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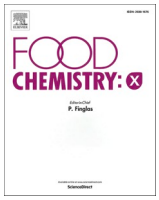

Effect of *Urtica dioica* L. Essential oil (forms of free and nanoliposome) on some inoculated pathogens (*Escherichia coli* and *Listeria monocytogenes*) in minced camel meat

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ABSTRACT

The goal of research was to investigate the impact of nanoliposome and free forms of nettle (*Urtica dioica L.*) essential oil (EO) on sensory, chemical and microbial properties of minced camel meat during storage at 4 °C. In our investigation, *Listeria monocytogenes* and *Escherichia coli* were inoculated into minced camel meat. The outcomes expressed the zeta potential, particle size, polydispersity index and efficiency of encapsulation of prepared nanoliposome were −17.5 mV to −12.8 mV, 143 to 158 nm, 0.77 ± 0.05 to 0.86 ± 0.07 Mw/Mn and 50.26–67.28 %, respectively. Also, according to the microbial analysis, the MIC of EO and nanoliposome-EO (*N*-EO) for *E.coli* was 25 ± 2.5 and 25 ± 2.1 mg/mL, respectively, and for *L. monocytogenes* was 12.5 ± 2.1 and 12.5 ± 2.1 mg/mL, respectively, and the MBC of EO and *N*-EO for *L. monocytogenes* was 50 ± 3.1 and 50 ± 3.2 mg/mL, respectively, and for *E. coli* was 50 ± 2.2 and 50 ± 2.2 mg/mL, respectively. The highest of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assay were detected in the BHT 200 (94.7 ± 2.7 and 95.6 ± 3.2, respectively) and lowest of them were detected NEO1% (33.7 ± 12.2 and 22.37 ± 0.22, respectively). After 18 days, the minimum value of pH was identified in the *N*-EO 2 % group incubated with *L. monocytogenes* (with pH = 6.9) and *E. coli* (with pH = 6.87). Furthermore, after 18 days of storage, the minimum TVB-N (total volatile basic nitrogen) value was observed in the *N*-EO group (26.89 mg N/100 g) and the maximum TVB-N value was observed in the control group (33.78 mg N/100 g). Finally, the *N*-EO and control treatment (during the experiment) had the highest and lowest sensory evaluation score, respectively. Finally, the *N*-EO group got a highest sensory score, whilst the group of control got the lowest acceptance score, after 18 days of storage. Based on the outcomes obtained from this research, using nettle (*Urtica dioica* L.) EO (in nanoliposome form) increases the storing time of minced camel meat.

1. Introduction

One of the important factors in evaluating the food security situation of the society is the amount of daily protein, which is usually supplied from plant sources (mainly bread, grains and legumes) and animal sources (mainly meat, chicken, fish, milk and dairy products and eggs) (Karimi, Hamidian, Behrouzifar, Mostafazadeh, Ghorbani-HasanSaraei, Alizadeh, et al., 2022). Compared to vegetable protein, animal protein has a higher biological value and usability for the body (Ghorbani-HasanSaraei, Rafe, Shahidi, & Atashzar, 2019). The meat and egg group is one of the key food groups in the food pyramid. This group comprises red meat (sheep and veal, camel), white meat (chicken, fish, birds, turkey and ostrich) and eggs. Each unit of low-fat meat contains 55 kcal, medium-fat meat 75 kcal, and high-fat meat 100 kcal. This group contains protein, iron, zinc and other nutrients (Kadim, Al-Amri, Al Kindi, & Mbaga, 2018).

The chemical composition of camel meat is not much different from the chemical composition of the meat of other domesticated animals, especially cattle. Camel meat than beef, has fewer protein and fat and more moisture. The global consumption of camel meat has rised in last

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years, especially in the Middle East and Asian countries due to its characteristics such as good aroma and taste. Since camel meat is rich in blood proteins such as myoglobin, this substance can act as a pro-oxidant agent in the lipid oxidation. Also, meat and meat products (such as camel meat) provide a suitable environment for pathogenic agents due to their nutritious factors and high water activity (Kadim, Al-Amri, Al Kindi, & Mbaga, 2018).

Listeria monocytogenes and *Escherichia coli* are among the bacteria that mainly cause meat contamination. All forms, especially *Escherichia coli*, are considered to be one of the most important causes of gastro-enteritis and a microbial indicator of water and food contamination. *Escherichia coli*, abbreviated as *E. coli*, is a type of gram-negative bacillus of the *Enterobacteriaceae* family, which is commonly found in the intestines of warm-blooded animals. Most strains of *Escherichia coli* are harmless; But some serotypes cause food poisoning and diarrhea. *Listeria* bacteria are gram-positive, microaerophilic, rod-shaped, sporeless, motile, and catalase positive. This genus has seven species, the main species that causes listeriosis in humans and animals is *Listeria monocytogenes*. These bacteria are widely scattered in the environment and can be found in soil, water and different food. Resistance to cold, drought and stability against osmotic stress has increased their survival and dispersion, so they can easily grow in the food in the refrigerator. Listeriosis is very dangerous in susceptible people who have immune system defects. Listeriosis causes different symptoms in humans, including abortion in pregnant women, neonatal septicemia, intrauterine infection in the form of granulomatosis, encephalitis, liver necrosis, and skin and digestive complications (Anvar, Nateghi, Shariatifar, & Mousavi, 2023; Pouryousef, Ahmady, Shariatifar, Jafarian, & Shahidi, 2022). The emergence of food-borne diseases, as well as economic and social difficulties, has resulted in a broad investigation into the production of healthy foodstuffs and the creation of novel agents of antimicrobial (Khademi, Raeisi, Younesi, Motamedzadegan, Rabiei, Shojaei, et al., 2022). Consequently, there is a pressing requirement to decrease/remove pathogenic microorganisms in foodstuff using various procedures (Raeisi, Ghoddusi, Boll, Farahmand, Stuer-Lauridsen, Johansen, et al., 2018). Furthermore, the desire for fewer synthetic preservatives from consumers has led to the study and use of natural derivatives possessing characteristics of antimicrobial. Notably, plant EOs and extracts are examples of such natural compounds.

