



Efficiency of Free and Nano-Encapsulated Ginger essential oil (*Zingiber officinale*) on Survival of *Staphylococcus aureus*, Microbial Shelf-Life Indicators, and Deterioration Criteria in Minced Beef Meat Model

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Abstract

The food industry is grappling with contamination issues, and natural antimicrobials can potentially enhance food safety by reducing the microbiological risk of minced beef. In this study, we investigated the ability of ginger (*Zingiber officinale*) essential oil (GEO) (40 µL/ml) and its nano-encapsulated form (GEO-Nps) (5 µL/ml) to control the deterioration and microbiological growth in raw minced beef meat as an actual food model. Ginger essential oil was nano-encapsulated in a mixture of chitosan and arabic gum to improve its stability and solubility. The size, and morphology of the obtained nanoparticles were studied with a zeta-sizer and TEM. The GEO-Nps showed a particle size of 233nm with a spherical shape. The antibacterial activity of GEO and GEO-Nps were evaluated using disk diffusion against *S. aureus* with a diameter of 12.5 and 16 mm, respectively. Two experiments were conducted to determine safety and spoiling characteristics, one with pathogen-inoculated samples and the other with non-inoculated samples. The minced beef meat samples were examined for their keeping quality by aerobic bacterial, *Staphylococcal* and *Enterobacteriaceae* counts, as well as measuring pH, TBA, and TVBN immediately after preparation (zero time) and throughout 14 days of chilled storage (4±1°C). The study reveals that GEO-Nps, when combined with original GEO, effectively inhibits *Staphylococcus aureus* and extends the shelf-life of minced beef meat from 6 to 14 days. In all sensory analyses, nanoencapsulation was the most effective form followed by GEO.

Keywords Ginger essential oils · Nanoencapsulation · *Staphylococcus aureus* · Antimicrobial · Antioxidant · Minced beef meat

Introduction

Meat, a high-quality food rich in protein, minerals, and vitamins, is more susceptible to microbial deterioration due to its high moisture, protein, and fat content [19]. Consequently, Meat products like minced meat are highly perishable during processing, transportation, and storage, making

preservation techniques essential to extend their shelf-life [5]. Microbial contamination of food during production causes foodborne diseases. Additionally, some bacteria are reported to produce toxins [60].

Staphylococcus aureus is a significant public health concern due to its significant role in foodborne illness, posing numerous risks to both the food industry and global consumers [43]. Furthermore, *S. aureus* produces heat-stable enterotoxins, leading to enteritis, septicemia, and resistance to some antibiotics [1, 22].

Researchers suggest that natural antioxidants and antimicrobial compounds can effectively control lipid oxidation and microbial spoilage in fresh meat during cold storage [6, 10, 11, 56]. Several studies have focused on discovering new bioactive compounds with antioxidant and antimicrobial properties from natural products and evaluating their potential use in food preservation [12]. Recently, consumers

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preferred using natural preservatives rather than synthetic preservatives, which have unfavorable side effects [31].

Essential oils (EOs) are volatile aromatic substances extracted from various plant parts, commonly used in food as flavoring agents and food additives [11, 66]. But due to their potent antioxidant and antimicrobial properties, they are also known as natural preservatives, which are an excellent source of bioactive compounds that have been shown to a special antimicrobial, antifungal, and antioxidant activities [40, 50]. In the food industry, their bioactive capabilities are primarily used to delay oxidative product deterioration and combat foodborne pathogens, which increases food shelf life, enhances sensory quality, and increases safety for consumers [58, 72]. Their main antibacterial action derives from impairing membrane integrity, which alters membrane potential, impacts permeability, and results in the leakage of essential ions and metabolites. Enzymatic and respiratory processes are hampered by this disturbance, which ultimately results in cell death. The cytoplasmic membrane is the target of phenolic chemicals such as carvacrol and thymol, which upset cellular equilibrium [11, 29, 41]. Additionally, small molecules of essential oils have a practical application in extending the shelf-life of food through their Anti-*Aspergillus* mechanisms [28].

Ginger essential oil (GEO) is a volatile oil that is obtained from ginger roots, and US FDA has listed ginger as "Generally Recognized as Safe" (GRAS) [57]. In addition, ginger is known to have a potent antimicrobial effect against *S. aureus* [57, 71]. Alternatively, some EOs can negatively impact the sensory qualities of food products depending on the primary EOs concentration and type of food [13]. One approach to remove these obstacles and enable EOs to display their preservative potential is encapsulating them by nanoparticles [55]. Such approach enables researchers to by-pass many obstacles in using EOs such as interactions with fat, protein, and starch components of the food matrix, low solubility in the aqueous phase, alteration of sensory properties of food at high concentration, sensitivity to oxygen, light, and heat during processing, utilization, and storage [32]. Nano-encapsulation of EOs improves controlled release, bioavailability, solubilization, volatility reduction, chemical and thermal stability, and distribution regulation, overcoming issues in their use [30]. The nanoencapsulation of EOs enhances their biological activity by increasing their surface area, enabling lower doses and improved sensory quality [50]. One of the nano-based delivery systems for the enclosure of GEO is nanoemulsions that bring the active compounds to the desired site, enhance their efficiency, and protect them from the environmental factors [20]. Also, nanoemulsions can be used to regulate the functional components delivery, and add bioactive compounds to the solid surface [14]. As mentioned before, other previous studies

deal with the essential oil's effect on microbial viability. Therefore, the novelty in the current study is to test that hypothesis in an actual food model, which is a minced meat model that hasn't been tested before. This was targeted through evaluation of the potential effect of using GEO in its free or its nano-form as a natural antimicrobial and antioxidant in the minced beef model.

Materials and Methods

Materials

GEO was purchased from the Unit for Pressing and Extracting Natural Oils, of the National Research Center, Giza, Egypt. Baird Parker Agar base (CM 0275), was purchased from Oxoid, Hampshire, England, Peptone water (PW, CM009), Plate Count Agar (PCA, CM0325B); Violet Red Bile Glucose agar plates (VRBG, CM1082B) were purchased from (Oxoid, Hampshire, England). Nutrient broth was purchased from (Difco, Sparks, Maryland).

Low molecular weight chitosan (CS), Arabic gum, and Sodium Tripolyphosphate (STPP) were bought from Siga-Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and Tween 80 were purchased from (Siga-Aldrich, Singapore).

Gram-positive *Staphylococcus aureus* reference strain (ATCC13565) was obtained from Microbiological Resource Center (MIRCEN), Ain Shams University, Cairo, Egypt. Stocks of *S. aureus* strain was kept in nutrient broth and 10% glycerol in the freezer. Cultures were propagated twice in nutrient broth and incubated for 24 h at 37°C before being used in further experiments.

