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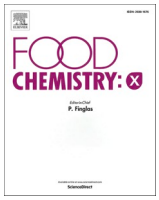

The effect of essential oil of *Anethum graveolens* L. seed and gallic acid (free and nano forms) on microbial, chemical and sensory characteristics in minced meat during storage at 4 °C

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ABSTRACT

The aim of this study was to evaluate the effect of gallic acid (GA) and essential oil (EO) of *Anethum graveolens* L. seed (forms of nanoliposome and free) on bacteriological, chemical and sensory properties of minced meat during storage. In this research, *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram positive) were used to examine the effect of these compounds on meat. The particle sizes (z-average diameter) of prepared nanoliposomes of EO and GA were in the range of 141 to 165 nm and 146–160 nm, respectively and the efficiency of encapsulation (EE %) in the current research was 51.76–69.8% in nano EO (NEO) and 53.23–67.07% in nano gallic acid (*N*-GA). Also, the outcomes indicated the treatment containing nano-liposomes had a better antimicrobial effect in both of bacteria. In present study, the Minimum Inhibitory Concentration (MIC) of GA, *N*-GA, EO and NEO for *S. aureus* was 0.62 ± 0.01, 0.62 ± 0.02, 0.62 ± 0.01 and 0.62 ± 0.01 mg/mL, respectively, and for *E. coli* was 0.62 ± 0.01, 0.62 ± 0.01, 1.25 ± 0.1 and 1.25 ± 0.1 mg/mL, respectively. Also, the results showed MBC (The Minimum Bactericidal Concentration) of GA, *N*-GA, EO and NEO for *S. aureus* was 0.62 ± 0.02, 0.62 ± 0.03, 1.25 ± 0.1 and 1.25 ± 0.1 mg/mL, respectively, and for *E. coli* was0.62 ± 0.01, 1.25 ± 0.1, 2.5 ± 0.2, 2.5 ± 0.2 mg/mL, respectively. The highest and lowest of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging were detected, in the Butylated hydroxytoluene (BHT) 200 and EO1%, respectively. Furthermore, after 18 day, minimum pH and Total volatile basic nitrogen (TVB-N) value were related to the N-GA2% on *S. aureus* with pH = 6.5 and NEO group (27 mg N/100 g), respectively. Finally, the treatment of NEO showed a higher acceptance score of sensory evaluation after 18 days. According to the outcomes of current investigation, the use of nanocapsulated EO and GA are effective (as a coating for food storage) and can increase the shelf life of minced meat.

1. Introduction

The increase of food diseases along with social and economic problems has led to extensive research for the production of healthy food and the development of new antimicrobial agents. Therefore, there is a need to reduce or eliminate pathogenic microorganisms caused by food using different methods. On the other hand, consumers' request for less use of synthetic preservatives has led to the research and consumption of natural derivatives with antimicrobial properties. Among these natural compounds, EOs and plant extracts can be mentioned (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahidi & Hossain, 2022; Taheri, Fazlara, Roomiani, & Taheri, 2018).

Medicinal plants have two major roles in food: first, to create a good taste in food, second, to preserve food due to its antimicrobial and antioxidant properties, delaying spoilage. Plant EO and various plant secondary metabolites are known as substances with antimicrobial properties that have little toxic effects.

The EO of plant are volatile secondary metabolites, which are made by plants for their own need. These compounds may have 20 to 60 compounds (aromatics) and this advanced mixture of compounds gives the EO its unique flavor and fragrance. EOs are extracted from many species of aromatic plants that grow around the world (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, &

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Jahed, 2018; Shahidi & Hossain, 2022; Taheri, Fazlara, Roomiani, & Taheri, 2018). EOs are extracted from many species of aromatic plants that grow around the world (Alexander, Lopez, Fang, & Corredig, 2012; Asdagh et al., 2021; Hussain et al., 2021; Javid, Raza, Hussain, & Rehman, 2014). One of these plants whose essential oil is used is *Anethum graveolens* L. (dill).

Anethum graveolens L., also known as dill, is a highly valuable essential oil-bearing medicinal plant and spice. Its flowers, seeds, leaves, fruits and stem all contain EO, making it a crucial herb for human use. To ensure its proper maintenance and medicinal benefits, it is essential to update our knowledge about its usefulness based on scientific studies (Jirovetz, Buchbauer, Stoyanova, Georgiev, & Damianova, 2003; Peerakam, Wattanathorn, Punjaisee, Buamongkol, Sirisa-ard, & Chansakaow, 2014; Radulescu, Popescu, & Ilies, 2010).

This plant is an annual aromatic herb belonging to the Umbelliferae family, originally from the West Asia and Mediterranean. The name *Anethum* is generic name and comes from the Greek word anethon, while dill comes from the Norse word dilla or dylla, which means to soothe. Dill is known by various names in different languages and cultures. It has a long antiquity of cultivation and usage as a medicinal herb and culinary in many parts of the world (Jirovetz, Buchbauer, Stoyanova, Georgiev, & Damianova, 2003; Peerakam, Wattanathorn, Punjaisee, Buamongkol, Sirisa-ard, & Chansakaow, 2014; Radulescu, Popescu, & Ilies, 2010). Also, several therapeutic effects in EO obtained from dill seeds such as anti-spasm, anti-cholesterol, anti-inflammatory, antioxidant, anti-fungal, anti-microbial, insecticidal, anti-cancer and anti-diabetic have been seen due to its biologically active compounds (Babri, Khokhar, Mahmood, & Mahmud, 2012; Jirovetz, Buchbauer, Stoyanova, Georgiev, & Damianova, 2003; Peerakam, Wattanathorn, Punjaisee, Buamongkol, Sirisa-ard, & Chansakaow, 2014; Radulescu, Popescu, & Ilies, 2010).

On the other hand, one of the polyphenolic compounds that is generally present in nature is gallic acid (3,4,5-trihydroxybenzoic acid). GA is a natural preservative (according to the USEPA standard, GA consumption of 5 mg/kg of body weight is non-toxic) is basically a secondary polyphenolic metabolite. Due to its antioxidant, anti-tumor and other properties, it has wide use in industries of chemical, pharmaceutical and food (Sorrentino et al., 2018).

However, its sensitivity to extreme temperatures, light and oxygen, and creating an unpleasant taste cause a series of problems in its potential applications. Its short half-life and rapid elimination from the body are other problems of using it as food. Therefore, there is a need to modify a formula that can have a protective effect in the process of production, storage and consumption and maintain its levels in the body for a longer period of time. Using the microencapsulation method is one of the ways to protect compounds and increase their shelf life in the body (Al-Sagheer, Daader, Gabr, & Abd El-Moniem, 2017; Alfei, Oliveri, & Malegori, 2019; Lee, Mahmud, Pillai, Perumal, & Ismail, 2012; Mancini et al., 2015).

Nanoliposomes are orbicular vesicles formed by hydrating surfactants such as phospholipids. Liposomes are flexible carriers that can transport both hydrophobic and hydrophilic materials simultaneously, either a bilayer membrane or inside a vesicle. Nanoliposomes are more solubilizing, provide better control over the release, have a higher surface area, and target encapsulated compounds more effectively than liposomes (Homayounpour et al., 2021; Mehdizadeh, Shahidi, Shariatifar, Shiran, & Ghorbani-HasanSaraei, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Tometri, Ahmady, Ariaii, & Soltani, 2020).

Meat is susceptible to lipid oxidation, color change and microbial spoilage owing to its specific composition and low stability of oxidative. Active coating is an effective method to postponement growth of bacterial at the surface of meat and preserve quality of meat for longer time, and also extending its shelf-life (Abdollahzadeh, Rezaei, & Hosseini, 2014; Kanatt, Rao, Chawla, & Sharma, 2013; Malinowska-Pańczyk & Kołodziejska, 2016; Taheri, Fazlara, Roomiani, & Taheri, 2018).

So far, no research has been done regarding the preservation of minced meat using the EO of *Anethum graveolens L.* seeds and GA (free and nano form) in Iran and other countries, and on the other hand, due to the high consumption of meat in Iran and the world, doing this Research seemed necessary. With these explanations, the objective of current research was to assess the influence of GA and EO of *Anethum graveolens* L. (forms of nanoliposomes and free) on some incubated foodborne pathogenic (*Staphylococcus aureus* and *Escherichia coli*) inoculated in minced meat at refrigerated temperatures during storage time.