In different types of food, plants (medicinal) play 2 chief roles: one is to create flavor and the other is to preserve food by delaying spoilage, due to their characteristics of anti-microbial and anti-oxidation (Davarnia, Shahidi, Karimi-Maleh, Ghorbani-HasanSaraei, & Karimi, 2020). Essential oils and secondary metabolites found in plants are recognized for their antimicrobial effects and no/minimal toxicity (S.-A. Shahidi, 2022). These oils containing 20 to 60 aromatic combinations that give them their unique smell and flavor. From numerous species of aromatic plant, essential oils are extracted, which these plants found across the globe, including nettle (*Urtica dioica* L.), which is a popular source of essential oil (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

Urtica dioica.L (Nettle) belonging to the Urticaceae family. It is native to Europe, much of temperate western North Africa and Asia (such as Iran, Turkey, etc.), but it can now be found all over the world. The uses of this plant include use in food, traditional medicine and drinking such as tea (Mahjoorian, Jafarian, & Fazeli, 2021). Nettle is a perennial, herbaceous and dioecious plant, in the summer have 0.9 to 2 m tall. Fresh leaves of this plant comprise fat (0.7 to 3.3 %), protein (about 5.5 %), carbohydrates (about 7.1 %), water (about 82.4 %) and dry matter (about 17.6 %). Mature leaves of this plant comprise a valuable omega-3 acid and α- linolenic acid (approximately 40 %). Nettle plant extract is effective on Salmonella and Proteus resistant to antibiotics. The phenolic compounds in nettle, which include ferulic acid, sinapic acid, caffeic acid, myrstin and phocitin, have an effect on bacteria such as *Klebsiella*, *Proteus vulgaris*, *Pseudomonas* and *Escherichia coli*, and the antifungal effects of some compounds in nettle have also been confirmed. Among the tested extracts, extract of nettle seed had the most antibacterial efficacy on bacteria of gram-positive, flower extract had the most antifungal efficacy, and extract of plant leaf had the most antibacterial efficacy on bacteria of gram-negative (Khan, Azad, Jan, Safdar, Bibi, Majid, et al., 2023).

While nettle has potential applications in food, its sensitivity to light, high temperatures, O_2, and its unpleasant taste, create obstacles. Furthermore, its rapid elimination and short half-life from the body make it less than ideal as a food source. To overcome these challenges, a modified formula (microencapsulation) that can protect it during consumption, production and storage is needed (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016).

Microencapsulation of EO and extracts of plant is increasing due to the important features that this technology creates in these microencapsulated materials (Mehdizadeh, Shahidi, Shariatifar, Shiran, & Ghorbani-HasanSaraei, 2022). Among these characteristics, it is possible to raise the permanency of microcoated materials by defending them from environmental, enzymatic and chemical changes (improvement in material buoyancy), providing a buffer state against pH changes, dealing with thermal changes and ionic changes, protecting against flavors and unpleasant odors, controlled release of encapsulated material and thorough mixing of immiscible materials (Hematian, Baghaei, Mohammadi Nafchi, & Bolandi, 2023; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

Phospholipid-based surfactants can form nanoliposomes, which are spherical vesicles with a bilayer membrane shaped by the hydrophobic tails joining together. These carriers are flexible and can transport simultaneously both materials of hydrophilic and hydrophobic, either within an inside a membrane of vesicle or bilayer. Compared with liposomes, forms of nanoliposome greater control over release, offer better solubilization, higher area of surface, and more effective targeting of encapsulated compounds (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016).

Camel meat is prone to spoilage of microbial, oxidation of lipid and changes of color due to its low oxidative stability and unique combinations (Kadim, Al-Amri, Al Kindi, & Mbaga, 2018). Nanoliposomes containing EO is an effective strategy to preserve the quality of camel meat for a longer period of time and to delay the growth of bacteria on its surface, thus prolonging its shelf life (Anvar, Nateghi, Shariatifar, & Mousavi, 2023).

Currently, there is no research available on the maintenance of camel minced meat by nettle EO in forms of nanoliposome or free in Iran or around the globe. Furthermore, owing to the increasing popularity of camel meat (in the country of Iran and around the globe), such research is warranted. Thus, the goal of this research was to consider the impact of nettle EO (nanoliposome and free forms) on two important pathogens of foodborne inoculated in camel minced meat (such as *L. monocytogenes* and *E. coli*) during refrigerated storage. For this purpose, microbiological analyzes (including minimum bacterial concentration (MBC), minimum inhibitory concentration (MIC) and antimicrobial effect against *L. monocytogenes* and *E. coli* inoculated in camel minced meat), physicochemical (nanoliposome size, zeta potential, encapsulation efficiency). polydispersity index, DPPH, TVB-N, FRAP and pH) and sensory were performed.

2. Materials and methods

2.1. Reagents and materials

Many materials, including, sodium acetate, acetic acid, cholesterol (95 %), glycerol (>97 % purity), dichloromethane, methanol ferric chloride, folin-Ciocalteu reagent, dimethyl sulfoxide, hydrochloric acid, and sodium carbonate were purchased from Merck Co. (Darmstadt, Germany). For nanoliposomes preparation, from Company of Across (USA) was bought, L-a-lecithin (with 99 % purity, granular phospholipid). Broth Heart Infusion (BHI) and CHROMagar™ Listeria culture were acquired from Merck Co.. Buffered peptone water and sterile

stomacher bag were acquired from Oxoid (Belgium) and VWR (Belgium), respectively. Also, Chromocult Coliform-agar and Triple Soy agar (TSA) were acquired from Merck (Germany). Finally, other solvents and materials were acquired from Company of Merck (Darmstadt, Germany), which were grade of analytical or better existing pureness.

2.2. Maintenance and preparation of bacteria

The strains of *Listeria monocytogenes (*ATCC 19118*)* and *Escherichia coli* (ATCC 25922) were provided from the National Center of Genetic and Biological Resources in Iran. We used our previous study to reactivate and prepare the strains (Tometri, Ahmady, Ariaii, & Soltani, 2020). The population of bacterial cell was considered at 600 nm by evaluating the OD (optical density), with a 1×10^8 cells/mL population (according to the pretest, 0.08–0.1 OD = 1×10^8 cfu/mL). Eventually, after dilution, the camel's minced meat with both E. *coli* and L. *monocytogenes* (1×10^4 cfu/g), was inoculated (Tometri, Ahmady, Ariaii, & Soltani, 2020).

2.3. Preparation of aerial parts essential oil

Two kg of nettle aerial parts were attained from Baneh City local bazaars (Iran), and were authenticated at TUMS (Tehran University of Medical Sciences) by an expert of pharmacology. Other steps were done according to the previous studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.4. Identification and diagnosis of the EO's chemical combinations by GC–MS

In this study, the chemical combinations of nettle EO was identified by GC–MS (gas chromatography model Agilent model 7890A and detector of mass selective model 5975C VL MSD with Detector of Triple-Axis). The GC–MS conditions and temperatures was mentioned in other researches (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.5. Preparation of nanoliposome of EO

In our study, by thin layer hydration and ultrasound procedures, preparations of EO nanoliposomes was performed based on the previous studies in four ratios (20:40, 30:30, 50:10 and 60:0) of lecithin/cholesterol in terms of mg (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.6. Minced camel meat's preparation and treatment groups' preparation

Fresh meat of camel (*Camelus dromedarius*) (raw meat, 10 kg) was bought from of Tehran's local markets. All other steps was performed according to the previous studies (Pouryousef, Ahmady, Shariatifar, Jafarian, & Shahidi, 2022; Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020). Minced meat of camel with *E. coli* and *L. monocytogenes* (1×10^4 CFU/g) (separately) were inoculated and after that they were applied to prepare all the treatments. Our treatments includes control groups (without bacteria and without EO) and EO treatments in levels of one and two percent (in the forms of nanoliposome and free), and finally they were analyzed (in triplicate) on 0, 3, 6, 9, 12, 15, and 18 days, during storage at 4 °C.