Methods

Preparation of Ginger Essential Oil Nanoparticles (GEO-Nps)

GEO-Nps were prepared by a method previously described by Ahmadi, Saber, Bagheri, and Mahdavinia [4] with some modifications. Chitosan was dissolved in acetic acid (1% v/v) with magnetic stirrer at 1500 rpm for 15 min. Then, 1 ml of tween ® 80 was added to the chitosan solution as an emulsifier with stirring for 15 min. GEO was added to chitosan- tween ® 80 mixture at a concentration of 20 µl/ml with stirring for 1 h at 1500 rpm. The Arabic gum was dissolved in distilled water and incorporated into the mixture, followed by the addition of (STPP) solution with stirring at 1000 rpm for 15 min. Particle size and particle dispersion index (PDI) for the obtained nanoparticles were determined by Zetasizer (Malvern, UK). Transmission electron microscope (TEM) measurement for the nanoparticles was

obtained using JEOL (JEM-1400). The following is a schematic diagram showing the process of nanoencapsulation:

Antimicrobial Activity of GEO and GEO-Nps

Disk Diffusion Assay The antimicrobial activity of GEO and GEO-Nps against *S. aureus* was investigated using a disk diffusion assay [36]. *S. aureus* was cultured in nutrient broth at 37 °C for 24 h and prepared to a turbidity of 0.5 McFarland Standard. The bacterial suspension was added to the top of the agar, and the agar was punched with the addition of antimicrobial compounds. The plates were subsequently incubated for 24 h at 37 °C, and the zone of inhibition was calculated by measuring the diameter of the clear zone in mm.

Determination of Minimum Inhibitory Concentration (MIC) The stock solution was prepared in DMSO and transferred to 10 ml of nutrient broth to obtain a final concentration of (40, 20, 10, 5, 2.5 µl/ml) and (20, 10, 5, 2.5, 1.25 µl/ml) for GEO and GEO-Nps, respectively. Subsequently, 50 µl/ml (1×10^7 CFU/mL) of bacterial suspension was added to the mixture and incubated for 24 h at 37 °C. MIC was identified by taking out 100 µl from each concentration and plating it over the Baird parker agar plate, and incubated at 37 °C for 24 h. MIC is the lowest concentration with no visible growth [38].

Experimental Design

The Effect of Using Oil as a Natural Preservative Fresh minced beef meat was purchased from the butcher shop and transferred quickly to an icebox to the laboratory, where the treatments were applied. To evaluate the effect of using oil as a natural preservative, minced beef meat was randomly divided into 3 groups G1 (control without any treatment), G2 (minced meat treated with GEO “40 µL/ml”), and G3 (minced meat treated with GEO-Nps “5 µL/ml”).

Challenge Study with *S. aureus* For challenge study with *S. aureus*, minced meat sample were inoculated with 10^4 CFU/mL of *S. aureus* then it was divided into 3 groups as following; G4, (control, minced meat inoculated with *S. aureus* only), G5 (minced meat inoculated with *S. aureus* and treated with GEO), and G6 (minced meat inoculated with *S. aureus* and treated with GEO-Nps). Samples from each group were placed individually into sterile plastic petri dishes. These were sealed using oxygen-impermeable polyethylene film and refrigerated at 4 °C.

All group treatments (G1 – G6) were performed in triplicate, and samples from each treatment group were examined for their bacteriological, chemical, and sensory criteria.

Microbiological Evaluation of Meat Samples

The Effect of Using Oil as Natural Preservative on Shelf – Life of Beef Minced Meat For determination of the aerobic plate count, 10 g from each sample of G1, G2, and G3 were aseptically weighed into 90 ml of 0.1% peptone water in a sterile plastic bag and then blended in a stomacher for 2 min to obtain a dilution of 10^{-1} . Then, 0.1 mL from the diluted tubes was plated over the surface of Plate Count Agar and incubated at 35 °C for 48 h [62]. To enumerate the psychrotrophic bacteria, another set from the inoculated plate count agar was incubated at 7 °C for 7–10 days [70].

For *enterobacteriaceae* bacterial counts, samples were enumerated after incubation on VRBG agar plates at 37 °C for 24 h [42].

Challenge Study with *S. aureus* For enumeration of *S. aureus* in G4, G5, and G6, an aliquot from the samples was inoculated into Baird-Parker agar plates and incubated at 37 °C for 48 h [16, 17].

Physicochemical and Sensory Evaluation of Minced Beef Meat Samples

Five g from each group of minced meat samples were aseptically removed and homogenized with 20 mL DW for the determination of pH value using a pH meter (Lovibond Senso Direct).

While the Thio barbituric acid value (TBA) was evaluated by measuring the absorbance of supernatant, measured at 531 nm using a spectrophotometer (Unico 1200 series), and the TBA value was obtained by multiplying the reading by 7.8 according to the method described by Du, and Ahn [21].

The total volatile Basic Nitrogen was evaluated using macro-Kjeldahl distillation apparatus following the methodology described by Kearsley et al. [39].

To evaluate the sensory characteristics, a number of experienced panelists (9) from both males and females were selected from the Department of Food Hygiene and control at Faculty of Veterinary Medicine, Cairo University, Egypt. Panelists were chosen based on their previous experience in evaluating sensorial items of minced meat, however, a preparatory session was performed for all the panelists to be familiar with the measured sensory items. For all panelists, informed consent was obtained for experimentation with human subjects. Randomized samples were provided to the panelists without knowing the treatment type for the provided samples, then each panelist was asked to assess each group and to give a numerical value based on a hedonic scale from 1 to 9, where 1 means (extremely unacceptable) and 9 means (extremely acceptable) for the following

characteristics: color, flavor (smell), and overall acceptability. The sensory evaluation of minced meat was performed according to guidelines outlined by AMSA [7].

Statistical Analysis

The bacterial count was converted into a $\log_{10}$ CFU/g value. Then the variance among mean $\pm$ standard deviation was determined using SPSS (Statistical Package for Social Sciences) Version 26. (IL, Chicago, USA) software at three different replicates (3 samples/treatment for each occasion) using ANOVA “One-way Analysis of Variance”. Meanwhile, for the sensory assessment, the Generalized Linear Mixed Model (GLMM) was adopted to test the variance among different treatments where the explanatory variables were the panelists and the sessions (random effect), besides the oil treatment (fixed effect). Furthermore, multiple comparisons of the means for all tested parameters were performed using Post Hoc (least significant differences “LSD”) procedures between the mean values at a 95% level of confidence, where the value of $P < 0.05$ was considered significant.