2. Materials and methods

2.1. Materials

Ferric chloride, glycerol (>97% purity), dimethyl sulfoxide, sodium acetate, folin-Ciocalteu reagent, sodium carbonate, anhydrous, dichloromethane, cholesterol (95%), acetic acid, hydrochloric acid, and methanol were obtained from Merck Co. (Darmstadt, Germany). Also, from Across Company (USA), L-a-lecithin (granular phospholipid) (with pure of 99 %) was obtained, for nanoliposomes preparation. Furthermore, other solvents and reagents with high purity (analytical grade) were purchased from Merck (Darmstadt, Germany). GA with code 27,645 was purchased from Sigma-Aldrich Company. Medians of Broth Heart Infusion (BHI) and Baird Parker agar were bought from Company of Scharlua (Spain) and Merck Co. (Germany), respectively. Sterile stomacher bag, buffered peptone water, Triple Soy agar (TSA) and Chromocult Coliform-agar were purchased from VWR (Belgium), Oxoid (Belgium), Merck (Germany) and Merck (Germany), respectively.

2.2. Preparation and maintenance of bacteria

The National Center of Genetic and Biological Resources in Iran provided the strains of *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 25922). To reactivate the strains, they were inoculated into glass vials containing 20% (w/v) glycerol in BHI and stored at −20 °C. The cultures were then grown in 15 mL of BHI for 24 h (37 °C) at 150 rpm with two consecutive passages. The bacterial cells were separated from the BHI by triple centrifugation at 6000 rpm for 5 min. During the last 2 steps of centrifugation, the supernatant was separated and replaced with physiological serum. The bacterial cell count was determined by measuring the optical density (OD) at 600 nm, with a population of 1×10^8 cells/mL (based on pretest, 0.1–0.08 OD was equal to 1×10^8 cfu/mL). Finally, the minced meat was inoculated with 1×10^4 cfu/g of both E. *coli* and S. *aureus* after dilution (Pouryousef et al., 2022a; Tometri, Ahmady, Ariaii, & Soltani, 2020).

2.3. Preparation of seed EO

From local-market in Tehran (Iran), 2 kg of *Anethum graveolens* L. seeds were acquired and by a pharmacology expert at Tehran University of Medical Sciences (TUMS) were authenticated. In this study, the seeds were dried (after washing with di-stilled water) at room temperature (in the shade). Next, the dried seeds of the plant were powdered by an electric grinder and 100 g of it were mixed with five times distilled water. After that, EO was extracted using Clevenger in different time periods (from 0.5 to 5 h with half hour intervals). After extracting the EO, it was dehydrated with $Na_2S_2O_3$ (sodium thiosulfate) and stored in glass vials (dark) and sealed at 4 °C until experimental time (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Mehdizadeh, Shahidi, Shariatifar, Shiran, & Ghorbani-HasanSaraei, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shariatifar, Pirali-Hamedani, Moazzen, Ahmadloo, & Yazdani, 2019).

2.4. Identification of the chemical compounds of the EO by GC–MS

Since the compounds in EOs are known as volatile substances in terms of molecular weight and polarity, therefore, the process of

separating and identifying the compounds of EOs obtained from seeds was evaluated by gas chromatography with a mass spectrometer (GC–MS, Agilent Technologies 7890A and Mass Selective Detector: 5975C VL MSD with Detector of Triple-Axis). One microliter of plant EO was injected into a gas chromatography equipment (with a 30 m length of column, 0.25 μm thickness of inner layer and 0.25 mm inner diameter) connected to a mass spectrometer to identify compounds of EO. The column primary temperature was fixed at 40 °C, which increased to 250 °C with a growth rate of 2.5 °C/min. The gas of helium (as a carrier) was applied with a 1.1 mL/min speed rate and an ionization energy of 70 electron volts. By using the normal range of alkanes and their inhibition index the types of compounds that make up the seed EOs were identified (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Pouryousef et al., 2022a).

2.5. Preparation of nanoliposome seed EO

In this part, in order to obtain suitable nanoliposomes, 4 diverse ratios of lecithin and cholesterol (20:40, 30:30, 50:10 and 60:0) in terms of mg was used. Different molar ratios of lecithin to cholesterol (20:40, 30:30, 50:10 and 60:0) were applied to prepared nanoliposomes based on thin layer hydration and ultrasound techniques. After accurate weighing, in a glass flask (50 mL), lecithin/cholesterol's different ratios were dissolved in 10 mL methanol and 10 mL dichloromethane. 60 mg seed EO was separately dissolved in 10 mL methanol and blended with the mentioned blend. Afterward, by a rotary evaporator, the various solvents of the blend were separated and then on the flask walls, a layer of thin film-like was formed. By 15 mL deionized distilled water, the obtained film was hydrated and homogenized in a homogenizer at 19,000 rpm/min for 20 min at 30 °C. To obtain nanoliposomes, in a bath of ice water, the suspension was sonicated using a suitable ultrasonic device for 6 min with a sequence of one second of ultrasound and one second of ultrasound rest (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Pouryousef et al., 2022a). In this method, according to previous studies, after adding the solution, ultrasound treatment was used to reduce the size of the particles. Considering that ultrasound may affect their size and functional properties, it is suggested to use ultrasound before adding the solution.

2.6. Preparation of nano liposome of GA

Gallic acid (GA, 100 g) was stored at 2 to 8 °C until use. Nano gallic acid (*N*-GA) was prepared by thin layer hydation method. In this part, 4 diverse ratios of lecithin and cholesterol (20:40, 30:30, 50:10 and 60:0) were used in order to obtain suitable nanoliposomes. Different molar ratios of lecithin to cholesterol (20:40, 30:30, 50:10 and 60:0) were applied to prepared nanoliposomes based on thin layer hydration and ultrasound techniques. After precise weighing, in a glass flask (50 mL), lecithin/cholesterol's different ratios were dissolved in 10 mL ethanol and 10 mL dichloromethane. Then, 5 mg/g of GA was separately dissolved in 10 mL methanol and then blended with the mentioned blend. After that, by a rotary evaporator, blend were evaporated into a thin film at 40 °C. Then, with saline phosphate buffered (PBS, pH 7.0 and 0.05 M), the dried lipid film was hydrated. Then it was homogenized in a homogenizer at 19,000 rpm/min for 20 min at 30 °C. The liposomal suspension was sonicated in an ice bath (in order to avoid applying too much energy into the solution and to prevent lipid hydrolysis and oxidation) at 160 W for fifteen minutes with a sequence of one second of ultrasound and one second of ultrasound rest. By lipid hydration, liposomes loaded with GA (1% and 2 %) were prepared. The film will be separated by ultracentrifugation with PBS comprising GA and unencapsulated GA (Al-Sagheer, Daader, Gabr, & Abd El-Moniem, 2017; Alfei, Oliveri, & Malegori, 2019; Lee, Mahmud, Pillai, Perumal, & Ismail, 2012; Mancini, et al., 2015).

2.7. Preparation of minced raw beef and preparation of treatments

Ten kilograms of fresh beef (raw) was acquired from super-markets of Tehran City (Iran). The meat was transported to the laboratory in a cold box. Firstly, to destroy all microorganisms (M.O) of surface, the whole raw beef was exposed to the UV lamp for 15 min, then the raw beef was separated in a sterile state with a sterile knife and sterile dishes. The raw beef (without skin and bones) was homogenized twice by a sterile blender. Minced raw meat was divided into 100 g pieces in sterile zip packs. Minced meat samples were inoculated with 1×10^4 CFU/g of *S. aureus* and *E. coli* (separately) and this inoculated meat was used to prepare all the treatments. At the same time, GA in concentrations of 2% and 1% and seed EO in levels of 1 and 2% (in free and nano form), each one separately, were added to raw ground beef containing bacteria *S. aureus* and *E.coli* 1×10^4 CFU/g was added separately and then mixed. The control treatment without bacteria and containing treatment of minced meat containing GA and EO in free and nanoliposome forms were considered separately. After that, the inoculated treatments were homogenized for 3 min at 200 rpm. Next, it was placed in polyester containers and covered with stretch film (polypropylene) and kept at 4 °C. These treatments were tested on days 0, 3, 6, 9, 12, 15, and 18 to perform microbial and chemical tests (the tests were repeated three times) (Pouryousef et al., 2022a; Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020).