2.7. Measurement of encapsulation efficiency (EE %), zeta potential, nanoliposomes z-average diameter and polydispersity index (PDI)

Based on the previous study, to evaluate the PDI, backscattered light was applied by utilizing a DLS (dynamic light scattering) system equipped with a detector of backscatter and a laser diffractometer (Nano ZS, Malvern Instruments Ltd., the UK) (Keykhosravy, Khanzadi, Hashemi, & Azizzadeh, 2020). Using a UV spectrophotometer (Pharmacia biotech ultraspec 2000, UK) was evaluated the nanoliposome's EE % (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Also, the nanoliposomes zeta potential was considered by a Malvern Zeta sizer Nano ZS (Malvern Panalytical Company, Worcestershire, UK) (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021). Lastly, by DLS (dynamic light scattering) procedure, the nanoliposomes z-average diameter was evaluated using a analyzer of particle size of Shimadzu (SALD 2101, Japan) (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.8. Analysis of TEM (transmission electron microscopy) and SEM (Scanning electron microscopy)

In this study, we applied SEM analyzer (KYKY-EM 3200; KYKY Technology Development Ltd., Beijing, China), which analyzed the nonoliposomes' morphology and structure. The SEM conditions was mentioned in previous studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Also, we applied TEM microscope analyzer (Philips Bio- Twin, the Netherlands) to examine the characterization of nanoliposomes. The TEM conditions was mentioned in previous studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021).

2.9. Analysis of microbiological

2.9.1. Analysis of MBC and MIC

In this study, 10^8 CFU/g of bacteria level (*L. monocytogenes* and *E. coli*) were added to microbial examination tubes, alongside with a solutions of nettle EO (0.2 mL each) in distilled (double) water and DMSO (dimethyl sulfoxide). Then, they were incubated at 37 °C for 24 h. The MIC and MBC was evaluated according to the prior study (Rashidaie Abandansarie, Ariaii, & Charmchian Langerodi, 2019).

2.9.2. Enumeration microbial test

Minced meat of camel (ten grams) was homogenized (to enumerate *E. coli*) in buffered peptone water (100 mL) for two minutes by a sterile stomacher bag (filter 0.5 mm pore size). Next, for 24 h at 37 °C, incubated in Chromocult Coliform-agar (Van Haute, Raes, Van Der Meeren, & Sampers, 2016). Using physiological serum (45 mL), 5 g of minced meat of camel (to enumerate *L. monocytogenes*) were homogenized. L. *monocytogenes* was enumerated by spreading serial dilutions (0.1 mL) on CHROMagar™ Listeria culture, then all plates were incubated for 24 h at 37 °C. To enumerate the growth of *L. monocytogenes* in heat-treated minced camel meat, plates were cultured (every 3 days) on CHROMagar™ Listeria culture. Also, bacterial colonies with blue color and white halo were counted on the plates (Abdollahzadeh, Rezaei, & Hosseini, 2014).

Finally, for *E. coli* and *L. monocytogenes*, the bacteria number/gram of camel's minced meat was expressed as log cfu/g.

2.10. Analysis of chemical

2.10.1. DPPH and FRAP assay

According to the prior studies, DPPH assay (by UV spectrophotometry) (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018) and FRAP assay (Stratil, Klejdus, & Kubáň, 2006) were evaluated in minced camel meat.

2.10.2. pH and TVB-N assay

Based on the prior studies, to assess the value of pH, ten grams of minced camel meat samples were mixed in of distilled water (100 mL) and then the pH value (by digital pH analyzer of HANNA, Germany) was evaluated (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021). The TVB-N value (by the procedure of micro diffusion) (Tometri, Ahmady, Ariaii, & Soltani, 2020) were assessed in minced camel meat. For 2 min, the samples (ten grams) were homogenized in perchloric acid 6 % (100 mL) and then filtrated. Next step, with sodium hydroxide solution (20

%), it was alkalized and finally steam distillation of the extract is done. By an acid receiver, the composites of volatile base were absorbed and evaluated by titration.

2.11. Sensory evaluation

Based on the prior research, we selected six panelists of semi-trained (that had a practice in meat sensory assessment) (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). In this study similar to the prior research, by questionnaire using five point (five to one) descriptive scale, the items of "red color", "discoloration'' and "off-odor'' of treated samples were valued. In this test, the value of one has the maximum quality or the maximum score and the value of five has the minimum quality or the minimum score. Also, when the sensory characteristics were higher than the value of three, the sample was no longer of good quality and was rejected (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

2.12. Analysis of statistical

All experimentations was accomplished in triplicate. All consequences were publicized as mean ± SD. In this study, the findings (with SPSS V.24) were estimated by test of ANOVA (analysis of variance), followed by the test of Duncan's post hoc to calculate the impact of storing period and treatments on parametric data (DPPH, TVB-N, sensory analysis, FRAP and pH). Finally, $p < 0.05$ was the sign of significance between treatments in all tests.

3. Results and discussion

3.1. Compounds identification of Urtica dioica L. EO

According to the results obtained from Table 1., 21 compounds of nettle essential oil were identified in the collection, which constituted 97.64 %, most of which included phytol, α-Limonene, p-Cymene, β-Ionone, γ-Terpinene, Carvacrol, β-Pinene, α-Copaene-8-o and α-Ionone. Our findings was confirmed by other researches but their percentages were different, which could be due to differences in species of plant, weather and water conditions, type of soil, and geographic region (Gül, Demirci, Başer, Akpulat, & Aksu, 2012; Keshavarz, Rezaeipour, & Asadzadeh, 2014; Khan, et al., 2023).

Table 1
The amount of combinations in the EO of *Urtica dioica* L. (aerial parts).

Peak NO.	Compound	RT (min)	A%
1	Benzaldehyde	9.35	0.1
2	α-Pinene	9.46	1.65
3	β-Pinene	10.21	2.22
4	β-Myrcene	10.63	1.1
5	Carvacrol	11.29	0.85
6	α-Limonene	13.33	15.27
7	p-Cymene	14.77	12.35
8	γ-Terpinene	15.69	7.24
9	β-Caryophyllene	16.32	1.60
10	Decanal	18.21	1.1
11	Camphene	19.62	0.13
12	Bornyl acetate	20.75	0.21
13	Thymol	21.58	0.39
14	Carvacrol	22.39	3.12
15	α-Ionone	2265	2.12
16	Geranyl acetate	23.12	0.1
17	β-Ionone	24.14	10.19
18	α-Copaene-8-o	24.65	2.22
19	Isopropyl dodecanoate	25.19	1.21
21	Phytol	26.35	34.47
total			97.64

3.2. Assessment of nettle EO nanoliposome properties

Based on the results shown in Table 2, the particle size (z-average diameter) of EO nanoliposome varied from 143 to 158 nm. For nanoliposomes to be effective and stable in releasing the combinations trapped in the nanoliposome core, z -average diameter is a chief item. Bimodal z-average diameter distribution was confirmed by tests of DLS. In a similar study, Haghju et al. analyzed chitosan combined with extract-loaded nanoliposomes of nettle (*Urtica dioica* L.) and expressed the z-average was ranged from 107.41 to 136.82 nm (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). In other study, Pabast et al. expressed the nanoliposom particles size was ranged from 93 to 96 nm (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018) and in Homayonpour et al. stated the particle size was varied from 140 to 164 nm (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021).