Results and Discussion

Ginger essential Oil Nanoencapsulation

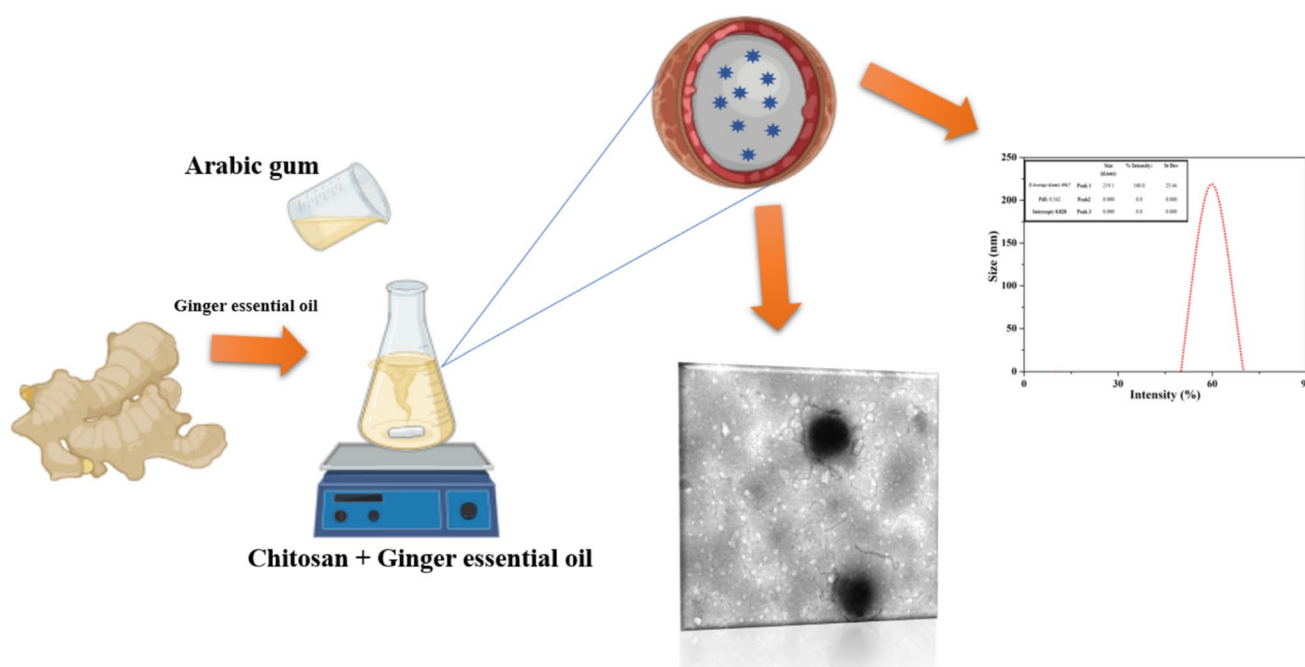
The encapsulation of essential oils is an alternative to improve solubility and enable their controlled release [8]. Chitosan and Arabic gum are non-toxic, food-grade, and

biodegradable polymers, with chitosan becoming water-soluble in acidic pH and Arabic gum opening easily at neutral pH [27, 59]. All these properties make these polymers suitable for food applications.

GEO-Nps is formed with an ionic gelation reaction, which begins with the interaction of chitosan with oil, forming nanoencapsulation as shown in (Scheme 1). The stabilization will occur after the addition of Arabic gum. The Arabic gum group (COO^-) will bind with the (NH_3^+) group of chitosan to form a polyelectrolyte complex bond [25]. Combining two polymers with different charges leads to an ionic interaction forming a polyelectrolyte complex bond, which strengthens the wall of nanoparticles and reduces the release of the oil [69]. In our study, the mean particle size of GEO-Nps was 219 nm, as shown in Fig. 1. According to the study of [74], the mean particle size of GEO encapsulated in chitosan nanoparticles was 233 nm. Moreover, the morphology of GEO-Nps indicated a spherical shape of the formed nanoparticles as presented in Fig. 1.

In-vitro Antimicrobial Activities of GEO and GEO-Nps on the Growth of *Staphylococcus aureus*

The antibacterial activity of GEO and its nanoencapsulated form was evaluated using disk diffusion assay and the microdilution method. The zone of inhibition of GEO and GEO-Nps against *S. aureus* was 12.5 and 16 mm, respectively. While at a concentration of 40 and 5 $\mu\text{L}/\text{mL}$ of GEO and GEO-Nps, respectively, the growth of *S. aureus* was significantly reduced (Fig. 2). Moreover, it is evident that the



Scheme 1 Experimental representation for ginger oil nanoencapsulation

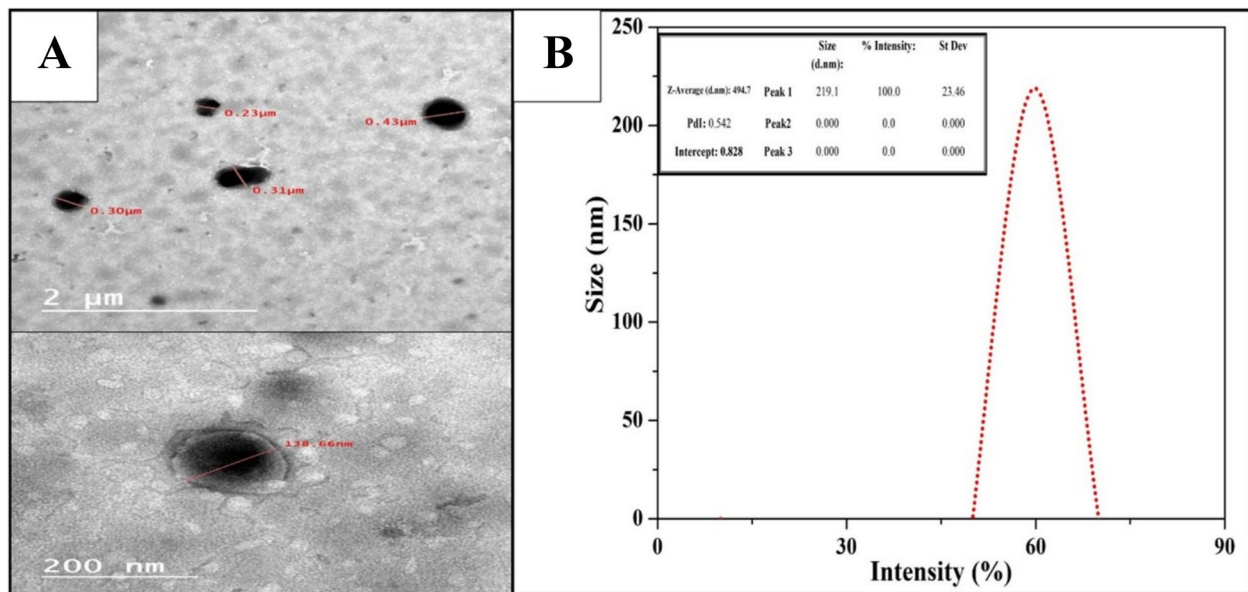
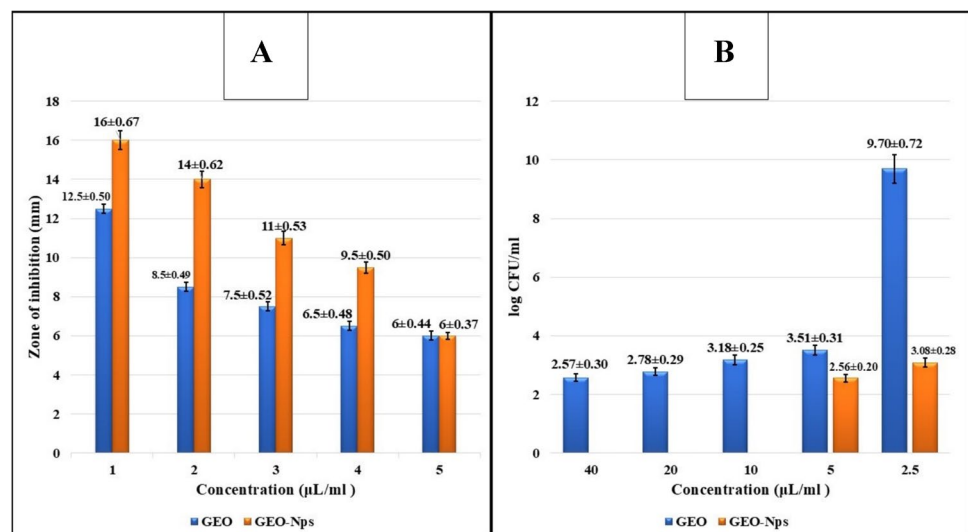