2.8. Size of nanoliposomes and EE (encapsulation efficiency %) assessment

According to the study of Pabast et al. a particle size analyzer (Shimadzu, SALD 2101, Japan) with DLS method (dynamic light scattering) was applied to assess the distribution of particle size and mean diameter of liposomes (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). And, according to Pabast et al.'s study, by a spectrophotometer of UV (Pharmacia biotech ultraspec 2000, UK) at 750 nm, the EE % of the liposome was measured (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.9. Scanning electron microscopy (SEM) assay

By SEM analyzer (KYKY-EM 3200; KYKY Technology Development Ltd., Beijing, China), the nonoliposomes' structure and morphology of GA and EO were assessed. The prepared samples were visualized at a 25 KV excitation voltage (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Pouryousef et al., 2022a).

2.10. Transmission electron microscopy (TEM) assay

The TEM image were done by electron microscope (Philips Bio-Twin, the Netherlands) to assay the nanoparticles characterization. These images were taken at 75 kV (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pouryousef et al., 2022a; Pouryousef et al., 2022b).

2.11. Microbiological analysis

2.11.1. MIC and MBC analysis

We followed the NCCLS recommendation and employed the technique of micro dilution to evaluate the impact of EO and GA (free and nonoliposome forms) on *E. coli* and *S. aureus*. Approximately 108 CFU/g concentration of the bacteria were added to experimental tubes, along with solutions of the EO and GA (0.2 mL each) in dimethyl sulfoxide (DMSO) and double distilled water. The tubes were then incubated for 24 h at 37 °C, and the MIC was determined as the lowest concentration level where no turbidity was observed. To determine MBC, 0.1 mL of clear tubes (without turbidity after 24 h) was cultured as surface plate on TSA agar and was incubated at 37 °C for 24 h. The first level where no growth of bacteria was considered in MBC (Rashidaie Abandansarie,

Ariaii, & Charmchian Langerodi, 2019).

2.11.2. Enumeration microbial test

A mixture of minced meat (10 g) and 90 mL of sterile sodium chloride (NaCl) solution was homogenized, and 1 mL of the desired dilution (on the Baird Parker) was cultured and incubated for 24 to 48 h at 37 °C. The formation of black shiny colonies with a thin white edge and a transparent zone around them is characteristic of *S. aureus*. Two plates were considered for each dilution (Tometri, Ahmady, Ariaii, & Soltani, 2020). To enumerate *E. coli*, 10 g of meat sample was homogenized in 100 mL buffered peptone water (Oxoid, Belgium) using a sterile bag of stomacher (filter 0.5 mm pore size) (VWR, Belgium) for 2 min. The spreading plate method was used with Chromocult Coliform-agar for incubation at 37 °C for 24 h (Van Haute, Raes, Van Der Meeren, & Sampers, 2016). For *S. aureus* and *E. coli*, the bacteria number/gram of minced meat was reported as log cfu/g.

2.12. Chemical analysis

2.12.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

The basis of a common antioxidant assay is DPPH free radical evaluation. This experiment was done according to the Pabast et al.'s research. Then, by UV spectrophotometry, the control and all treatments samples were read at 517 nm (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.12.2. pH assay

By using pH meter (digital from HANNA, Germany), the pH value of treatments and control sample was evaluated, according to the Homayonpour et al.'s research (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021b).

2.12.3. Total volatile base nitrogen value (TVB-N)

According to research of Tometri et al. the TVB-N value of the minced meat was measured by using the micro diffusion procedure (Tometri, Ahmady, Ariaii, & Soltani, 2020). The results were indicated as mg N/100 g of minced meat.

2.13. Sensory evaluation

According to Pabast et al. in this research, all treatment samples were considered by six panelists semi-trained (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). All panelists experts had an experience in meat sensory evaluation at TUMS, and in past had participated in Nouri et al.'s research (Noori, Zeynali, & Almasi, 2018). Based on the previous study, by questionnaire using 5 point (5 to 1) descriptive scale, the traits of ''off-odor'', "discoloration'' and "red color" were valued (number 1 had the highest score (highest quality) and number 5 had the lowest score (lowest quality), when the sensory attributes augmented above 3, a rejection would be happen) (Djenane et al., 2001; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.14. Statistical analysis

All results were reported as mean ± SD. By SPSS Ver.24 (Chicago, IL), the outcomes were evaluated with ANOVA test (analysis of variance), followed by the test of Duncan's post hoc to measure the influences of treatments and storage time on parametric data (sensory analysis, TVB-N, pH and DPPH). Significant differences were evaluated ($p < 0.05$). All assays were done at least in triplicate.

3. Results and discussion

3.1. Compounds identification of Anethum graveolens L. EO

In this study, 21 EO compounds constitute 97.82 % of the identified compounds (Table 1). According to this table, the principal components were dodecenyl acetate, *N*-octyl 2-methyl butyrate, octyl butyrate, Geraniol, Anethole, Geranyl acetate, *n*-Octyl acetate, *trans*-Anethole and octyl ester. The compounds in the present research have been found in other studies with different percentages, which is probably the reason for this difference, weather conditions, soil type, plant species and geographical region (Babri, Khokhar, Mahmood, & Mahmud, 2012; Jirovetz, Buchbauer, Stoyanova, Georgiev, & Damianova, 2003; Peerakam, Wattanathorn, Punjaisee, Buamongkol, Sirisa-ard, & Chansakaow, 2014; Radulescu, Popescu, & Ilies, 2010).

3.2. Evaluation of nanoliposomes characteristics of Anethum graveolens L. EO and GA

The z-average diameter (particle sizes) of prepared nanoliposomes of EO and GA were presented in Table 2. For nanoliposomes to be stable and effective in releasing compounds trapped in the liposome core, particle size is an important factor. By Dynamic light scattering (DLS) analysis, bimodal particle size distribution was confirmed. The results showed that the particle size of EO and GA were in the range of 141–165 nm and 146–160 nm, respectively. In similar research by the Pabast et al. the nanoliposom particles size was 93 to 96 nm (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018) and in Homayonpour et al.'s study the particle size was ranged from 140 to 164 nm (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a), which were somewhat similar to our study.

To influence the EE percentage, 3 factors are important that including the type, inner volume of vesicles and lipid ratio (Fan, Xu, Xia, & Zhang, 2008). According to the Table 2, the efficiency of encapsulation (EE %) in present research was 51.76–69.8% for NEO and 53.23–67.07% for *N*-GA. There are two parts in the liposome structure, which include hydrophobic and hydrophilic. Between the phospholipid layers (two), hydrophobic composites are surrounded and hydrophilic composites are located in the aqueous environment of liposomes, so the phospholipid layers (for EO and GA) act as a reservoir. Our results was similar to the Shahbazi's study (Shahbazi, 2017), Pabast and *et al.*'s study (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018) and Tometri *et al.*' study (Tometri, Ahmady, Ariaii, & Soltani, 2020).

3.3. SEM assay

Fig. 1 shows SEM assay of *N*-EO and *N*-GA. The SEM assay was

Table 1
The analysis of *Anethum graveolens L.* seed EO compounds by GC/MS.

Peak No.	Compound	RT (min)	A%
1	Hexanol	4.58	0.07
2	α-Pinene	9.29	3.23
3	Camphene	10.41	0.52
4	β-Pinene	6.75	3.97
5	β-Myrcene	7.03	0.25
6	limonene	8.61	1.14
7	δ -Terpinene	9.43	4.27
8	p-cymene	12.953	3.35
9	Linalool oxide	13.234	1.36
10	Linalool	13.374	63.41
11	a-Terpinolene	13.78	1. 1
12	Citral	14.312	0.06
13	*n*-Octyl acetate	16.128	0.04
14	*trans*-Anethole	16.823	1.57
15	octyl ester	17.277	0.03
16	*N*-octyl 2-methyl butyrate	18.323	1.73
17	octyl butyrate	20.132	3.3
18	Geraniol	24.392	2.98
19	Anethole	26,327	2.75
20	Geranyl acetate	29.211	3.32
21	dodecenyl acetate	30.17	0.47
Total			97.82

Table 2
Characterization of nanoliposomes of EO and GA.