The zeta potential (ZP) is a main items in determining the surface charge of particles (electrical), depiction of colloidal systems and determining constancy of prepared nanoliposomes. By increasing the absolute ZP (due to high forces of repulsive that reduce the rate of aggregation and fusion), colloidal suspensions have higher stability of chemical and physical (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). Based on the results shown in Table 2, the ZP value varied from −17.5 mV to −12.8 mV. According to the results of Table 2, the zeta potential was negative in all ratios of lecithin to cholesterol, which is attributed to the existence of lipid terminals. In this study, when the cholesterol's ratio increased, the total quantity of nanoliposome's negative charge increased significantly. Adding more cholesterol to nanoliposome's membranes stabilizes chains of lipid by rising the density of phospholipid molecules and by filling in the holes of vesicle molecular in an area where phospholipids are able to complexing with cholesterol. In a similar study, Haghju et al. analyzed chitosan combined with extract-loaded nanoliposomes of nettle (*Urtica dioica* L.) and expressed the zeta potential was varied from −53.60 to −52.50 mV (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). Also, our findings was confirmed by others studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

The PDI value evaluates the molecule heterogeneity or sizes of particles and this value varied from zero to one. Based on the results shown in Table 2, the PDI value in this study was varied from 0.77 ± 0.05 to 0.86 ± 0.07 Mw/Mn. In similar study, Haghju et al. analyzed chitosan combined with extract-loaded nanoliposomes of nettle and expressed the PDI was varied from 0.26 to 0.28 Mw/Mn (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). Also, our findings was confirmed by others studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

Based on the results shown in Table 2, the EE % was varied from 50.26 to 67.28 % for nanoliposomes. To impact EE % of nanoliposomes, three parameters are important, which comprise type, ratio of lipid and the inner volume of vesicles (Ebrahimi, Nafchi, Bolandi, & Baghaei, 2022). There are 2 segments in the structure of nanoliposome that comprise hydrophobic and hydrophilic. Amid the 2 layers of phospholipids, combinations of hydrophobic are surrounded and combinations of hydrophilic in the nanoliposomes' aqueous medium, so the phospholipid bilayers (for EO) act as a reservoir. In an analogous study, Haghju et al. analyzed chitosan combined with extract-loaded nanoliposomes of *Urtica dioica* L. and expressed the EE% was varied from 69.13 to 71.11 % (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). Our results also confirmed by other studies with other essential oils (Abdollahzadeh, Rezaei, & Hosseini, 2014; Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

3.3. SEM image

Fig. 1(A) indications *N*-EO SEM image. The SEM image (according to

Table 2
Determination of particle size, ZP, PDI and EE of *Urtica dioica* L. aerial parts essential oil.

Code	Lecithin: Cholesterol	Polydispersity Index (Mw/Mn)	z-average diameter (nm) of NEO	Encapsulation Efficiency% Of NEO	Zeta potential (mV)
1	60:00	0.80 ± 0.06^{b}	156 ± 0.22^{a}	62.08 ± 0.63^{b}	-12.8 ± 0.32^{a}
2	50:10	0.77 ± 0.05^{c}	143 ± 0.32^{c}	67.28 ± 0.42^{a}	-16.4 ± 0.38^{a}
3	40:20	0.79 ± 0.04^{a}	150 ± 0.43^{b}	59.58 ± 0.88^{c}	-17.5 ± 0.41^{b}
4	30:30	0.86 ± 0.07^{a}	158 ± 0.53^{a}	50.26 ± 0.59^{d}	-16.9 ± 0.32^{c}

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

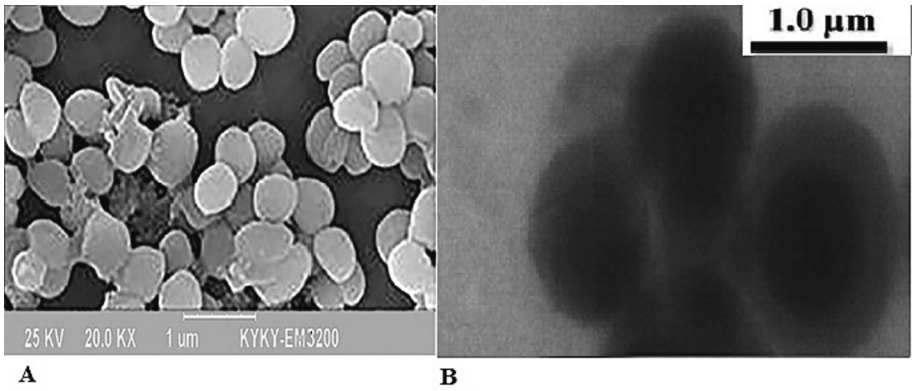


Fig. 1. (A): SEM assay of *N*-EO. (B): TEM image of *N*-EO.

the previous findings) was accomplished to show the morphology and structure of EO nanoliposomes with the maximum EE% and the minimum droplet size (60:00 lecithin/cholesterol) (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). As revealed in mentioned image, hemispherical and spherical nanoliposome of EO were shaped. Our finding in this study confirmed in previous studies by other researchers (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

3.4. TEM image

Fig. 1(B) indications TEM image of EO nanoliposome. The TEM image was accomplished to show the morphology of *N*-EO with the minimum size of droplet and the maximum EE% (60:00 lecithin/cholesterol), based on the prior findings (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). As revealed in this figure, the appearance of nano-sized of EO loaded nanoliposomes exposed an ostensible structure of core–shell, which proved the nanoliposomes formation. Our finding in this study confirmed in previous studies by other researchers (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