Fig. 1 **A** Transmission electron microscope (TEM) micrographs and **B** particle size distribution for the prepared ginger oil nanoparticle (GEO-Nps)

Fig. 2 **A** Zone of inhibition of GEO and GEO-Nps against *Staphylococcus aureus* using disk diffusion assay, **B** Determination of MIC of GEO and GEO-Nps against *Staphylococcus aureus* using broth dilution method



bacterial inhibition increased with the increasing concentration of GEO and GEO-Nps. In this regard, da Silva et al. [16, 17] reported a lower number with an inhibition zone of (8.9 mm), while higher results (17.1 mm and the MIC value was 1.0 mg/mL) were recorded by Wang et al. [71]. However, Yousefi et al. [74] reported higher results in GEO-loaded chitosan nanoparticles and free GEO against *S. aureus* (35 mm and 19 mm, respectively). Lei et al. [43] showed that GEO exhibited good antibacterial activity against *S. aureus*.

Effect of GEO and GEO-Nps on Shelf – Life of Beef Minced Meat Model

The Effect of GEO and GEO-Nps on total bacterial count, psychrotrophic bacterial count and *Enterobacteriaceae*

count in minced beef meat was studied. A variety of food preservatives can be utilized as antibacterial agents for food protection during storage.

Results in Table 1 represent the changes in total bacterial count (log CFU/g) of the treated and untreated meat samples. The total bacterial count was not significantly ($P > 0.05$) different between groups at the beginning of the storage, i.e., on day zero of the study, where the mean total bacterial count (log CFU/g) for (G1) was 4.44 log CFU/g while those values were 4.00 and 4.08 for (G2) and (G3), respectively. Additionally, after two days of storage, GEO (G2) and GEO-Nps (G3) showed a significant ($P < 0.05$) decrease in total bacterial count with mean values of 3.9 and 4.00, respectively, while the control treatment (G1) was 5.00 log CFU/g. By the 8th day of storage, both GEO

Table 1 Effect of ginger on total bacterial count expressed as mean±standard deviation (log₁₀ CFU/g)

Storage day	G1 (Control)	G2 (GEO)	G3 (GEO-Nps)
Zero	4.44±0.54 ^{a,I}	4.00±0.17 ^{a,I}	4.08±0.35 ^{a,I}
2nd	5.00±0.19 ^{b,I}	3.90±0.38 ^{a,II}	4.00±0.23 ^{a,II}
5th	6.00±0.17 ^{c,I}	4.04±0.31 ^{a,II}	4.26±0.26 ^{a,II}
8th	7.03±0.52 ^{d,I}	5.48±0.17 ^{b,II}	5.52±0.19 ^{b,II}
10th	NE	6.30±0.35 ^{c,I}	6.40±0.52 ^{c,I}
12th	NE	6.62±0.61 ^{c,I}	6.60±0.17 ^{c,I}
14th	NE	7.90±0.87 ^{d,I}	7.83±0.76 ^{d,I}

^{a-d}Values of mean±standard deviation with different superscripts within the same column are significantly ($P<0.05$) different

^{I-II} Values of mean±standard deviation with different superscripts within the same row are significantly ($P<0.05$) different

NE Not examined as it reached the spoilage limit in 8th day i.e., 7.03 log CFU/gram

G1 Control treatment (minced meat), G2 Minced meat+40 µL/ml of ginger essential oil (GEO), G3 Minced meat+5 µL/ml of nano encapsulated ginger essential oil (GEO-Nps)

and GEO-Nps had significantly ($P<0.05$) lower mean values of 5.48 and 5.52 log CFU/g, respectively, than the control treatment 7.03 log CFU/g. Furthermore, on day 8th of chilled storage, the control samples were fully ruined and putrefied, and they were removed from the course of study. Correspondingly, till the 12th day of storage, it was noticed that samples of (G2) and (G3) are on the borderline. Accordingly, it was clear that there is an increase in the shelf life of meat by five days during the chilled storage when using GEO (G2) and GEO-Nps (G3) compared to control (G1). This indicates that (GEO) and GEO-Nps are effective in delaying microbial spoilage during the storage period, where GEO-Nps It was shown to be a preferred preservative ingredient because it gives the same effect at very low concentration. Similar findings for comparison between the effect of EO emulsions and nanoemulsions on the microbial quality of different products, regardless of the type of natural oil, were reported by several authors [9, 44, 73]. Nazzaro, Fratianni, De Martino, Coppola, and De Feo [49] found that the GEO and GEO nano-based can affect both internal and external bacterial structures. They further added that the hydrophobic nature of the EOs might increase their permeability to the bacterial cell and be responsible for cell destruction. Reduction of droplet size to the nanomaterial scale promotes contact with microbial pathogens, resulting in cell death and lysis. Also, the components of EOs can easily access through the cell membrane [3]. Previous studies reported that the hydrophobicity of EOs interferes with the synthesis of proteins, enzymes, and bacterial cell membrane [15].

