *E. coli* and *S. aureus*, followed by ginger and thyme NEs. Moreover, *S. aureus* revealed higher sensitivity to the used NEs than *E. coli.*

Furthermore, all of the used NEs had a preservative potential appeared through extension of the overall sensory acceptability of the treated samples in relation to the control samples; which appeared signs of decomposition after the 6^{th} day of refrigerated storage.

Discussion

Meat products are a vital component of human nutrition and have been integral to diets worldwide for centuries. Meat is an excellent source of complete protein and fats, providing all essential amino acids and fatty acids necessary for muscle growth, repair, and overall health. In addition, meat, particularly red meat, is rich in B vitamins, including B12, iron and zinc which is essential for nerve function and the formation of red blood cells[16].

Bacterial contamination of meat is a significant public health concern, primarily arising from sources such as improper handling, inadequate cooking, and cross-contamination during food preparation. Common pathogens, including *E. coli* and *S. aureus*, can enter meat products at various stages—from slaughtering to processing and distribution[17]. These bacteria can proliferate if meat is stored at incorrect temperatures or left unrefrigerated for extended periods. The consumption of contaminated meat poses serious health risks, leading to foodborne illnesses characterized by symptoms such as nausea, vomiting, diarrhea, and abdominal pain. In severe cases, these infections can result in long-term health complications or even death, particularly in vulnerable populations like children, the elderly, and individuals with weakened immune systems[18]. To mitigate these risks, proper food safety practices, including thorough cooking, proper storage, and hygiene during food handling, are essential.

Efforts to mitigate foodborne bacteria in meat products increasingly focus on the use of natural compounds, which offer a promising alternative to synthetic preservatives and chemical treatments[19]. Researchers are exploring the antimicrobial properties of various natural substances, such as essential oils, plant extracts and spices that can be incorporated into marinades, coatings, or even injected into the meat to enhance its safety and shelf life[20].

Natural and synthetic meat preservatives differ significantly in terms of safety, effectiveness, and antibacterial effects. Natural preservatives, such as herbs and spices, have been shown to enhance the shelf life of meat while posing fewer health risks compared to synthetic options. For example, thyme and rosemary can extend meat's shelf life significantly, with thyme showing effectiveness for up to 60 days, while also minimizing health concerns associated with synthetic preservatives like butylated hydroxytoluene (BHT), which has been linked to various health issues including cancer risks and hyperactivity in children. In terms of effectiveness, synthetic preservatives are often favored for their guaranteed antibacterial properties and longer shelf-life extension; however, they may compromise the sensory qualities of the meat and introduce potential side effects. Additionally, natural preservatives possess inherent antibacterial properties that can effectively inhibit foodborne pathogens without the adverse effects commonly associated with synthetic chemicals, making them a preferable choice for enhancing food safety. Overall, while both types of preservatives can be effective in extending meat shelf life and ensuring safety, natural options are increasingly recognized for their health benefits and lower risk profile[3].

Application of nanotechnology in food preservation represents a cutting-edge approach to enhance food safety and extending shelf life. Incorporating essential oils in nanoemulsion form, which consist of tiny droplets of essential oils suspended in a liquid, for meat preservation has emerged as an innovative approach to enhance food safety and extend shelf life through improving the bioavailability and antimicrobial effectiveness of these natural compounds[21]. Essential oils such as ginger, thyme and lemongrass have demonstrated significant antibacterial properties, effectively inhibiting pathogens like *E. coli* and *S. aureus*. When applied to meat products, these nanoemulsions can create a protective barrier that not only reduces microbial growth but also helps retain the meat's sensory qualities, including flavor and freshness[22]. This method offers a dual benefit: it aligns with consumer preferences for natural preservation techniques while providing a sustainable solution to combat foodborne pathogens and minimize spoilage, ultimately contributing to safer and longer-lasting meat products[23].

Referring to the current recorded results, all of the used nanoemulsions revealed significant antibacterial effect on *E. coli* and *S. aureus* in minced meat samples; which appeared to be dose dependent; where, higher additive concentration had higher reduction effects on the examined foodborne bacteria; which agree with the recorded results of Noori *et al.* [24], Bhat and Bhat [25] and Bakheet *et al.* [12] who recorded significant reductions in the treated foodborne bacterial counts post-treatment with ginger, thyme and lemongrass essential oil based nanoemulsions with concentrations ranged from 0.5% to 5.0% without adverse affecting the sensory characters of the treated meat samples.

The tested NEs have demonstrated significant antibacterial effects, particularly against *S. aureus* and *E. coli*. Their potency may be attributed to its nanoscale size, which enhances the bioavailability and stability of its active compounds. This sustained release enhances the antibacterial activity over time, effectively disrupting microbial cell membranes and leading to cell lysis[25].

The mechanism of action involves the penetration of gingerol of ginger; thymol and carvacrol of thyme; citral of lemongrass and other active components into the phospholipid bilayer of bacterial membranes, increasing permeability and causing leakage of cellular contents, which ultimately results in microbial death[23, 27, 28].