3.5. Analysis of microbiological

3.5.1. Analysis of MIC and MBC

As exposed in Table 3, the outcomes exhibited the MIC of EO and NEO for *E.coli* was 25 ± 2.5 and 25 ± 2.1 mg/mL respectively, and for *L. monocytogenes* was 12.5 ± 2.1 and 12.5 ± 2.1 mg/mL, respectively. Furthermore, the outcomes exhibited the MBC of EO and NEO for *L. monocytogenes* was 50 ± 3.1 and 50 ± 3.2 mg/mL, respectively, and for *E. coli* was 50 ± 2.2 and 50 ± 2.2 mg/mL, respectively (Table 3). The outcomes exhibited the MIC numbers in bacteria of gram nagative were higher than bacteria of gram positive. Bacteria of gram positive (*L. monocytogenes*) is sentient to combinations of antibacterial owing to the nonexistence of a layer of lipo-polysaccharide in their walls of cell. Conversely, bacteria of gram nagative (*E. coli*) have layer lipo-polysaccharide that can avert active combinations from incoming the membrane of cytoplasmic (Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020). Likewise, in gram nagative bacteria, the hydrophilic surface of the outer membrane can make them resistant to combinations of antibacterial. The reason is the presence of the lipopolysaccharide cell wall layer, which prevents the penetration of different antibiotic molecules and different enzymes that break down the molecules entering the periplasmic space (Shahbazi, 2017; Tometri, Ahmady, Ariaii, & Soltani, 2020). Prior research has shown that phenolic and flavonoid compounds are associated with antibacterial activity. The antibacterial activity of plant essential oils seems to be influenced by these compounds (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Tometri, Ahmady, Ariaii, & Soltani, 2020). Furthermore, this research approves that the essential oil activity of antimicrobial is connected with changes in membranes of cell caused by the permeation of phenolic combinations and a cell membranes' electrical imbalance leading to leakage of intracellular combinations and ultimately death of cell. Subsequently, an intensification in the phenolic compounds level propelled to an intensification in 1,8-cineol, which augmented significantly the impact of antimicrobial (Noori, Zeynali, & Almasi, 2018; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; Shahbazi, 2017). In a similar study, Pouryousef et al. analyzed impacts of nanoliposome and free forms of *Mentha pulegium* L. Eo and nisin on inoculated bacterial (*S. aureus*, *V. parahaemolyticus* and *L. monocytogenes*) in minced fish (silver carp), and expressed MIC was ranged from 0.010 to 0.052 mg/mL and MBC was ranged from 5 to 20 mg/mL for EO, nisin, NEO and *N*-nisin treatments against 3 stated bacteria (Pouryousef, Ahmady, Shariatifar,

Table 3
The MBC and MIC of the *Urtica dioica* L. EO and NEO.

		L. monocytogenes	*E. coli*
EO	MIC	12.5 ± 2.1^{c}	25 ± 2.5^{a}
NEO	MIC	12.5 ± 2.1^{c}	25 ± 2.1^{b}
p value		0.00	0.00
EO	MBC	50 ± 3.1^{c}	50 ± 2.2^{c}
NEO	MBC	50 ± 3.2^{c}	50 ± 2.2^{d}
p value		0.00	0.00

Jafarian, & Shahidi, 2022). In other similar research by Shahbazi, analyzed effect of four different of EO (*Mentha longifolia*, *Falcaria vulgaris, Tragopogon graminifolius* and *Allium rotundum*) against 6 different pathogenic bacteria (*L. monocytogenes, S. typhimurium, B. subtilis, E.coli, S. aureus,* and *B. cereus*) and stated that the MBC and MIC values in bacteria of gram nagative were higher than bacteria of gram posetive (Shahbazi, 2017). Our results also confirmed by Tometri *et al.*'s research that analyzed the effect of *Laurus nobilis* leaf extract against two different bacteria (include *S. aureus* and *E. coli*) (Tometri, Ahmady, Ariaii, & Soltani, 2020) and by Hajlaoui *et al.* that analyzed effect of *Mentha longifolia* EO compared with gentamycin antibiotic against some pathogenic bacterial and stated EO had an acceptable impact on growth of bacterial (*Listeria monocytogenes* and *Staphylococcus aureus*) (Hajlaoui, Snoussi, Jannet, Mighri, & Bakhrouf, 2008).

3.5.2. Antimicrobial impact of EO (forms of nanoliposome and free) on some inoculated bacteria (L. Monocytogenes and E. coli)

According to the Fig. 2A and 2B, the growth of 2 mentioned bacteria (*L. monocytogenes* than *E. coli*) in treatments of EO and NEO (during 18 days of storage) was reduced ($p < 0.05$), but it was raised in the group of control. Our findings stated, the treatments (EO and NEO) had a better effect (during 18 days of storage) on the *L. monocytogenes* than *E. coli*, so that in the NEO2% treatment, the growth of *L. monocytogenes* has reduced from 4.6 (day 0) to 2 (day 18) log CFU/g and the growth of *E. coli* has reduced from 4.2 (day 0) to 2 (day 18) log CFU/g. According to our findings, with the raise in the amount of EO from 1 % to 2 % (in both forms of nanoliposome and free), the antimicrobial effect has enhanced, which can be owing to the increased EO antimicrobial effect. Besides, in 2 mentioned bacteria (*L. monocytogenes* than *E. coli*), the treatments comprising EO in the form of nanoliposome (NEO) had a preferable effect. Nano form of combinations like nanoemulsion or nanoliposomes tend to compounds of encapsulated (bioactive) owing to their higher areas of surface and closer nearness to cells of bacterial, generating a greater influence of antimicrobial and quantum-size influence in the formulations of EO (Noori, Zeynali, & Almasi, 2018). So, coatings containing nanoliposome may be better for extending of camel meat storage time. In a similar study, Mahjoorian et al. analyzed effect of nettle EO on *P.aeruginosa, E. coli*, *L. monocytogenes* and *S. aureus*, and stated that the NEO treatment had better antimicrobial effect compared to the group of control (Mahjoorian, Jafarian, & Fazeli, 2021). Another research by Haghju et al. that they analyzed chitosan combined with extract-loaded nanoliposomes of nettle and expressed all treatments had better effect of