Changes in psychrotrophic bacterial count of the treated and untreated meat samples were presented in Table 2. Similar to the results obtained in the total bacterial count,

Table 2 Effect of ginger on psychrotrophic bacterial count expressed as mean±standard deviation (log₁₀ CFU/g)

Storage day	G1 (Control)	G2 (GEO)	G3 (GEO-Nps)
Zero	4.24±0.35 ^{a,I}	3.63±0.19 ^{a,II}	3.69±0.36 ^{a,II}
2nd	4.00±0.17 ^{a,I}	3.69±0.55 ^{a,I}	3.88±0.23 ^{a,I}
5th	6.14±0.26 ^{b,I}	4.85±0.69 ^{b,II}	4.20±0.48 ^{a,II}
8th	6.41±0.38 ^{b,I}	4.92±0.21 ^{b,II}	4.95±0.17 ^{b,II}
10th	NE	5.60±0.87 ^{b,I}	5.08±0.19 ^{b,I}
12th	NE	6.48±0.35 ^{c,I}	5.30±0.71 ^{b,II}
14th	NE	7.90±0.29 ^{d,I}	6.83±0.95 ^{c,II}

^{a-d}Values of mean±standard deviation with different superscripts within the same column are significantly ($P<0.05$) different

^{I-II} Values of mean±deviation with different superscripts within the same row are significantly ($P<0.05$) different

NE Not examined as it reached the spoilage limit in 8th day i.e. 6.41 log₁₀ CFU/gram

G1 Control treatment (minced meat), G2 Minced meat+40 µL/ml of ginger essential oil (GEO), G3 Minced meat+5 µL/ml of nano encapsulated ginger essential oil (GEO-Nps)

generally, the psychrotrophic bacterial counts were increased throughout the refrigerated storage period, especially for the control sample (G1). Moreover, there were significant ($P>0.05$) differences between groups at the beginning of the storage on day zero day of the study, where the mean of psychrotrophic bacteria (log CFU/g) for (G1) was 4.24 log CFU/g while those values were 3.63 and 3.69 for (G2) and (G3), respectively. The control sample had the highest progress rate of psychrophilic bacteria. By reaching the 8th day of chilled storage, and compared to control, the mean of psychrotrophic bacteria was significantly ($P>0.05$) lower for both GEO (G2) and GEO-Nps (G3), with mean values of 4.92 and 4.95, respectively, whereas (G1) (control) had a count of 6.41 (log CFU/g). After day 8th, the untreated control samples showed spoiling signs; therefore, it was withdrawn from the course of the experiment. In general, ginger-treated samples have results that are more effective in slowing down psychrotrophic bacterial growth, especially that coated in the GEO-Nps, which had the most effective inhibitory effect. In this regard, Noori et al. [50] observed that nanoemulsions-based ginger essential oil-based edible coat was more effective against bacterial growth on chicken breast fillet. The antimicrobial activity of EOs from spices and herbs is believed to be due to their rich content of phenolic compounds [61, 65]. The primary components of GEO, which is derived from ginger, are α -zingiberene and α -curcumene [64]. Furthermore, Wang et al. [71] reported that analysis of volatile components of GEO revealed the following components: α -Pinene, Camphene, Sabinene, α -Phellandrene, β -Phellandrene, Borneol, Farnesene, β -Bisabolene, Elemene, α -Selinene, γ -Elemene, α -Curcumene, Zingiberene, α -Farnesene, Zingiberone, Elemol, Geraniol, Geranialdehyde, 6-Gingerol,

Octadecadienoic acid, while the main components of the extracted GEOs were zingiberene and α -curcumene. Additionally, the GEO mechanism works by damaging the bacterial cell membrane, which allows macromolecules like bacterial proteins and nucleic acids to leak out. This, in turn, causes a decrease in the metabolic activity of the bacteria and, ultimately, bacterial cell death [71].

Concerning the *Enterobacteriaceae* count, results presented in Table 3 showed that, unlike the total bacterial count, the effect of ginger in inhibiting the *Enterobacteriaceae* count started immediately at the beginning of the storage (zero day), either for GEO (G2) or GEO-Nps (G3), when compared to control (G1). Where (G1) had significantly ($P < 0.05$) higher mean value of *Enterobacteriaceae* count of 4.37 log CFU/g, meanwhile it was 2.69 and 2.00 log₁₀ CFU/g in G2 and G3, respectively. The obtained results indicated that all treatments increased in their *Enterobacteriaceae* counts by storage; however, after two days of storage, the GEO (G2) and GEO-Nps (G3) showed a significant ($P < 0.05$) reduction in their *Enterobacteriaceae* counts with mean values of 3.26, 3.15 log CFU/g, respectively, compared to control (G1) 4.60 (log CFU/g). By the 8th day of storage, (G1) was totally spoiled and withdrawn from the experiment, while the *Enterobacteriaceae* counts were still 5.44 and 4.73 log CFU/g in (G2) and (G3), respectively. By the end of the storage period on 14th day of storage, it was noticed that the GEO (G2) showed a significantly ($P < 0.05$) higher *Enterobacteriaceae* counts compared to GEO-Nps (G), they were 7.6 and 6.45 log CFU/g, respectively. This finding agreed with those of [54, 75], who found that GEO is highly effective against G+ve and G-ve bacteria. Which makes ginger in its crude oil or nano-emulsion form a perfect natural preservative.

Table 3 Effect of ginger on *Enterobacteriaceae* count expressed as mean $\pm$ standard deviation (log₁₀ CFU/g)

Storage day	G1 (Control)	G2 (GEO)	G3 (GEO-Nps)
Zero	4.37 $\pm$ 0.52 ^{a, I}	2.69 $\pm$ 0.57 ^{a, II}	2.00 $\pm$ 0.78 ^{a, II}
2nd	4.60 $\pm$ 0.17 ^{a, I}	3.26 $\pm$ 0.19 ^{b, II}	3.15 $\pm$ 0.17 ^{b, II}
5th	6.82 $\pm$ 0.23 ^{b, I}	4.43 $\pm$ 0.17 ^{c, II}	4.58 $\pm$ 0.21 ^{c, II}
8th	7.26 $\pm$ 0.76 ^{b, I}	5.44 $\pm$ 0.59 ^{d, II}	4.73 $\pm$ 0.35 ^{c, III}
10th	NE	5.46 $\pm$ 0.21 ^{d, I}	5.46 $\pm$ 0.02 ^{d, I}
12th	NE	6.78 $\pm$ 0.88 ^{e, I}	6.00 $\pm$ 0.55 ^{e, I}
14th	NE	7.36 $\pm$ 0.42 ^{e, I}	6.45 $\pm$ 0.75 ^{e, II}

^{a-c}Values of mean $\pm$ standard deviation with different superscripts within the same column are significantly ($P < 0.05$) different