Although, the present results indicated that ginger and thyme NEs also possess antibacterial properties, though they are generally less potent than lemongrass; which was previously recorded by Gago *et al.* [29] who concluded superiority of the used LNE over the other examined oils; which may be attributed to the nanosize of the used emulsions, bioavailability of the active components of each essential oil and the extent of microbial sensitivity to the used material[30].

Moreover, *S. aureus* revealed higher sensitivity to the used NEs than *E. coli*. The higher sensitivity of Gram-positive bacteria to food preservatives compared to Gram-negative bacteria can be attributed to several structural and physiological differences; such as, cell wall composition, where Gram-positive bacteria possess only peptidoglycan layer; whereas in contrast, Gram-negative bacteria have an additional lipopolysaccharides outer membrane that acts as a barrier, making it more challenging for preservatives to penetrate and exert their effects[31].

Furthermore, all of the used NEs had a preservative potential appeared through extension of the overall sensory acceptability of the treated samples in relation to the control samples; which appeared signs of decomposition after the 6th day of refrigerated storage; which can be attributed to the noticed antibacterial and antioxidant effects of the used essential oils that was maximized in to nanoemulsion form.

Conclusion

The dual action of essential oils—acting as both antimicrobial agents and antioxidants—makes them valuable natural preservatives for meat products. All of the used essential oil based nanoemulsion revealed potent antibacterial effect, particularly against *E. coli* and *S. aureus*. Lemongrass nanoemulsion appeared to be more potent than ginger and thyme NEs, respectively. Their ability to reduce spoilage organisms and inhibit oxidative processes not only enhances food safety but also improves the overall quality of the meat during storage. As consumer demand for natural additives rises, the incorporation of essential oils based nanoemulsion into meat preservation strategies presents a promising alternative to synthetic preservatives.

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.

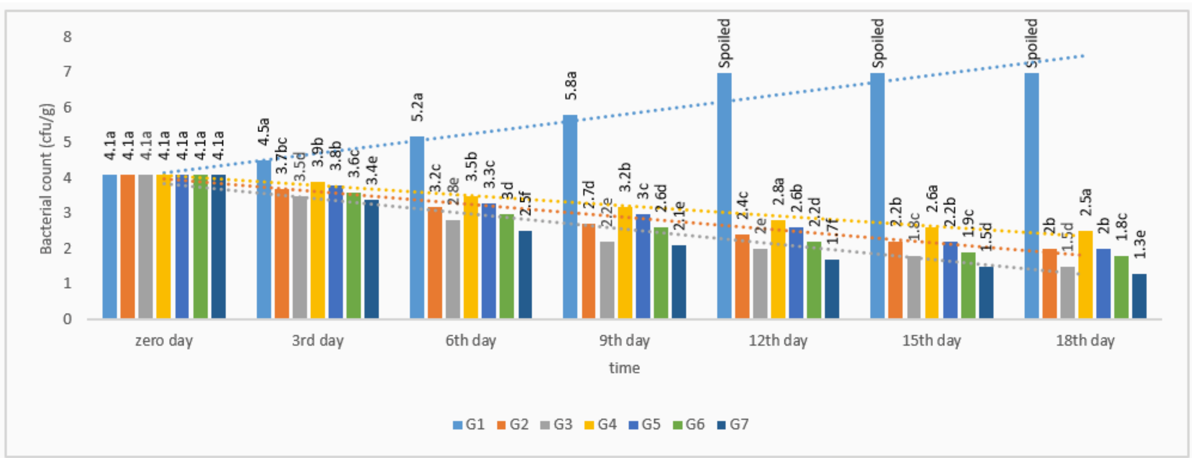

Fig. 1. Mean *E. coli* counts (log_{10} CFU/g) in the different groups of the experiment

[abcd] Different superscript letters within same day indicates significant difference ($P≤0.05$); S: spoiled apparently

TABLE 1. Reduction (%) of *E. coli* counts in the treated groups

Day	G2	G3	G4	G5	G6	G7
3rd day	9.8	14.6	4.9	7.3	12.2	17.1
6th day	22.0	31.7	14.6	19.5	26.8	39.0
9th day	34.1	46.3	22.0	26.8	36.6	48.8
12th day	41.5	51.2	31.7	36.6	46.3	58.5
15th day	46.3	56.1	36.6	46.3	53.7	63.4
18th day	51.2	63.4	39.0	51.2	56.1	68.3