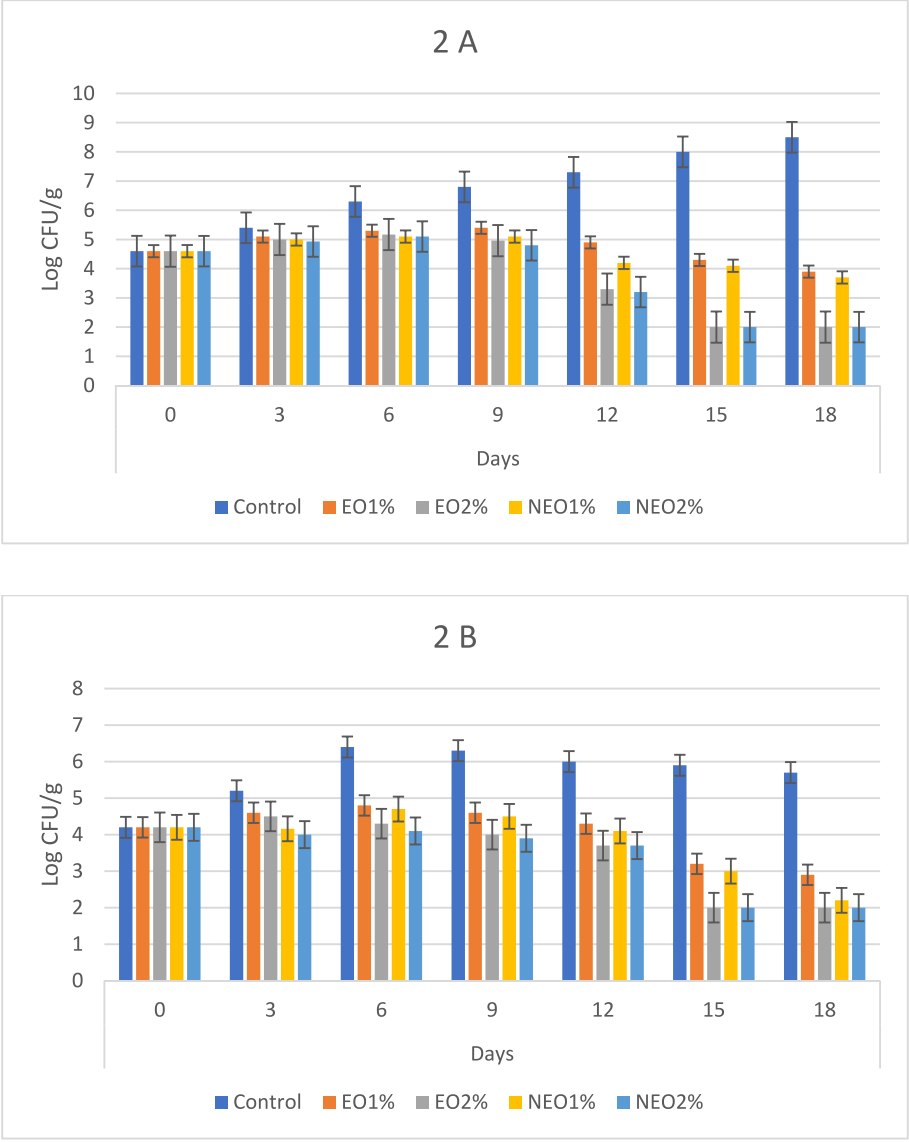


Fig. 2. (A) Antimicrobial impact of EO (forms of nanoliposome and free) against inoculated *L. monocytogenes* (log CFU/g) and (B) antimicrobial impact of EO (forms of nanoliposome and free) against inoculated *E. coli* (log CFU/g).

antioxidant against bacteria of *S. aureus* compared to the control samples (Haghju, Beigzadeh, Almasi, & Hamishehkar, 2016). Similarly, Abdollahzadeh et al. investigated the effect of coating containing thyme EO on the growth of *L. monocytogenes* in fish samples (during 12 days) and stated increasing the EO amount, increased the effect of antimicrobial (Abdollahzadeh, Rezaei, & Hosseini, 2014). Likewise, Van Haut et al. investigated the growth of *E. coli* in chicken samples (breast fillet and skin) and stated the counts of *E. coli* was lower in skin chicken samples mixed with thyme (one percent) compared to the samples of blank (without thyme) and but in samples of breast fillet, the counts of *E. coli* was constant in samples of blank and all treatments (Van Haute, Raes, Van Der Meeren, & Sampers, 2016). Forthermore, Mazhar *et al.* in an analogous experiment, analyzed different levels of mint EO on the growing of *Salmonella paratyphi* and *S. typhimurium* in fish (minced) and expressed increasing the quantity of EO (0.1 % to 0.5 %) rises the effect of antimicrobial (Mazhar, Aliakbari, Karami, Morshedi, Shariati, & Farajzadeh, 2014).

3.6. Chemical analysis

3.6.1. Analysis of DPPH and FRAP

Based on the results obtained from Table 4, the DPPH value raised with increasing EO levels from 1 % to 2 %. Plant essential oils have antioxidant activity due to the presence of phenolic compounds in their matrix. The phenolic compounds antioxidant properties is mainly owing to their properties of redox oxidizing, so it acts as an agent of reducing, scavenger of oxygen (O_2) and donor of hydrogen (H_2) (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Tometri, Ahmady, Ariaii, & Soltani, 2020). The maximum and minimum of DPPH value were detected in the BHT200 (94.7 ± 2.7) and NEO1% (33.7 ± 12.2), respectively. Almasi et al. (in similar study) anlayzed chitosan combined with extract-loaded nanoliposomes of nettle and they stated as the amount of extract increased, the amount of DPPH increased, which TBHQ was upper than all treatments (Almasi, Zandi, Beigzadeh, Haghju, & Mehrnow, 2016). Our results also confirmed by Gülçin et al. that analyzed antimicrobial, antioxidant, analgesic and antiulcer effects of nettle (Gülçin, Küfrevioğlu, Oktay, & Büyükokuroğlu, 2004). Similarly, Polatoğlu *et al.* expressed the *Lathyrus ochrus L.* (Cyprus Vetch, Luvana) EO had lesser effect of antioxidant compared to α-tocopherol and BHT (Polatoğlu, Arsal, Demirci, & Başer, 2015). Also, Hassani et al. stated thyme EO (in the forms of free and nono) had activity of antioxidant and NEO treatment had better impact compare to the free form of EO and BHT treatment had the best (almost) activity of antioxidant (Hassani & Hasani, 2018).

FRAP value is an assay of antioxidant capability, which usages Trolox as a standard. FRAP examine is applied (often) to evaluate the antioxidant capability of beverages, foodstuffs, and supplements of nutritional comprising polyphenols. The highest and lowest of FRAP value were detected, in the BHT 200 (95.6 ± 3.2) and NEO1% (22.37 ± 0.22), respectively. Our results was confirmed by other studies (Mamadalieva, Sharopov, Satyal, Azimova, & Wink, 2017; Thomas, Essien, Ntuk, & Choudhary, 2017).

