Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-022-01436-9

ORIGINAL PAPER

Preparation and evaluation of *Mentha spicata* L. essential oil nanoemulsion: physicochemical properties, antibacterial activity against foodborne pathogens and antioxidant properties

Sahar Zamaniahari[1] · Abdollah Jamshidi[1] · Mir-Hassan Moosavy[2] · Seyed Amin Khatibi[2]

Received: 11 February 2022 / Accepted: 24 April 2022

Abstract
In this study, nanoemulsion of *Mentha spicata* L. essential oil (MSEO) was successfully formulated and characterized. The chemical composition of MSEO was analyzed by gas chromatography-mass spectrometry. MSEO nanoemulsion was prepared by ultrasonication method and mixing the MSEO (10% w/w) with surfactant (5% w/w) and water (85% w/w). Damaging effect of MSEO nanoemulsion was evaluated on the cell membrane of common foodborne pathogens (*Salmonella* Typhimurium, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes*) using scanning electron microscopy and it was compared with free oil. Also, the activity of MSEO nanoemulsion was studied on the growth behavior of bacteria during storage at 35 °C for 72 h. The antioxidant activity of pure oil was compared with its nanoemulsion using 2,2-Diphenyl-1-picrylhydrazyl. The mean droplet size (51.46 nm), Polydispersity index (PDI) value (0.296), and Zeta-potential (− 13.2 mv) of oil nanoemulsion were determined. The bacterial cells were remarkably disintegrated and the levels of cell membrane damages by nano-encapsulated oil were higher than free oil. The activity of MSEO nanoemulsion at sub-inhibitory concentrations against the growth behavior of pathogenic bacteria was better than pure oil. Also, the radical scavenging activity of oil was significantly ($p < 0.05$) increased after incorporation into nanoemulsions and the IC_{50} value was decreased by $17.05 \pm 0.03\%$. In the present study, a food-grade nanoemulsion of MSEO was successfully produced by the ultrasonic emulsification method. The results demonstrated that nanoemulsions containing MSEO can be used, as a promising natural preservative, to increase the shelf life of food products and beverages.

Keywords *Mentha spicata* · Essential oil · Nanoemulsion · Antimicrobial · Antioxidant

Introduction

Foodborne diseases caused by pathogenic bacteria remain a challenge for food safety and public health, which imposes many financial losses to societies annually [1, 2]. The pathogenic microorganisms such as *Salmonella* spp., *Staphylococcus aureus, Escherichia coli*, *Listeria monocytogenes* and *Bacillus cereus* can affect the quality of foods, representing a serious risk to human health in terms of foodborne infections and intoxications [3–5]. On the other hand, oxidative reactions have been known as one of the main causes of deterioration leading to the decrease in the quality of food products [6, 7]. Oxidation of lipids and proteins can negatively affect the color, flavor and overall quality of food products.

The chemical preservatives with antimicrobial and antioxidant activities are usually used by producers to improve the safety of food products [8, 9]. Nowadays, the increasing of the consumers' awareness about the potential health risks of synthetic preservatives have increased the trends in the use of plant-based antimicrobial and antioxidant compounds as natural preservatives. Essential oils (EOs) have been recognized as a class of natural preservatives, and their antimicrobial and antioxidant effects have been shown in the literature [10–14]. EOs are secondary metabolites of plants that can be extracted from various parts of plants such as

✉ Abdollah Jamshidi
jamshidi39@gmail.com

✉ Mir-Hassan Moosavy
mhmoosavy@gmail.com; moosavy@tabrizu.ac.ir

[1] Department of Food Hygiene and Aquaculture, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, P.O. Box: 5166614779, Iran

Published online: 23 May 2022

roots, seeds, leaves, flowers, peels and fruits [2]. The EOs are aromatic hydrophobic liquid products, composed of many volatile and nonvolatile natural compounds [4, 5]. Among the wide range of components in EOs, the phenolic compoundsare thought to be responsible for their antioxidant and antimicrobial properties [2, 15].

The poor solubility of EOs in water, susceptibility to oxidative degradation, high volatility and intensive flavor has limited their usage in food products [2, 15, 16]. Therefore, finding a solution to these challenges can increase their usage in the food industry. Nowadays, emulsion-based technology has been emerged to consolidate hydrophobic compounds like EOs in a water system [17]. This system can improve the solubility of hydrophobic compounds, help to their homogeneous dissipation all over the food matrix, and increase their physical stability, as well as enhance their antimicrobial and antioxidant activities [10]. The other benefits of this system are the controlled release of their active ingredients and the prevention of the loss of volatile compounds [5, 18, 19].

Recently, nanoemulsions have gained much attention due to some advantages over conventional emulsions. Nanoemulsion is a colloidal system with monodisperse particles that is kinetically stable [20]. Oil-in-water nanoemulsion consisted of oil droplets in nanometric sizes (10 to 100 nm in diameter) dispersed in an aqueous solution, presenting as transparent or slightly turbid liquid [21–23]. Nanoemulsions are commonly prepared by a surfactant or co-surfactants (to stabilize the oil droplets in aqueous phase) and a special instrument (for example a sonicator, micro-fluidizer, high-pressured homogenizer, and high shear homogenizer) [2, 5, 17, 23].