^{I-III} Values of mean $\pm$ deviation with different superscripts within the same raw are significantly ($P < 0.05$) different

NE Not examined as it reached the spoilage limit in 8th day i.e. 7.26 log₁₀ CFU/gram

G1 Control treatment (minced meat), G2=minced meat + 40 μ L/ml of ginger essential oil (GEO), G3 Minced meat + 5 μ L/ml of nano encapsulated ginger essential oil (GEO-Nps)

Effect of GEO and GEO-Nps on *Staphylococcus aureus* Count in Minced Meat Model

Results in Table 4 show the mean (log CFU/g), count of the surviving *Staphylococcus aureus* among G4 (control), G5 (GEO), and G6 (GEO-Nps), during the chilled storage period. It was observed that after two hours of chilled storage (0-time), there was no significant ($P > 0.05$) difference between all treatments, where the mean *Staphylococcus aureus* count for (G4) was 4.1 (log CFU/g) while those values for G5 and G6, were 4.69 and 4.41, respectively. This could be attributed to the short storage period in 0-time (only two hours), which was very short for either GEO or GEO-Nps to express their antimicrobial effect against *Staphylococcus aureus*. After two days of chilled storage, GEO-Nps showed a significant difference ($P < 0.05$) in higher inhibition of *Staphylococcus aureus*, where the mean value was 3.54 (log CFU/g) in comparison to 4.7 for control (G4) and 4.73 for Geo (G5). While after five days of storage, both GEO and GEO-Nps had significantly ($P < 0.05$) lower mean values of 2.69 and 2.60 (log CFU/g), respectively, than the control 5.04 (log CFU/g). By the end of the 6th day of storage, it was noticed that *Staphylococcus aureus* could not be recovered from minced meat treatments either for GEO or GEO-Nps. *Staphylococcus aureus* mean count in control treatment (G4) significantly ($P < 0.05$) increased till it reached 5.17 (log CFU/g), which is an unacceptable limit for minced meat according to [26]. It was obvious that there is an inverse relationship between *Staphylococcus aureus* survival and increasing the storage period of treatments containing ginger, either in its original essential oil form or its nanoencapsulated form, which ensures the antimicrobial effect of ginger against *Staphylococcus aureus* in the minced meat model, as Ginger's active ingredients prevent bacterial growth by disrupting membranes. These results were consistent with [50, 57, 71, 76] who reported that the GEO extracted from the ginger

Table 4 Effect of ginger on *Staphylococcus aureus* count expressed as mean $\pm$ standard deviation (log₁₀ CFU/g)

Storage day	G4 (Control)	G5 (GEO)	G6 (GEO-Nps)
Zero	4.10 $\pm$ 0.29 ^{a, I}	4.69 $\pm$ 0.87 ^{a, I}	4.41 $\pm$ 0.52 ^{a, I}
2nd day	4.70 $\pm$ 0.17 ^{b, I}	4.73 $\pm$ 0.69 ^{a, I}	3.54 $\pm$ 0.17 ^{b, II}
5th day	5.04 $\pm$ 0.19 ^{c, I}	2.69 $\pm$ 0.87 ^{b, II}	2.60 $\pm$ 0.69 ^{c, II}
6th day	5.17 $\pm$ 0.14 ^{c, I}	0.00 $\pm$ 0.00 ^{c, II}	0.00 $\pm$ 0.00 ^{d, II}

^{a-d}Values of mean $\pm$ standard deviation with different superscripts within the same column are significantly ($P < 0.05$) different

^{I-II}Values of mean $\pm$ standard deviation with different superscripts within the same raw are significantly ($P < 0.05$) different

G4 Control treatment (control inoculated with *S. aureus* only), G5 (minced meat treated with GEO) and G6 (minced meat treated with GEO-Nps)

has great antimicrobial activities on *Staphylococcus aureus*. In this regard, Wu et al., [73] reported that encapsulating bioactive compounds in nanoemulsions enhances the antibacterial efficacy of EOs, improving transport mechanisms through microorganism cell membranes. Chitosan's antibacterial activity is attributed to its interaction with anionic groups. Additionally, Wang et al. [71] tested the efficacy of GEO against *Staphylococcus aureus*, and the results revealed that GEO significantly inhibited the growth of *Staphylococcus aureus* [18, 24]. Moreover, Nano-encapsulation of essential oils (EOs) improves their use by controlling release, improving bioavailability, solubilization, reducing volatility, boosting chemical and thermal stability, and regulating distribution [30]. To our knowledge, very limited studies have tested the effect of GEO-Nps against *Staphylococcus aureus* in a minced meat model. However, Nouri & Khodaiyan [51] reported that nanoencapsulation has been effective in increasing the antioxidant activities of ginger as well as its antibacterial effect, especially against *Staphylococcus aureus* in vitro. Furthermore, Tang et al. [68] reported that the nano-encapsulated GEO prepared by a low-energy self-assembly method had a good inhibitory effect on the common spoilage bacteria, such as *S. aureus*, in chilled chicken. One of the main challenges and limitations of using nano-encapsulated essential oils as a preservation strategy for meat is the inherent instability of essential oil compounds. Research indicates that GEO contains volatile components that are sensitive to environmental factors, including temperature, light, oxygen, and pH variations. These environmental sensitivities can alter the antioxidant and antimicrobial efficacy of the encapsulated oil, leading to inconsistent results across different storage conditions and study durations [52].

Physicochemical Analysis and Sensory Evaluation

Results outlined in Fig. 3 illustrate the effect of adding GEO (G2) or GEO-Nps (G3) to minced beef meat on the shelf-life stability and antioxidant activity compared to non-treated samples (G1). pH has a significant ($P < 0.05$) impact on microbiological development that affects the shelf life of beef products [47]. Figure 3-A demonstrates the changes in pH of meat samples over the course of refrigeration. The pH of all samples markedly increased during the refrigeration period for 14 days. The initial pH of meat samples ranged from 5.82 to 5.6 among all treatments. However, the control treatment showed an unacceptably high pH level of 6.92 at the 9th day of storage. Meanwhile, both GEO and GEO-Nps treatments had lower values of 6 and 6.3, respectively, on the same storage day, then these values slightly elevated to 6.1 and 6.5 by the end of 14th day of chilled storage. These obtained results clearly indicate that ginger controlled the