3.6.2. Analyze of pH

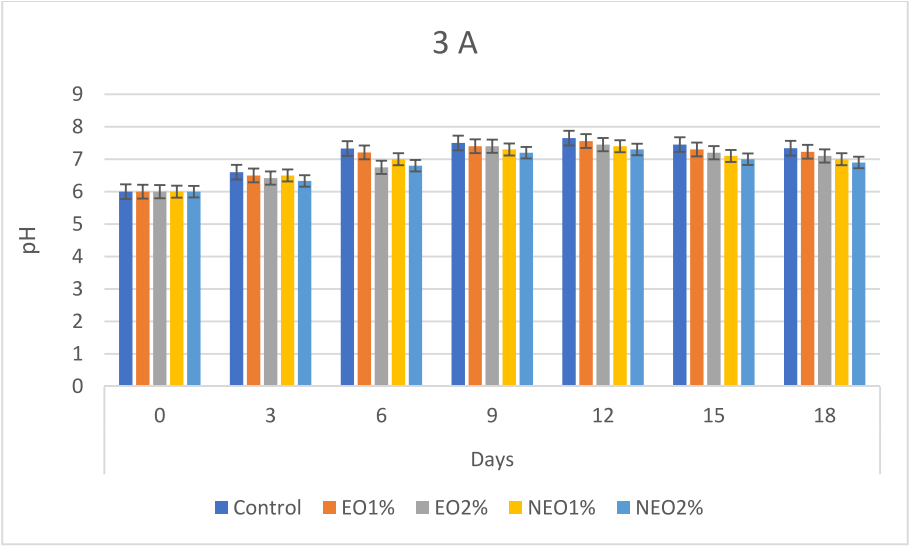
Based on the findings obtained from Fig. 3A and 3B, at the beginning of the test, the pH increased first in both bacteria and afterward decreased (a little) on the eighteenth day. The maximum obtained value of pH was detected in the control samples on the 12th day of testes, owing to the growth of *L. monocytogenes*, with pH = 7.65 and *E. coli* with pH = 7.79. After 18 days, the NEO2% treatments incubated with *L. monocytogenes* (with pH = 6.9) and *E. coli* (with pH = 6.87) had lowest pH value. Our findings also indicated compared to the free form of EO, nanoliposome of EO cause a greater decrease in pH value, because they are made to deal with the instability and volatility of essential oils (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; F. Shahidi & Hossain, 2022). The endogenous enzymes (like proteases and lipases) autolysis process, the alkaline materials production (like indole, histamine, ammonia and trimethylamine), or activity of microorganism enzymes leading to a raise in volatile bases, can be mentioned among the reasons for the increase in pH value during long-term storage. Moreover, the increase in pH during storage at cold temperatures (in the refrigerator) is probably due to the decarboxylation of amino acids, resulting in the formation of amines (Kılıç, Şimşek, Claus, & Atılgan, 2014). In our study, compared to the control samples, the growth graph of the pH value (during storage) remained almost constant in all treatments, which is probably due to the lower growth of bacteria and their protective activity (Kılıç, Şimşek, Claus, & Atılgan, 2014). In a similar study, Milani et al. analyzed impact of bioactive edible coating of Hydroxypropyl-β-Cyclodextrin/Gelatin comprising with EO nanoemulsion of nettle on the storage time of turkey meat, and expressed nanoemulsion of nettle EO had lowest pH value compared to the group of control and other treatments (Adeli Milani, Ghobadi Dana, Ghanbarzadeh, Alizadeh, & Ghasemi Afshar, 2020). Also, the results obtained in this study were similar to other studies, for example Anvar et al. analyzed impact of *Anethum graveolens* L. EO and gallic acid (forms of nanoliposome and free) against *E.coli* and *S. aureus* incubated in minced meat (Anvar, Nateghi, Shariatifar, & Mousavi, 2023) and also by study of Pouryousef et al. that analyzed impact of *Mentha pulegium* L. EO and nisin (forms of nanoliposome and free) against some bacterial (*L. monocytogenes, S. aureus* and *V. parahaemolyticus*) incubated in minced silver carp fish (Pouryousef, Ahmady, Shariatifar, Jafarian, & Shahidi, 2022) and both studies confirmed our results. Furthermore, in study by Pabast *et al.* that analyzed pH value in treatment of Satureja EO (free and nano form) and expressed nano form had better impact on the pH value of lamb meat (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

Table 4
Determining the antioxidant effect of the free and nano form of *Urtica dioica* L. aerial parts essential oil using DPPH and FRAP test.

Test	EO 1 %	EO 2 %	NEO 1 %	NEO 2 %	BHT 100	BHT 200
DPPH	35.6 [a] ± 2.3	38.7 [a] ± 1.5	33.7 [a] ± 12.2	37.3 [a] ± 2.5	93.5[b] ± 3.2	94.7[b] ± 2.7
FRAP	26.44 [a] ± 0.44	29.38 [a] ± 0.28	22.37 [a] ± 0.22	28.31 [a] ± 0.29	94.3[b] ± 3.3	95.6 [a] ± 3.2

3.6.3. Analyze of TVB-N

Based on the findings obtained from Fig. 4, during 18 days of test, amount of TVB-N was raised (in all samples of treatments and control). This raise could be owing to degradation of nucleotides, microbial activity, deamination of free amino acid and some processes of enzymatic like amines oxidation (Tometri, Ahmady, Ariaii, & Soltani, 2020). On the 18th day of the experiment, the minimum amount of TVB-N was detected in the treatment of NEO2% (26.89 mg N/100 g) and the maximum amount of TVB-N was detected in the control samples (33.78 mg N/100 g). The lower amount of TVB-N in the treatment compared to the control samples was probably owing to the reduction of the population of bacterial or their oxidative impact to remove amines from combinations of volatile nitrogen. Nanoliposome may have also contributed to preserving the impact of antibacterial of EO for a lengthier duration time. In similar study, Milani et al. analyzed impact of bioactive edible coating of 2-hydroxypropyl-β-cyclodextrin/gelatin comprising EO nanoemulsion of nettle on the storing time of turkey meat, and expressed nanoemulsion of nettle essential oil had lowest TVB-N value compared to the samples of control and other treatments (Adeli Milani, Ghobadi Dana, Ghanbarzadeh, Alizadeh, & Ghasemi Afshar, 2020). Also, Bagheri et al. analyzed impact of chitosan combined with EO of nettle and gum of basil seed on the shelf-life of beef burger and confirmed our TVB-N results (Bagheri, Ariaii, & Motamedzadegan, 2021). Forthermore, Hasani *et al.* analyzed lemon EO (in form of free



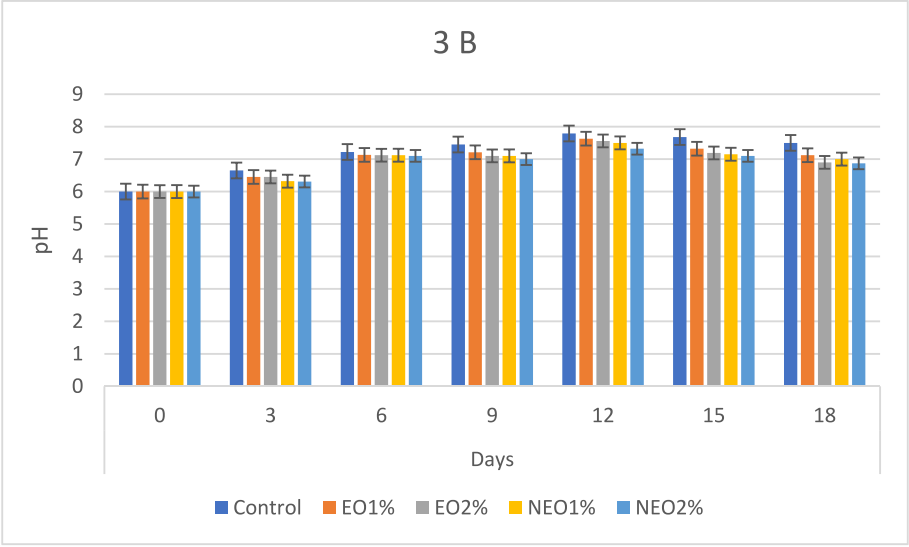


Fig. 3. (A) The value of pH of treatments (forms of nanoliposome and free) and control group on *L.monocytogenes* and (B) the value of pH of treatments (forms of nanoliposome and free) and control group on *E. coli.*

and nano) and expressed nano form had better impact in assay of TVB-N (Hasani, Ojagh, Ghorbani, & Hasani, 2020).