Code	Lecithin: Cholesterol	z-average diameter (nm) of EO	z-average diameter (nm) of GA	Encapsulation Efficiency% Of EO	Encapsulation Efficiency% Of GA
1	60:00	165 ±	159 ±	64.56 ± 0.96^{b}	62.34 ± 0.98^{b}
2	50:10	0.95^{a}	0.43^{a}	69.08 ± 0.65^{a}	68.07 ± 0.87^{a}
3	40:20	141 ± 0.75^{d}	146 ± 1.21^{c}	60.22 ± 1.23^{c}	56.21 ± 0.76^{c}
4	30:30	150 ± 0.74^{c}	152 ± 0.95^{b}	51.76 ± 0.88^{d}	53.23 ± 0.65^{d}
		156 ± 0.86^{b}	160 ± 1.13^{a}		

Data are means ± SD.
Means with different letters within a column indicate significant differences ($p < 0.05$).

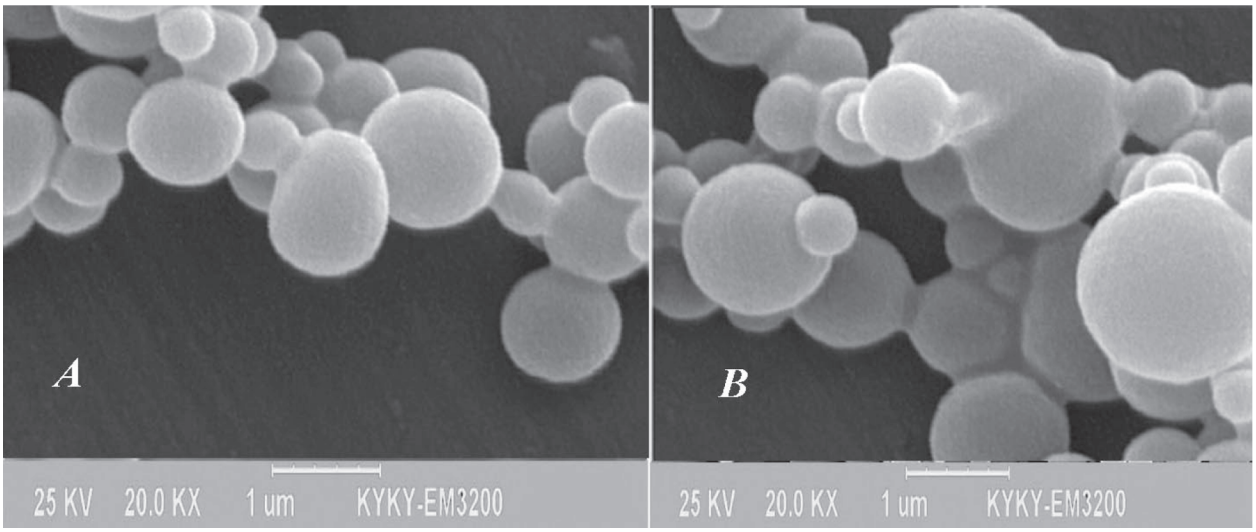

Fig. 1. Scanning electron microscope (SEM) image of *Anethum graveolens* NEO (A) and *N*-GA (B).

performed to assess the morphology of loaded nanoliposomes of GA and EO with the lower droplet size and the highest percent of EE (60:00 cholesterol: lecithin molar ratio), according to the previous results (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Pouryousef et al., 2022a; Pouryousef et al., 2022b). As shown in this figure, spherical and hemispherical nanoliposomes were formed from GA and EO.

3.4. TEM assay

Fig. 2 shows TEM assay of NEO and *N*-GA. The TEM assay was

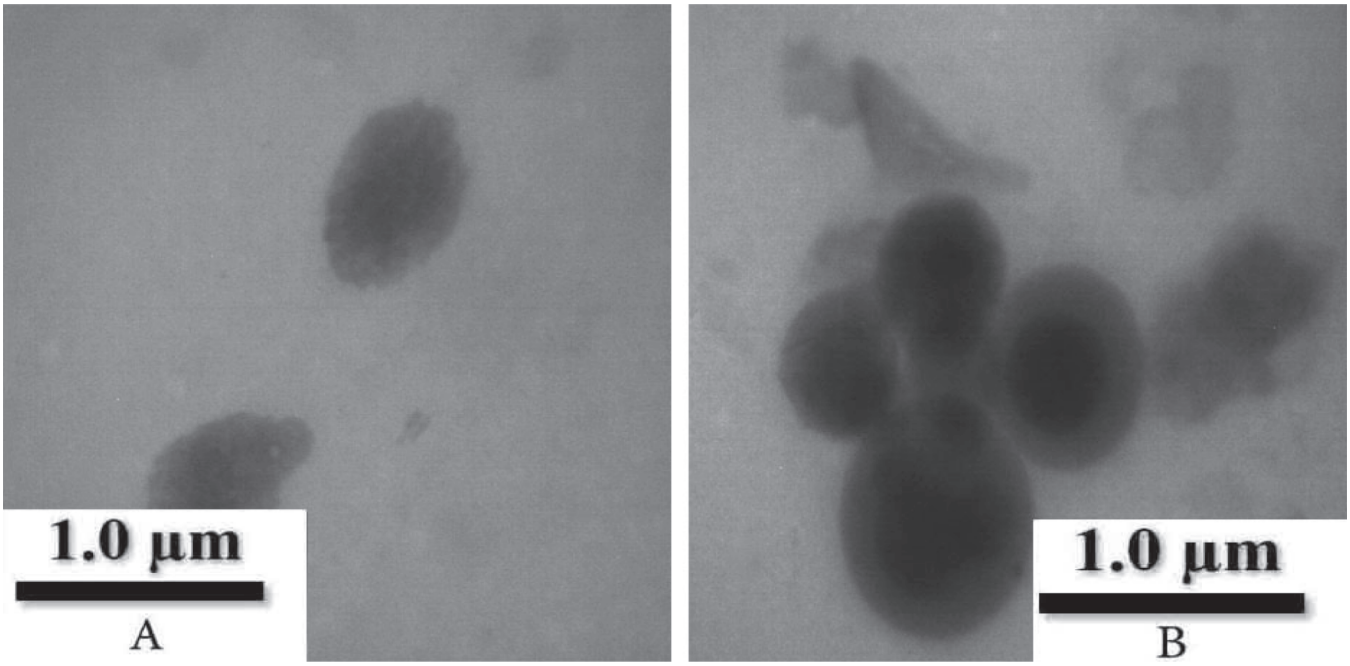

Fig. 2. Transmission electron microscope (TEM) image of *Anethum graveolens* NEO (A) and *N*-GA (B).

performed to evaluate the *N*-EO and *N*-GA morphology with the lower droplet size and the highest percent of EE (60:00 lecithin/cholesterol molar ratio), according to the previous results (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Pouryousef et al., 2022a; Pouryousef et al., 2022b). As shown in this figure, the appearance of capsules containing nano-sized EO and GA showed the apparent core–shell structure, which proved the formation of capsules.

3.5. Microbiological analysis

3.5.1. MIC and ABC

As presented in Table 3, the results showed that the MIC of GA, *N*-GA, EO and NEO for *S. aureus* was 0.62 ± 0.01, 0.62 ± 0.02, 0.62 ± 0.01 and 0.62 ± 0.01 mg/mL, respectively, and for *E. coli* was 0.62 ± 0.01, 0.62 ± 0.01, 1.25 ± 0.1 and 1.25 ± 0.1 mg/mL, respectively. Also, the results showed that the MBC of GA, *N*-GA, EO and NEO for *S. aureus* was 0.62 ± 0.02, 0.62 ± 0.03, 1.25 ± 0.1 and 1.25 ± 0.1 mg/mL, respectively, and for *E. coli* was0.62 ± 0.01, 1.25 ± 0.1, 2.5 ± 0.2, 2.5 ± 0.2 mg/mL, respectively (Table 3). Our results showed that the GA and *N*-GA more effective on two mentioned bacteria than EO and NEO. Furthermore, the results showed that the values of MIC and MBC in gram positive bacteria were lower than gram negative (bacteria. Gram-positive bacteria (such as *S. aureus*) are sensitive to antibacterial compounds due to the absence of a lipopolysaccharide layer in their cell walls. Contrariwise, gram-negative bacteria (such as *E. coli*) possess this layer, which can prevent active compounds from entering the cytoplasmic membrane (Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020). Moreover, the hydrophilic surface of the outer membrane in gram-negative bacteria can make them resistant to antibacterial compounds. This is because the cell wall lipopolysaccharide layer prevents the penetration of different enzymes and antibiotic molecules that break down molecules imported to the periplasmic space (Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020). Previous research has shown that flavonoid and phenolic compounds are associated with antibacterial activity. It seems that the antibacterial activity of plant EOs is influenced by these compounds (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Tometri, Ahmady, Ariaii, & Soltani, 2020). In addition, this study confirms that the antimicrobial activity of EOs is related to the changes in the cell membrane caused by the penetration of phenolic compounds and the electrical imbalance of the cell membranes, which leads to the leakage of intracellular compounds and finally cell death. As a result, an increase in the level of phenolic compounds leads to an increase in 1,8-cineol that significantly enhances the antimicrobial effect. Other studies have also reported that the antimicrobial activity of 1,8-cineole (Noori, Zeynali, & Almasi, 2018; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahbazi, 2017). In the study of Shahbazi, 4 kinds of EO (*Allium rotundum, Falcaria vulgaris, Mentha longifolia* and *Tragopogon graminifolius*) was evaluated against six pathogenic bacteria (*S. typhimurium, B. subtilis, B. cereus, S. aureus, L. monocytogenes,* and *E.coli*) and stated that the MIC and MBC tests in Gram-positive bacteria were lower than Gram-negative bacteria that was the same analogous to current findings (Shahbazi, 2017) these findings approved also by research of Tometri *et al.* with assess the leaf extract of *Laurus nobilis* against *E. coli* and *S. aureus* (Tometri, Ahmady, Ariaii, & Soltani, 2020).