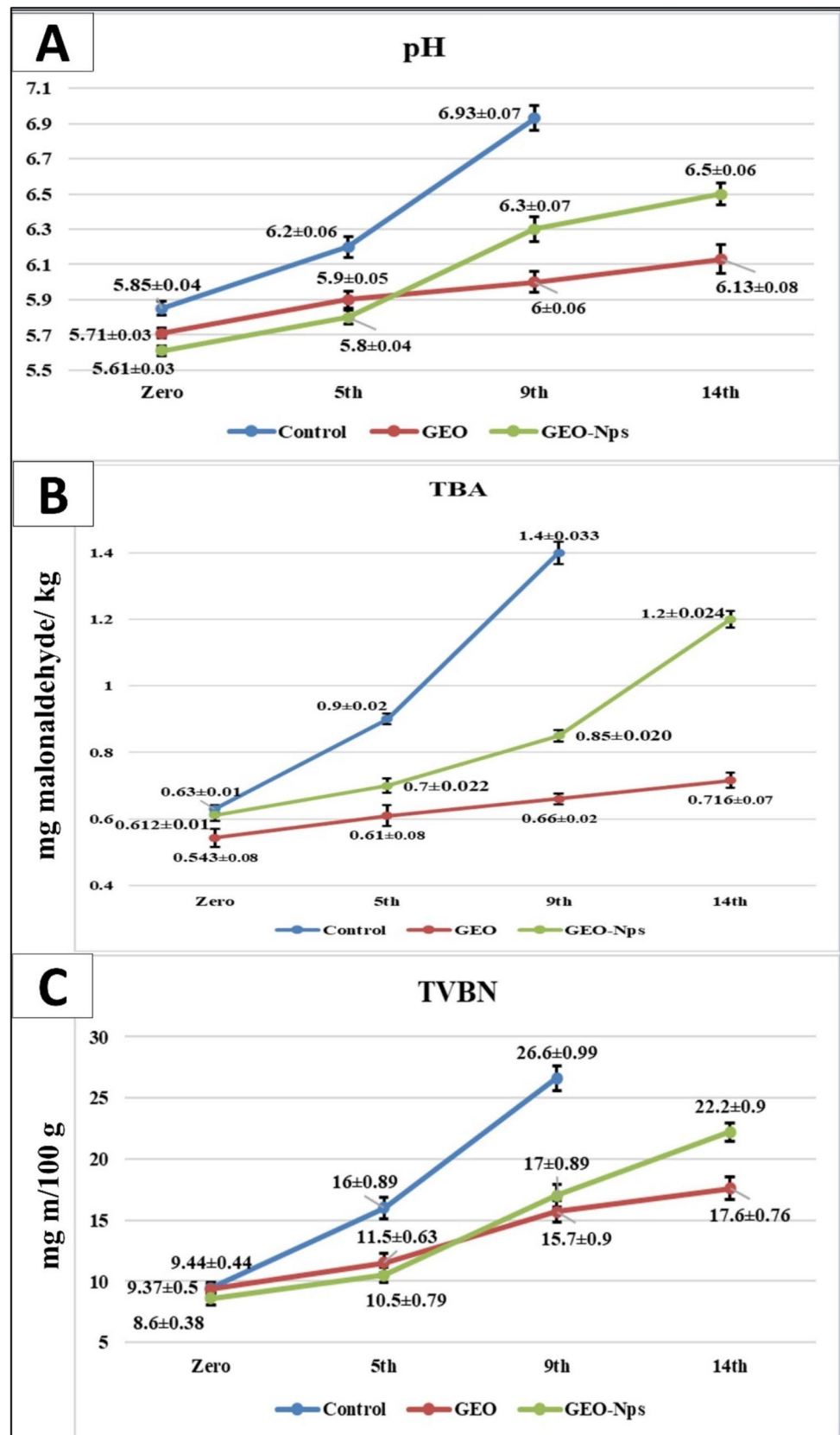
elevation in the pH level throughout the storage period in all ginger-treated samples compared to non-ginger-treated samples. In the study of Olatidoye, Sobowale, Oluwafemi, and Alabi [53], pH value of the control compared to treated minced meat with ginger extract was significantly ($P < 0.05$) and gradually increased during storage time (2–8 days). The increase in pH value occurred due to metabolites accumulation, like ammonia from the bacterial action [37].

Lipid autooxidation and growth of microorganisms lead to the formation of undesirable compounds, rancidity, off-flavor, and spoilage development, rendering the product unacceptable for human consumption [48].

As indicated in Fig. 3, TBA values in both GEO and GEO-Nps treatments were significantly ($P < 0.05$) lower than the control at the start of the storage. Furthermore, GEO treatment expressed the lowest TBA value throughout all storage periods from the start until the end of the storage. At the 9th day of chilled storage, the control treatment showed an unacceptable elevated value of 1.4 mg malonaldehyde/kg, while the values of both GEO and GEO-Nps were within the acceptable limit (0.65 and 0.86 for GEO and GEO-Nps) according to the Egyptian standard specifications [26]. These results agree with those of Stoilova, Krastanov, Stoyanova, Denev, and Gargova [67], who revealed that samples containing 0.05% ginger extract had lower TBA values than the control samples.

Total volatile Basic Nitrogen (TVBN) is frequently used as an indicator for the freshness and quality of meat. Numerous volatile nitrogen molecules, including dimethylamine, trimethylamine, and ammonia, are created as a result of microbial activity and their damaging effect on dietary proteins [45]. Results presented in Fig. (5-C) showed the TVBN content of fresh minced meat samples under refrigerated storage period. The level of TVBN gradually increased during the storage period in all treatments. The initial TVBN value of control treatment was 9.44 mg/100 g, but by the 9th day of storage, the control samples exhibited a TVBN values of 26.6 mg/100 g which exceeded the marginal acceptable limit (20 mg/100 g) outlined by Egyptian standard specifications [26] mean- while the values of both GEO and GEO-Nps were within the acceptable limit (15.9 and 17.5, respectively). The detected differences between the control group and other treated samples could be assigned to the antibacterial effects of GEO and GEO-Nps on the number of microorganisms that cause protein degradation [63]. The obtained TVBN results of the current study were in agreement with those obtained by El-Sheikh et al. [23] who found that adding ginger nanoemulsions to minced meat by concentration of 1% kept the values of TVBN under the permissible limits up to 12th day of chilled storage with values of 19.3 mg/100 g while using a concentration of 2% increased the storage days into the 18th day with TVBN values of 19.8 mg/100 g compared to

Fig. 3 Changes in storage stability indicators including pH **A**, thio-barbituric acid reactive substances values **B** and Total volatile Basic Nitrogen **C** contents during refrigerated storage of control, GEO and GEO-Nps treatments for 14 days



the controle which exceeded the permissible limit at the 9th day of storage. Additionally, essential oils nanoemulsions have been recorded to reduce the volatile compounds related to protein degradation through suppressing the formation of TVBN, therefore, ginger nano-emulsion helps maintain the quality and freshness of the meat during chilled storage [23, 34].