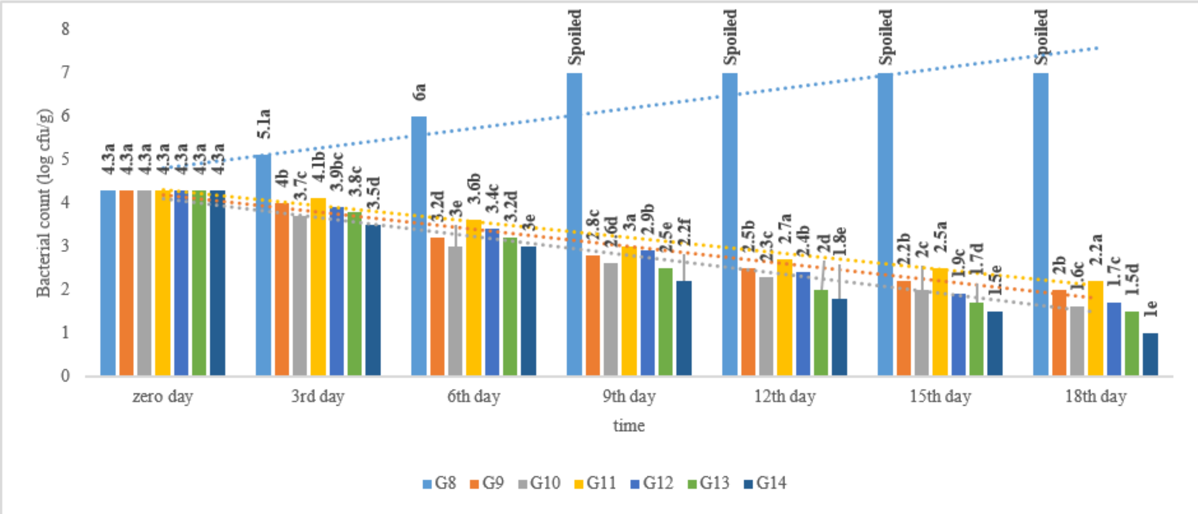

Fig. 2. Mean *S. aureus* counts (log_{10} CFU/g) in the different groups of the experiment

[abcd] Different superscript letters within same day indicates significant difference ($P \leq 0.05$); S: spoiled apparently

TABLE 2. Reduction (%) of *S. aureus* counts in the treated groups

Day	G9	G10	G11	G12	G13	G14
3rd day	7.0	14.0	4.7	9.3	11.6	18.6
6th day	25.6	30.2	16.3	20.9	25.6	30.2
9th day	34.9	39.5	30.2	32.6	41.9	48.8
12th day	41.9	46.5	37.2	44.2	53.5	58.1
15th day	48.8	53.5	41.9	55.8	60.5	65.1
18th day	53.5	62.8	48.8	60.5	65.1	76.7

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التأثير المضاد للبكتيريا لبعض المستحلبات النانوية المستخلصة من الأعشاب ضد بعض البكتيريا المنتقلة عبر الغذاء

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الملخص

ظهرت مستحلبات الزيوت العطرية النانوية كاستراتيجية واعدة لتحسين الخواص الظاهرية والجودة الميكروبيولوجية وإطالة مدة حفظ منتجات اللحوم من خلال الاستفادة من خصائصها المضادة للميكروبات ومضادات الأكسدة. لذلك، تمت دراسة التأثير المضاد للبكتيريا للمستحلبات النانوية من زيوت الزنجبيل والزعتر وعشبة الليمون على معزولات الايشرشيا كولاي والاستافيلوكوكس اوريس المعزولة من اللحم المفروم بتركيزات 1.0 و 2.0٪ لكل منهما: حيث تم حقن عينات اللحم المفروم ببكتيريا الايشرشيا كولاي والاستافيلوكوكس اوريس والمستحلبات النانوية وحفظها في الثلاجة بعد خلطها جيداً. أظهرت النتائج فروقاً معنوية بين المجموعات التي تمت معالجتها بالمستحلبات النانوية ظهرت من خلال الانخفاض الملحوظ في اعداد بكتيريا الايشرشيا كولاي والاستافيلوكوكس اوريس مع الحفاظ على خصائص الجودة الحسية المرغوبة اثناء فترة التخزين مقارنة بالمجموعة الضابطة التي أظهرت علامات الفساد بعد اليوم السادس من التخزين المبرد. كشفت النتائج عن وجود علاقة طردية بين تركيز المادة المُضافة ووقت التفاعل والعد البكتيري؛ حيث أظهر التركيز الأعلى (2.0%) نسبة انخفاض أعلى في العد البكتيري وبالتالي إطالة فترة صلاحية اللحوم المُعالَجة. وكشفت النتائج عن انخفاض في العد البكتيري اثناء الدراسة يتراوح بين 1.6-2.8 (لو$_{10}$ خلية/جرام) لبكتريا الايشرشيا كولاي، و2.1-3.3 (لو$_{10}$ خلية/جرام) لبكتريا الاستافيلوكوكس اوريس مما يكشف عن مستويات حساسية اعلى لبكتيريا الاستافيلوكوكس اوريس تجاه المستحلبات المستخدمة مقارنة ببكتيريا الايشرشيا كولاي. حيث تم تسجيل الحد الأقصى للانخفاض في العد البكتيري في المجموعة المُعالجة بمستحلب زيت عشبة الليمون (2.0%) 68.3% و76.7%، على التوالي). وعلاوة على ذلك، أظهرت النتائج قدرة واعدة للمستحلبات المسخدمة في إطالة العمر التخزيني لمجموعات اللحم المفروم المعالجة مع الحفاظ على الخصائص الحسية المرغوبة؛ لذلك، يمكن التوصية باستخدام المستحلب النانوية للزيوت العطرية لهذه الأعشاب التقليدية لحفظ اللحوم في التخزين المبرد مع الحفاظ على التركيزات المستخدمة.

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