Table 3
MIC and MBC of treatments.

		S. aureus	*E. coli*
GA	MIC	0.62 ± 0.01^{a}	0.62 ± 0.01^{a}
N -GA	MIC	0.62 ± 0.02^{b}	0.62 ± 0.01^{b}
EO	MIC	0.62 ± 0.01^{c}	1.25 ± 0.1^{c}
NEO	MIC	0.62 ± 0.01^{c}	1.25 ± 0.1^{c}
p-value		0.00	0.00
GA	MBC	0.62 ± 0.02^{a}	0.62 ± 0.01^{a}
N -GA	MBC	0.62 ± 0.03^{b}	1.25 ± 0.1^{b}
EO	MBC	1.25 ± 0.1^{c}	2.5 ± 0.2^{c}
NEO	MBC	1.25 ± 0.1^{c}	2.5 ± 0.2^{d}
p-value		0.00	0.00

Data are mean ± SD. The mean with different letters in a row indicates a significant difference ($p < 0.05$).

3.5.2. Antimicrobial effect of EO and GA (free and nano form) on *S. aureus* and *E. coli* inoculated in minced meat

As shown in Tables 4 and 5, during the experimental time (0–18 days), the growth of two bacteria in EO and GA treatments decreased ($p < 0.05$), while it enhanced in the control samples. The results showed that, during the experiment, the treatments had a greater effect on the *S. aureus* than *E. coli*, so that the growth of *S. aureus* in NGA2% treatment from day 0 to 18th has reached from 8 to 2 log CFU/g and for *E.coli* it has reached from 4.2 to 2 log CFU/g. Furthermore, the treatments containing nano form of GA and EO had a better influence (in both of the bacteria). Nanoform compounds such as nanoemulsions or nanoliposomes tend to encapsulate bioactive compounds due to higher surface area and greater proximity to bacterial cells and provide greater antimicrobial effect and quantum size effect in EO or GA formulations. (Noori, Zeynali, & Almasi, 2018). Therefore, nano-coatings may be ideal for extending the shelf life of meat. The findings of the present study were similar to the study of Tomtri *et al.*, whose results showed that during the storage period, the number of *S. aureus* colonies increased in the control treatment and decreased in most of the treatments containing *Laurus nobilis* leaf extract. Also, on the fourth day of storage with a concentration of 1500 ppm, no colony of *S.aureus* was observed in the nano extract, which indicates the better effect of nano treatments (Tometri, Ahmady, Ariaii, & Soltani, 2020). Haut et al. analyzed the counts of *E. coli* in chicken breast fillet and chicken skin mixed with 1% thyme and stated that the number of *E. coli* was lower compared to blank samples in chicken skin and also in chicken breast samples, it was constant in all treatments and blank samples (Van Haute, Raes, Van Der Meeren, & Sampers, 2016). Furthermore, Mazhar et al. evaluated of *Mentha polegium* EO at different levels in growth of *S. paratyphi* and *S. typhimurium* incubated in minced fish, and stated increasing the EO concentration (0.1 percent to 0.5 percent) enhances the antimicrobial effect in the fish samples that was similar to our results in present research (Mazhar, Aliakbari, KARAMI, Morshedi, Shariati, & Farajzadeh, 2014). Also, Abdollahzadeh et al. analyzed the coating of thyme EO on the *L. monocytogenes* growth during 12 days at 4 °C in fish samples and stated increasing the EO amount enhances the

Table 4
Antimicrobial effect of EO and GA (free and nano forms) on *E. coli* inoculated in minced meat (log CFU/g).

Treatments	first day	3th day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	$4.2^{Af} \pm 0.1$	$4.5^{Aa} \pm 0.2$	$5.2^{Ad} \pm 0.2$	$5.9^{Ab} \pm 0.2$	$6.2^{Aa} \pm 0.1$	$5.8^{Ab} \pm 0.2$	$5.7^{Ac} \pm 0.2$
EO1%	$4.2^{Aa} \pm 0.2$	$4.1^{Bb} \pm 0.1$	$3.8^{Bc} \pm 0.1$	$3.6^{Bd} \pm 0.1$	$3.3^{Be} \pm 0.1$	$3^{Bf} \pm 0.1$	$2.8^{Bg} \pm 0.1$
EO2%	$4.2^{Aa} \pm 0.2$	$4^{Bb} \pm 0.1$	$3.3^{Dc} \pm 0.1$	$3^{Ed} \pm 0.1$	$2.7^{Ce} \pm 0.1$	$2^{Ef} \pm 0.1$	$2^{Df} \pm 0.1$
NEO1%	$4.2^{Aa} \pm 0.1$	$4.1^{Bb} \pm 0.1$	$3.7^{Bc} \pm 0.1$	$3.5^{Bd} \pm 0.1$	$3.2^{Be} \pm 0.1$	$3^{Cf} \pm 0.1$	$2^{Dg} \pm 0.1$
NEO2%	$4.2^{Aa} \pm 0.1$	$4^{Bb} \pm 0.1$	$3.1^{Ec} \pm 0.1$	$2.9^{Dd} \pm 0.1$	$2.7^{Ce} \pm 0.1$	$2^{Ef} \pm 0.1$	$2^{Df} \pm 0.1$
GA1%	$4.2^{Aa} \pm 0.2$	$4.1^{Bb} \pm 0.2$	$3.8^{Bc} \pm 0.2$	$3.6^{Bd} \pm 0.1$	$3.3^{Be} \pm 0.1$	$3^{Bf} \pm 0.1$	$2.7^{Bg} \pm 0.1$
GA2%	$4.2^{Aa} \pm 0.1$	$4^{Bb} \pm 0.1$	$3.6^{Cc} \pm 0.1$	$3.3^{Cd} \pm 0.1$	$2.4^{Ee} \pm 0.1$	$2^{Ef} \pm 0.1$	$2^{Df} \pm 0.1$
NGA1%	$4.2^{Aa} \pm 0.2$	$4.1^{Bb} \pm 0.2$	$3.7^{Bc} \pm 0.1$	$3.5^{Bs} \pm 0.1$	$3^{De} \pm 0.1$	$2.8^{Df} \pm 0.1$	$2.5^{Cg} \pm 0.1$
NGA2%	$4.2^{Aa} \pm 0.2$	$4^{Bb} \pm 0.1$	$3.7^{Bc} \pm 0.2$	$3.3^{Cd} \pm 0.1$	$2.3^{Ee} \pm 0.1$	$2^{Ef} \pm 0.1$	$2^{Df} \pm 0.1$

Data in the same column followed by different capital case letters are significantly different ($P < 0.05$). Data in the same row followed by different lower letter are significantly different ($p < 0.05$).

Table 5
Antimicrobial effect of GA and EO (free and nano forms) on *S. aureus* inoculated in minced meat (log CFU/g).