3.7. Evaluation of sensory

As presented in Table 5, the sensory properties of treatments was assessed. In this sensory tests (red color, discoloration and off-odor), value of one had the maximum quality or maximum score and value five had the minimum quality or minimum score. Also, when the sensory properties were higher than the value of three, the sample was no longer of good quality and was rejected. Based on our findings, the storage period had an impact (significantly) on the all three sensory items of treatments and control samples ($p < 0.05$). On the 18th day of the experiment, the treatment of *N*-EO (because they are made to deal with the instability and volatility of essential oils) had a maximum score for all sensory items (red color, discoloration and off-odor), while the control sample was received the minimum acceptance score (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018; F. Shahidi & Hossain, 2022). This results confirmed by Milani et al. that analyzed impact of bioactive edible coating of 2-hydroxypropyl-β-cyclodextrin/gelatin comprising EO nanoemulsion of nettle on the storage time of turkey meat, and expressed nanoemulsion of nettle essential oil had better sensory properties compared to the samples of control and other treatments (Adeli Milani, Ghobadi Dana, Ghanbarzadeh, Alizadeh, & Ghasemi Afshar, 2020) and also mentioned by Bagheri et al. that analyzed impact of chitosan combined with EO of nettle and gum of basil seed on the sensory properties of beef burger (Bagheri, Ariaii, & Motamedzadegan, 2021). Our findings in this study also confirmed by prior studies (Homayonpour, Jalali, Shariatifar, & Amanlou, 2021; Pabast, Shariatifar, Beikzadeh, & Jahed, 2018).

4. Conclusion

According to our biological and chemical analysis, nettle EO (nanoliposome and free forms) can extend the shelf life and maintain the

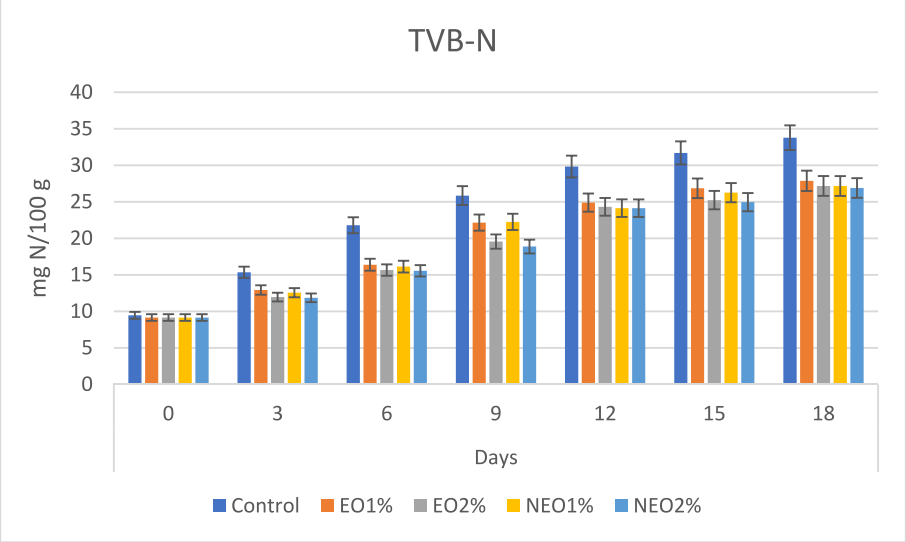


Fig. 4. TVB-N value of treatments.

Table 5
Sensory items evaluated in minced camel meat.

Parameter	group	Days of storage						
		0	3	6	9	12	15	18
Red color	Control	1 ± 0.01	2 ± 0.02	4 ± 0.03	5 ± 0.03	5 ± 0.04	5 ± 0.03	5 ± 0.04
	EO	1 ± 0.01	1 ± 0.02	2 ± 0.02	2 ± 0.03	3 ± 0.03	4 ± 0.02	4 ± 0.02
	N-EO	1 ± 0.01	1 ± 0.01	1 ± 0.02	1 ± 0.01	2 ± 0.02	2 ± 0.03	3 ± 0.03
Discoloration	Control	1 ± 0.02	3 ± 0.02	4 ± 0.02	5 ± 0.04	5 ± 0.03	5 ± 0.04	5 ± 0.03
	EO	1 ± 0.01	1 ± 0.01	2 ± 0.03	2 ± 0.02	3 ± 0.03	3 ± 0.02	4 ± 0.03
	N-EO	1 ± 0.01	1 ± 0.02	1 ± 0.01	2 ± 0.03	2 ± 0.01	2 ± 0.02	3 ± 0.02
Off-odor	Control	1 ± 0.01	3 ± 0.02	4 ± 0.02	4 ± 0.03	5 ± 0.04	5 ± 0.03	5 ± 0.04
	EO	1 ± 0.02	1 ± 0.01	1 ± 0.01	2 ± 0.03	3 ± 0.02	4 ± 0.03	4 ± 0.02
	N-EO	1 ± 0.01	1 ± 0.02	1 ± 0.02	1 ± 0.02	2 ± 0.03	2 ± 0.02	2 ± 0.01

quality of minced camel meat during storage at refrigerated temperature. Also, *N*-EO treatment had a better effect (in storage time) in terms of sensory, physical and chemical analysis of minced camel meat compared to the free form of EO. All treatments containing nettle EO (nanoliposome and free forms) reduced the microbial population compared to the control sample. In addition, antibacterial test results showed that *N*-EO had better results than free form of EO and control samples against both bacteria (*L. monocytogenes* and *E. coli)*. One of the limitations of this study is the lack of financial resources to evaluate the extract (aqueous and ethanol) of this plant on camel minced meat. As a result, according to the results of this research, it can be suggested to use nettle nanoliposome essential oil as a suitable and practical storage method in the camel meat industry.

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Ethics approval and consent to participate

The authors will follow the Ethical Responsibilities of Authors and COPE rules. All authors agree to publish.

In this research, the sensory panel participants have given permission to participate and use their data/answers.

Consent for publication

All the authors give their consent for the submitted manuscript to be published in the Journal of Food Chemistry: X.

CRediT authorship contribution statement

Masoudeh Shabani: Data curation, Investigation, Methodology, Writing – original draft. **Azade Ghorbani-HasanSaraei:** Conceptualization, Supervision, Validation, Writing – review & editing. **Nabi Shariatifar:** Supervision, Writing – review & editing. **Faezeh Savad-koohi:** Supervision, Writing – review & editing. **Seyed-Ahmad Shahidi:** Conceptualization, Supervision, Validation, Writing – review & editing.

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

All data are presented in the manuscript

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carried out by the researchers in the Tehran University of Medial Sciences.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.101050.

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