Sensory methods were employed to assess the freshness of a product based on its organoleptic features like odor, color, texture, and overall acceptability [33]. From the obtained results presented in Table 5, it was noticed that the study found no difference in color, flavor, or acceptability between control and GEO-Nps at zero-day storage. However, GEO-treated minced meat slightly lowered sensory scores, which means that nano encapsulation of ginger essential oil could mask the ginger flavor and allow using lower and more effective doses of ginger without affecting the sensorial quality. Ginger significantly ($P < 0.05$) enhanced the color, flavor, and overall acceptability scores

of minced meats during cold storage for both GEO and GEO-Nps treatments in comparison to control untreated samples that showed signs of deterioration and marked unacceptable spoilage signs by the 8th day of storage. Moreover, during the 8th, 12th, and 14th storage days, the GEO-Nps had higher sensory scores than GEO. Generally, the obtained results indicated that the best sensory quality was attained by the encapsulated GEO treatment, followed by the GEO-treated minced beef samples as compared to untreated samples. The adverse effect of GEO indicated in some reports that ginger may provide a peppery and strong pungent flavor, which affects the color parameters that can limit its use in food formulation [2, 46]. The obtained results agreed with those obtained by El-Sheikh et al. [23] who found that adding ginger nanoemulsions to minced meat either by concentration of 1% or 2% significantly improved the sensory score compared to the control samples where the control samples showed a lowered sensory scores at the 7th day while the GEO-Nps had an acceptable sensory score till the 13th and 18th day of storage for 1% and 2% GEO-Nps respectively. The improved sensory score upon using GEO & GEO-Nps was attributed to their antibacterial and antioxidant action which maintain the freshness of minced meat within the acceptable limit compared to control non treated samples [35, 40].

Table 5 Changes in sensory attributes including color, flavor and overall acceptability during refrigerated storage of control, GEO and GEO-Nps treatments for 14 days

Storage day	G1 (Control)	G2 (GEO)	G3 (GEO-Nps)
Color			
Zero	8.67 ± 1.00 ^{a,I}	7.00 ± 0.00 ^{a,II,I}	8.33 ± 1.90 ^a
2nd	6.67 ± 1.00 ^{b,I}	6.67 ± 1.00 ^{a,I}	8.33 ± 1.00 ^{a,II}
5th	4.67 ± 1.00 ^{c,I}	6.33 ± 1.00 ^{a,II}	7.33 ± 1.00 ^{a,b,II}
8th	1.67 ± 1.00 ^{d,I}	6.00 ± 1.73 ^{a,II}	6.67 ± 1.00 ^{b,II}
12th	NE	4.00 ± 1.73 ^{b,I}	4.67 ± 1.00 ^{c,I}
14th	NE	2.67 ± 1.00 ^{c,I}	4.67 ± 1.00 ^{c,II}
Flavor			
Zero	8.67 ± 1.00 ^{a,I}	6.33 ± 1.00 ^{a,II}	9.00 ± 0.00 ^{a,I}
2nd	7.33 ± 1.00 ^{b,I}	5.67 ± 1.00 ^{a,b,II}	7.67 ± 1.00 ^{a,b,I}
5th	4.33 ± 1.00 ^{c,I}	5.00 ± 1.73 ^{a,c,I}	7.00 ± 1.73 ^{b,c,II}
8th	1.33 ± 1.00 ^{d,I}	5.00 ± 1.73 ^{a,c,II}	6.67 ± 2.05 ^{b,c,II}
12th	NE	4.33 ± 1.90 ^{b,c,I}	5.67 ± 1.00 ^{c,d,I}
14th	NE	3.33 ± 1.90 ^{c,I}	5.00 ± 0.90 ^{d,II}
Overall acceptability			
Zero	8.67 ± 1.00 ^{a,I}	7.33 ± 1.00 ^{a,II}	8.67 ± 1.00 ^{a,I}
2nd	7.67 ± 1.00 ^{a,I}	6.33 ± 1.00 ^{a,b,II}	7.67 ± 1.00 ^{a,b,I}
5th	5.00 ± 1.73 ^{b,I}	5.33 ± 1.90 ^{b,c,I}	7.00 ± 1.72 ^{b,c,I}
8th	1.33 ± 1.00 ^{c,I}	5.00 ± 1.73 ^{b,c,d,II}	6.00 ± 1.73 ^{c,d,II}
12th	NE	4.33 ± 1.90 ^{c,d,I}	5.67 ± 1.00 ^{d,I}
14th	NE	3.33 ± 1.90 ^{d,I}	5.33 ± 1.00 ^{d,II}

^{a-d} Values of mean ± standard deviation with different superscripts within the same column are significantly ($P < 0.05$) different

^{I-II} Values of mean ± standard deviation with different superscripts within the same row are significantly ($P < 0.05$) different

NE Not examined as it reached the spoilage limit in 8th day i.e., 7.03 log CFU/gram

G1 Control treatment (minced meat), G2 Minced meat + 40 µL/ml of ginger essential oil (GEO), G3 Minced meat + 5 µL/ml of nano encapsulated ginger essential oil (GEO-Nps)

Conclusions

This study concluded that ginger essential oil is a promising natural antibacterial agent, especially against *Staphylococcus aureus*. As well as effectively improving the microbial stability, oxidative stability, and sensory quality during chilled storage. GEO proved to have a major role in retarding the deteriorative changes in the minced meat model in comparison with non-ginger-containing minced meat. Furthermore, nanoencapsulation of ginger essential oil proved to have the ultimate antibacterial effect trough enhancing the bioavailability and antimicrobial efficiency of ginger essential oil by using lower doses, that had the least deteriorative effect on the sensorial acceptability. Moreover, this nano-encapsulation helps to protect the ginger active principle to allow better delivery of its effect. Therefore, using ginger essential oil either in its crude (GEO) or nano-encapsulated form (GEO-Nps) increased the shelf-life storage period of the minced meat model during chilled storage. Using both GEO & GEO-Nps in minced meat offers a promising natural alternative to synthetic preservatives with a highly acceptable sensorial quality. However, challenges related to nanoparticle stability, cost-effectiveness, and regulatory approval should be considered before large-scale application. Therefore, future studies are recommended to evaluate

the economic feasibility of using GEO & GEO-Nps under industrial conditions. Also, future studies are suggested to reveal the effect of using ginger in combination with other natural essential oils for more synergistic action.

Author's Contribution Fathi A. M. El-Nawawi: Supervision, Conceptualization, Methodology, Investigation, Writing—review & editing; Sherifa A. Mohamed: Writing original draft, Investigation; Shaimaa A. Khalid; Writing original draft, Investigation, Methodology, Formal analysis; Hamdy M.B.A. Zaki: Writing—review & editing, Supervision, Conceptualization, Methodology, Formal analysis. All authors reviewed and approved the manuscript.

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Data availability Data will be available upon request.

Declarations

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