Treatments	first day	3th day	6 th day	9 th day	12 th day	15 th day	18 th day
Control	8^{Ad} ± 0.1	8.1^{Ab} ± 0.2	8.9^{Aa} ± 0.2	8.5^{Ab} ± 0.2	8.3^{Ac} ± 0.2	8.1^{Ad} ± 0.2	7.9^{Ae} ± 0.2
EO1%	8^{Ab} ± 0.0	7.9^{Aa} ± 0.0	7.3^{Da} ± 0.1	7.1^{Cc} ± 0.1	5.6^{Cd} ± 0.1	4.2^{Ce} ± 0.1	2.7^{Cf} ± 0.1
EO2%	8^{Ab} ± 0.1	7.8^{Aa} ± 0.2	7.4^{Da} ± 0.2	7.0^{Cc} ± 0.0	5.5^{Cda} ± 0.1	4^{De} ± 0.1	2.5^{Df} ± 0.1
NEO1%	8^{Ab} ± 0.1	8^{Ca} ± 0.1	7.2^{Eb} ± 0.0	6.9^{Cc} ± 0.1	5.5^{Cd} ± 0.2	4^{De} ± 0.2	2.3^{Ef} ± 0.1
NEO2%	8^{Ab} ± 0.1	8^{Db} ± 0.0	7.2^{Ba} ± 0.2	6.7^{Ec} ± 0.1	5.3^{Dd} ± 0.1	4.6^{Be} ± 0.0	2^{Ff} ± 0.2
GA1%	8^{Ac} ± 0.0	7.9^{Bb} ± 0.2	7.3^{Ba} ± 0.1	6.5^{Bd} ± 0.0	5.8^{Be} ± 0.1	4.3^{Cf} ± 0.1	3.1^{Bg} ± 0.1
GA2%	8^{Ac} ± 0.1	7.8^{Bb} ± 0.2	7.2^{Ca} ± 0.2	6.4^{Cd} ± 0.1	5.4^{De} ± 0.1	4^{Df} ± 0.1	2^{Fg} ± 0.1
NGA1%	8^{Ac} ± 0.1	7.9^{Bb} ± 0.1	7.1^{Ca} ± 0.1	6.2^{Cd} ± 0.2	5.5^{Ce} ± 0.0	4.1^{Df} ± 0.1	3^{Bg} ± 0.0
NGA2%	8^{Ac} ± 0.1	7.8^{Cb} ± 0.2	7.0^{Ca} ± 0.1	6.0^{Dd} ± 0.1	5.3^{De} ± 0.1	4^{Df} ± 0.1	2^{Fg} ± 0.1

-Different small letters in each column and different capital letters in each row indicate statistically significant differences ($p \geq 0.05$).

antimicrobial effect in fish samples that was similar to our results in present research (Abdollahzadeh, Rezaei, & Hosseini, 2014). Sorrentino et al. analyzed effect of GA in growth of *Pseudomonas* spp. during storage of fish and stated that the GA increases the shelf life of fish compared to control samples (so that after 28 days of storage, their microbial population was 8.77 log CFU/g logs in the control samples and 3.76 log CFU/g in the samples treated with 2.5 mg/mL GA), and the higher amount of GA, the better the effect (Sorrentino et al., 2018). Behbahani et al. evaluated that the effect of coating containing *Plantago major* seed mucilage (PMSM) and *Anethum graveolens* L. EO (D) on increasing the shelf life of beef and stated that after 18 days storage treatment of PMSM + 1.5% D had maximum antibacterial effect against *E. coli* and *S. aureus* (Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017).

3.6. Chemical analysis

3.6.1. DPPH assay

The results of present research showed that DPPH free radical scavenging activity (Table 6) also increased with increasing EO concentration. The plant EO has antioxidant activity due to its phenolic compounds. The antioxidant activity of phenolic compounds is mainly due to their redox oxidizing properties, thus acting as a reducing agent, oxygen scavenger and hydrogen donor (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Tometri, Ahmady, Ariaii, & Soltani, 2020). The highest and lowest inhibition of DPPH radical was observed in BHT 200 and EO1, respectively. These results were similar to the results of Pabast *et al.* and Homayonpour *et al.* who also reported by EO and NEO had antioxidant activity (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Sundararajan *et al.* also reported *Ocimum basilicum L.* free EO and nono form had antioxidant activity, and NEO form had better effect compare to the free EO (due to they have been developed to overcome the high volatility and instability of EOs (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahidi & Hossain, 2022; Taheri, Fazlara, Roomiani, & Taheri, 2018)), which was similar to the present findings (Sundararajan, Moola, Vivek, & Kumari, 2018). Hassani et al. confirmed the study by Sundararajan et al. and our study. They reported that thyme EO free and nono form had antioxidant activity and NEO had better influence compare to the EO free form. BHT had almost the best antioxidant activity similar to our study (Hassani & Hasani, 2018). Also, Polatoğlu *et al.* reported the *Lathyrus ochrus L.* (Cyprus Vetch, Luvana) EO had lower antioxidant activity compare to BHT and α-tocopherol (Polatoğlu, Arsal, Demirci, & Başer, 2015).

Table 6
DPPH assay of treatments.

EO1%	EO2%	NEO1%	NEO2%	BHT100	BHT200	p-value
33.8 ± 2	36.3 ± 1.2	32.5 ± 1.3	35.1 ± 2.1	93.9 ± 2.2	95.5 ± 1.8	0

3.6.2. pH assay

The pH values of the control and other treatments raised initially and then slightly reduced again on the 18th day, as shown in Figs. 3 and 4. The highest increase in pH value was observed on the 9th day in the control group due to the growth of *S. aureus*, with pH = 8. The minimum pH value was observed in the N-GA2% group (on the 18th day) with pH = 6.5 against *S. aureus*. The results also showed that the nano group treatments (*N*-GA and NEO) had a better effect against both bacteria due to they have been developed to overcome the high volatility and instability of EO (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahidi & Hossain, 2022; Taheri, Fazlara, Roomiani, & Taheri, 2018). The increase in pH value during long-term storage is generally attributed to the process of autolysis of endogenous enzymes (such as proteases and lipases), production of alkaline substances (such as histamine, trimethylamine, indole, and ammonia) and increased activity of microbial enzymes. In addition, it is assumed that the increase in pH value during the cold storage period is due to the decarboxylation of amino acids and thus the formation of amines (Kılıç, Şimşek, Claus, & Atılgan, 2014). Similar research has been done on the effect of EO treatments on reducing pH in in Nile tilapia (*Oreochromis niloticus*) (EL-Hanafy et al., 2011), tilapia (chen, Wu, Deng, Gao, Wang, & Liao, 2011) and shrimp fillets (Simpson, Gagne, Ashie, & Noroozi, 1997). During storage, the pH behavior of treatments with different levels of GA and EO (forms of nano and free) remained almost constant, likely due to their activity of protective alongside substrate decomposition and lower growth of bacterial compared to control sample (Kılıç, Şimşek, Claus, & Atılgan, 2014). This result is consistent with previous studies, such as Homayonpor *et al.*, who reported that the EO nano form of *Cuminum cyminum* L. had a better effect (during storage at 4 °C) than the EO free form (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a). Additionally, in a similar research on the rainbow trout characteristics (during storage for 15 days) coated with chitosan and thyme EO, Chamanara *et al.* stated that treatments with chitosan and EO had the lowest value of pH while the control (uncoated) had the highest value of pH (Chamanara, Shabanpour, Gorgin, & Khomeiri, 2012). Furthermore, Pabast *et al.* reported that *N*-EO of Satureja (during storage at 4 °C) had a better influence on the pH of meat (lamb) compared to the EO (free form) (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Behbahani et al. evaluated the effect of coating containing *Plantago major* seed mucilage (PMSM) and *Anethum graveolens L.* EO(D) on shelf life extension of beef and stated that after 18 days storage treatment of PMSM + 1.5 D had minimum pH value (Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017).

3.6.3. TVB-N assay

In all the treatments and control samples, TVB-N value raised during 18 days ($p < 0.05$), as shown in Fig. 5. This increase can be due to microbial activity, some enzymatic processes such as oxidation of amines, deamination of free amino acid (FAA) and destruction of nucleotides (Tometri, Ahmady, Ariaii, & Soltani, 2020). After 18 days, the highest value of TVB-N was related to the control sample (33.56 mg N/100 g) and the lowest value of TVB-N was related to the NEO treatment (27 mg N/100 g). This can be attributed to the reduction of the bacterial population or their oxidative ability to remove amines from volatile nitrogen compounds. Encapsulation may have helped to preserve the antibacterial properties of GA and EO for a longer time. These results are

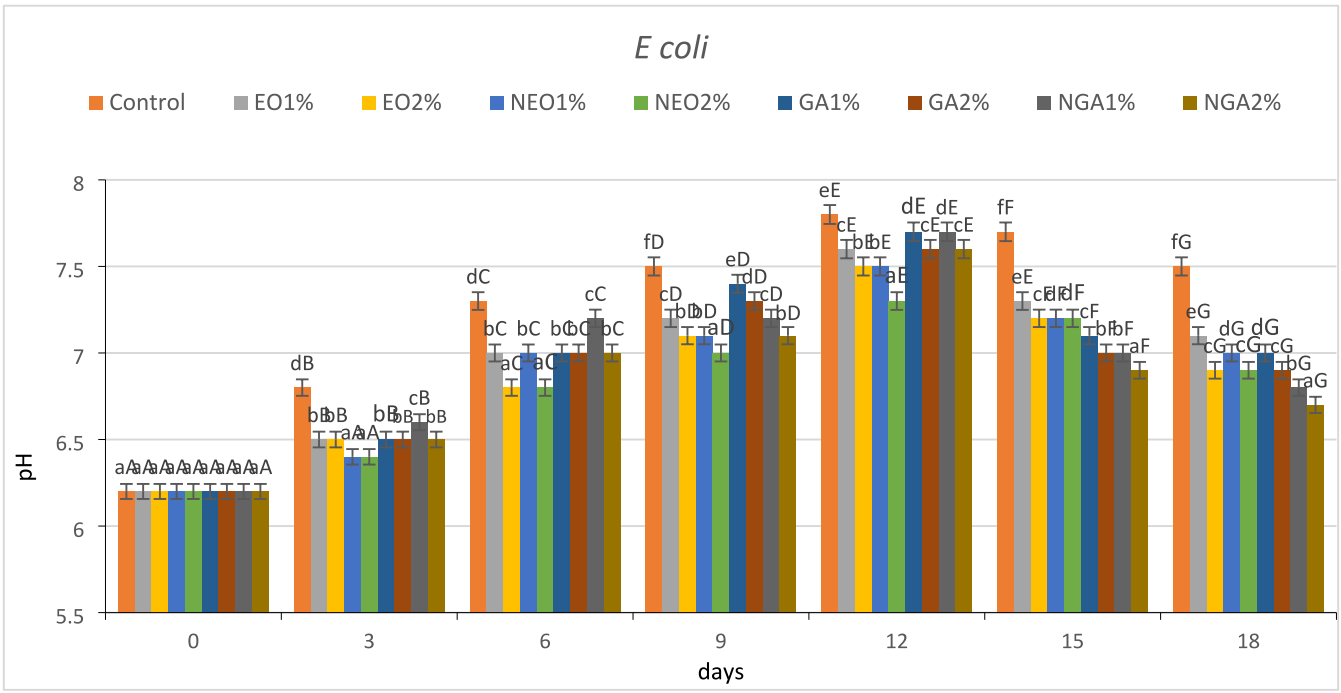

Fig. 3. The value of pH of GA and EO (forms of free and nano) during 18 days storing at 4 °C on *E. coli*.

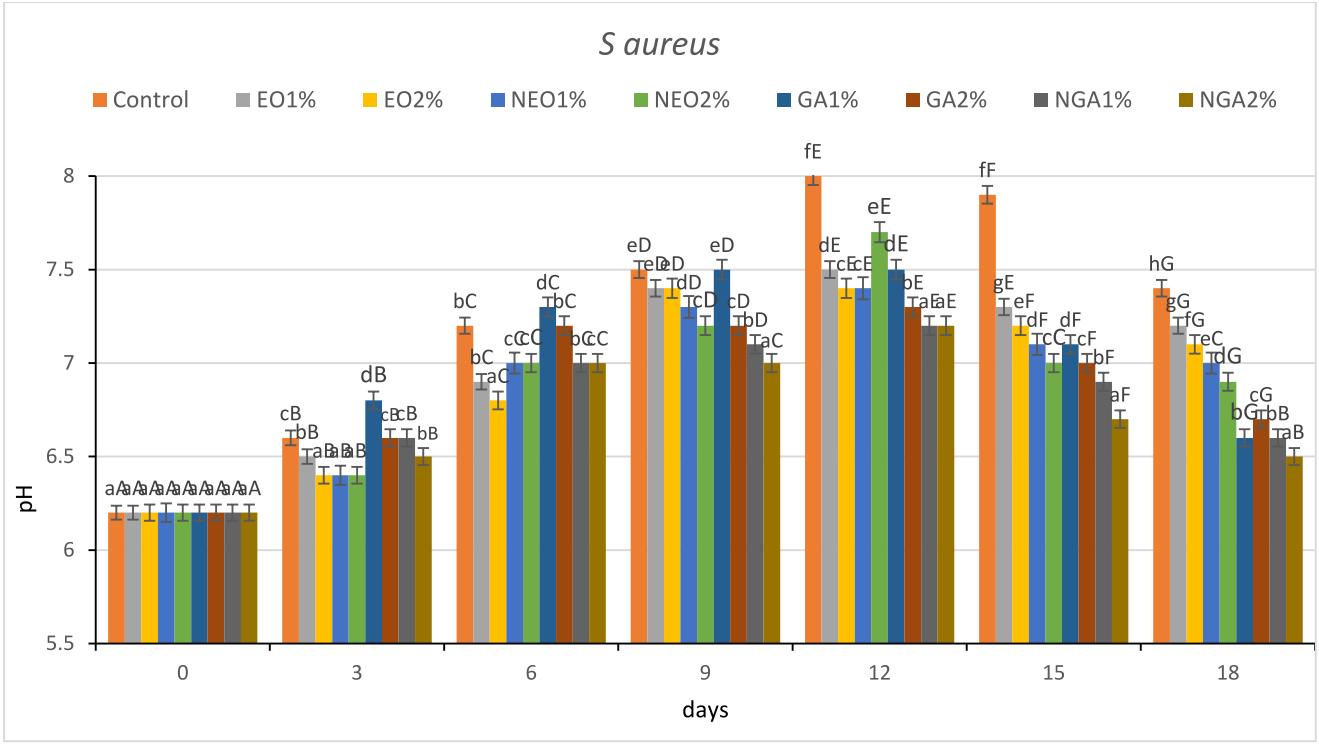

Fig. 4. The value of pH of GA and EO (forms of free and nano) during 18 days storing at 4 °C on *E. aureus*.

consistent with previous studies, such as Hasani et al., who reported that in TVB-N assay, the nano-encapsulation of lemon EO had a better effect than the free form, which supports our results (Hasani, Ojagh, Ghorbani, & Hasani, 2020). Like the mentioned study and our study, Tometri et al. also reported the nano form of Laurus nobilis leaf extract had a better effect in TVB-N assay compared to the free form of this extract (Tometri, Ahmady, Ariaii, & Soltani, 2020). Sayyari et al. also confirmed these results in TVB-N assay (during storage) by analyzing the *Foeniculum vulgare* EO (forms of free and nano) on fish fillets (Sayyari, Rabani, Farahmandfar, Esmaeilzadeh Kenari, & Mousavi Nadoshan, 2021).

3.7. Sensory evaluation

Table 7 shows the sensory evaluation of the evaluated treatments during the maintenance period. The scores for red color ranged from very faded red (score of 5) to so brilliant red of fresh-meat (score of 1),

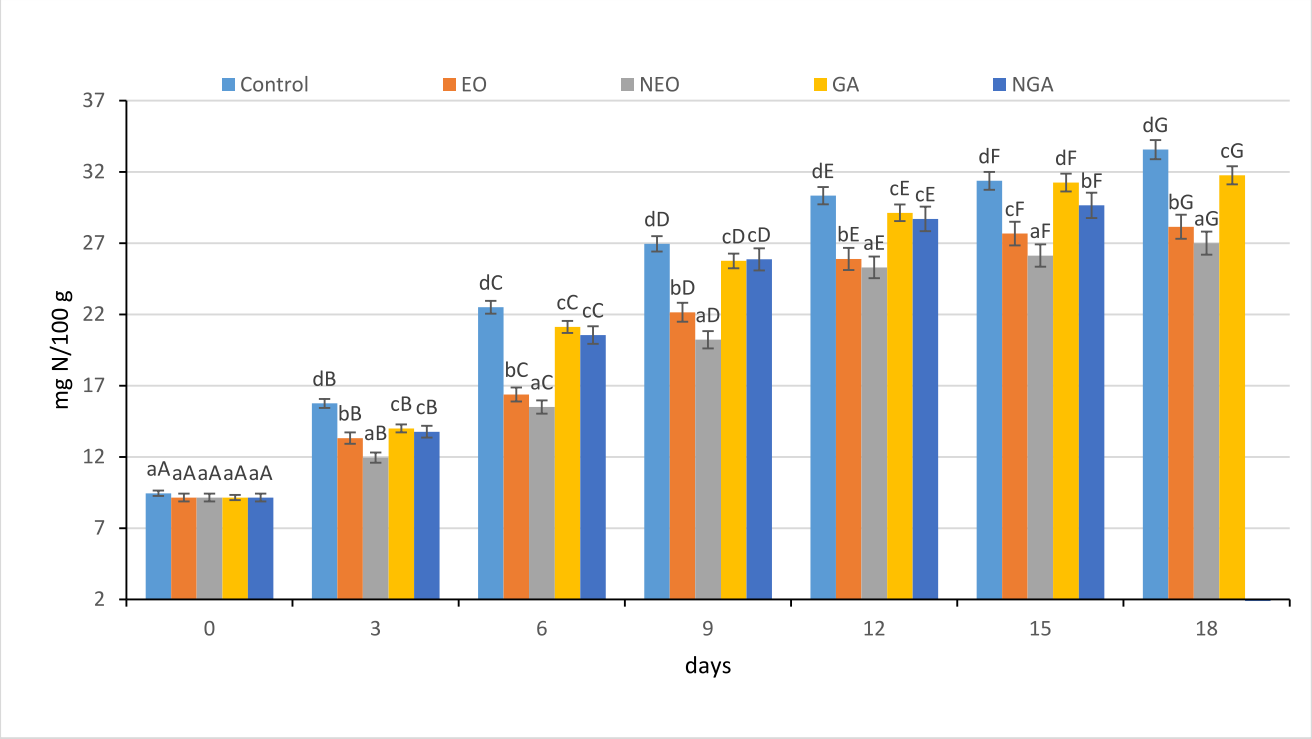

Fig. 5. TVB-N value of treatments.

Table 7
Sensory evaluation of minced meat samples storing at 4 °C during 18 days.

Parameter	group	Days of storage						
		0	3	6	9	12	15	18
Red color	Control	1 ± 0.00^{a}	2 ± 0.10^{b}	4 ± 0.10^{c}	5 ± 0.20^{d}	5 ± 0.20^{c}	5 ± 0.10^{d}	5 ± 0.20^{c}
	EO	1 ± 0.01^{a}	1 ± 0.00^{a}	1 ± 0.00^{a}	2 ± 0.11^{b}	2 ± 0.00^{a}	3 ± 0.11^{b}	4 ± 0.00^{b}
	N-EO	1 ± 0.01^{a}	$1 \pm 0.\ 10^{a}$	$1 \pm 0.\ 10^{a}$	$1 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$	$3 \pm 0.\ 10^{a}$
	GA	1 ± 0.00^{a}	2 ± 0.10^{b}	2 ± 0.10^{b}	3 ± 0.10^{c}	3 ± 0.10^{b}	4 ± 0.10^{c}	4 ± 0.10^{b}
	N-GA	1 ± 0.01^{a}	1 ± 0.00^{a}	1 ± 0.00^{a}	2 ± 0.11^{b}	3 ± 0.10^{b}	3 ± 0.21^{b}	4 ± 0.00^{b}
Discoloration	Control	1 ± 0.01^{a}	2 ± 0.10^{b}	4 ± 0.10^{d}	5 ± 0.10^{c}	5 ± 0.20^{d}	5 ± 0.10^{c}	5 ± 0.10^{c}
	EO	1 ± 0.00^{a}	1 ± 0.00^{a}	2 ± 0.00^{b}	2 ± 0.00^{a}	3 ± 0.00^{b}	4 ± 0.00^{b}	4 ± 0.00^{b}
		1 ± 0.0	$1 \pm 0.$	$1 \pm 0.$	$2 \pm 0.$	$2 \pm 0.$	$3 \pm 0.$	$3 \pm 0.$
	N-EO	1^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}	10^{a}
	GA	1 ± 0.00^{a}	1 ± 0.10^{a}	3 ± 0.10^{c}	3 ± 0.10^{b}	4 ± 0.10^{c}	4 ± 0.10^{b}	4 ± 0.10^{b}
	N-GA	1 ± 0.01^{a}	1 ± 0.00^{a}	2 ± 0.02^{b}	3 ± 0.20^{b}	3 ± 0.00^{b}	3 ± 0.22^{b}	4 ± 0.00^{b}
Off-odor	Control	1 ± 0.00^{a}	3 ± 0.10^{b}	4 ± 0.10^{c}	5 ± 0.20^{c}	5 ± 0.10^{d}	5 ± 0.10^{d}	5 ± 0.10^{d}
	EO	1 ± 0.01^{a}	1 ± 0.00^{a}	1 ± 0.00^{a}	2 ± 0.13^{a}	3 ± 0.00^{b}	3 ± 0.00^{b}	4 ± 0.00^{c}
	N-EO	1 ± 0.01^{a}	$1 \pm 0.\ 10^{a}$	$1 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$	$2 \pm 0.\ 10^{a}$
	GA	1 ± 0.00^{a}	1 ± 0.10^{a}	2 ± 0.10^{b}	3 ± 0.10^{b}	4 ± 0.10^{c}	4 ± 0.12^{c}	4 ± 0.13^{c}
	N-GA	1 ± 0.01^{a}	1 ± 0.00^{a}	2 ± 0.00^{b}	3 ± 0.12^{b}	3 ± 0.00^{b}	3 ± 0.22^{b}	3 ± 0.00^{b}

Data in the same column followed by different capital case letters are significantly different ($P < 0.050$).

while off-odor scores ranged from extreme (score of 5) to none (score of 1). Discoloration scores ranged from extreme (score of 5) to none (score of 1). According this table, the storage time had a significant influence on the sensory characteristics of all treatments ($p < 0.05$). After 18 days of storage, the NEO treatment received a highest score, whereas the control group had the lowest acceptance score. Our findings also showed that *N*-GA and NEO had better acceptability scores (due to they have been developed to overcome the high volatility and instability of EOs (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahidi & Hossain, 2022; Taheri, Fazlara, Roomiani, & Taheri, 2018)), which is consistent with the results of previous studies by Homayonpour et al and Pabast et al. (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021a; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Additionally, this results confirmed by EL-HANAFY et al., they stated GTE (green tea extract) treatment samples retained good quality characteristics in terms of sensory assessment compared to the group of control (EL-Hanafy et al., 2011). Sorrentino et al. analyzed effect of GA in sensory of fish during storage, and they stated that the samples treated with GA were far better than the control samples during storage for 28 days (Sorrentino et al., 2018). Alizadeh Behbahani et al. evaluated the effect of coting containing *Plantago major* seed mucilage (PMSM) and *Anethum graveolens L.* EO (D) on shelf life extension of beef and stated that after 18 days storage treatment of PMSM + 1.5 D had highest score of sensory evaluation (Behbahani, Shahidi, Yazdi, Mortazavi, & Mohebbi, 2017).

4. Conclusion

Our biological and chemical evaluations showed that GA and *Anethum graveolens L.* EO, in both free and nanoforms, can improve

biological safety, extend shelf life, and maintain minced meat quality during storage. The EO had a more positive effect on the chemical and sensory properties of minced meat compared to GA during 18 days of storage. Also, compared to the control samples, all treatments of GA and *Anethum graveolens* L. EO (forms of free and nano) decreases the count of microbial (significantly). Additionally, the nano forms of GA and *Anethum graveolens* L. EO (compared to the free form) showed that the highest antibacterial activity and enhanced the stability of minced meat samples during storage. One of the limitations of the study was not having enough resources to develop laboratory work. Finally, these findings suggest that the coating containing nano forms of *Anethum graveolens* L. EO and GA can be a useful method for maintaining meat quality in the meat industry.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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