Nanoemulsions have many beneficial effects for the incorporated materials such as improving the physicochemical properties, increasing the stability, enhancing dispersion of oil droplets in water, improving their efficiency by increasing the contact surface area, masking the smell or taste of incorporated compounds and thus lowering their effect on the organoleptic properties of food [20, 24].

Thus, design of an effective nanoemulsion-based delivery system to encapsulate EOs and investigate how this system can affect their antimicrobial and antioxidant properties is very important.

Mentha spicata L. (*M. spicata*) (Spearmint), an aromatic plant (*Lamiaceae* family), is one of the most commonly used fresh herb ingredients and flavoring agents in food products. Also, this plant is used in traditional medicine to treat nausea, bronchitis, anorexia and liver disorders due to its anti-inflammatory, anti-spasmodic, anti-spasmodic and stimulant properties [25–27]. The radical scavenging activity of *M. spicata* and its antimicrobial properties have already been reported [28, 29]. However, the effect of its incorporation into nanoemulsion has not been investigated on the levels of above activities. The present work aimed to study the antimicrobial effect of pure EO and its nano-emulsified form on the most important food-borne pathogens such as *Salmonella* Typhimurium, *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, and *Bacillus cereus*. Their effect was studied on the growth behavior and cell membrane of the treated microorganisms in broth culture medium. The antioxidant effect of pure oil was compared with its nanoemulsion. Also, the characterizations of nanoemulsion containing *Mentha spicata* L. essential oil (MSEO) such as mean particle diameter, polydispersity index (PDI) and zeta potential were determined.

Materials and methods

Extraction of essential oil

Aerial parts of *M. spicata* were collected from agricultural farms in the northwest of Tabriz city (Latitude: 38° 06′ N, Longitude: 46° 14′ E, Altitude: 1338 m) (East Azerbaijan province, Iran) during the summer season of 2020. Soil properties were as follows: Clay: 52.60% Silt: 34.32%, Sand: 10.08%, Electrical conductivity: 24.90 ds/m, pH 7.63, Lime: 10.40%, Available phosphorus: 10.80: mg/kg, Total nitrogen: 0.07%, Organic carbon: 0.78%. The taxonomic identification of plant was performed in the laboratory of Herbarium, Faculty of Pharmacy, Tabriz University of Medical Sciences. The leaves of the plant were separated from the stems.They were fully dried in shadow at room temperature and ground in a grinder with a 2 mm diameter mesh. To extract the EO, dried powder of leaves was subjected to steam distillation in a Clevenger-type apparatus for 3 h. The extracted EO was further dried using anhydrous sodium sulfate. The EO was filtered and stored at 4 °C until used for the next analyses.

Chromatographic analysis of essential oil

The composition of MSEO was analyzed using HP-6890 gas chromatography (Agilent Technologies, United States) equipped with a DB-624 column (30×0.25 mm ID, 0.25 µm film thickness) and an HP-5973 mass detector. The oven temperature was set at 60 °C and held at this temperature for 3 min. Then, it was increased to 220 °C at a rate of 5 °C/min and held under isothermal conditions for 1 min. The injector temperature was 250 °C. The carrier gas was helium (flow rate: 1.2 ml/min) and the split ratio was 1:4. The electronic ionization energy was set to 70 eV. The injection volume of sample was 1 µL of diluted oil in hexane. The components of EO were identified based on their relative retention time (RI) [relative to n-alkanes (C8–C24)] and mass spectra

fragmentation (using the NIST-MS and WILEY-MS libraries as well as literature data). The percentage of each EO component was calculated from GC peak areas.

Preparation of nanoemulsion

Oil-in-water nanoemulsion of *M. spicata* was prepared according to the method of Bhargava et al. [30] and Yazgan et al. [31] with minor modifications. The nanoemulsion of MSEO was prepared by mixing the EO (10% w/w) with Tween 80 (5% w/w) and water (85% w/w). The initial emulsion was formed using a magnetic stirrer (IKA, Germany) at 250 rpm for 10 min. The mixture was homogenized by an ultrasonic homogenizer (Hielscher, UP200H, Germany) for 15 min at an amplitude of 40%, output power of 200 W and ultrasound frequency of 24 kHz. During the sonication process, increase in the temperature of nanoemulsion was prevented using ice around the beaker.

Properties of nanoemulsion

The mean droplet size of the nanoemulsion and the PDI value was determined using the dynamic light scattering technique (Nano-ZS Zetasizer, Malvern, UK). To minimize the multiple scattering effects, the nanoemulsion was diluted with deionized water in the ratio of 1:30, before each experiment. Also, the Zeta potential of EO particles was detected by the electrophoretic mobility technique (Zetasizer Nano-ZS, Malvern, UK). The results were calculated as the mean ± standard deviation from three different experiments. All measurements were carried out at 25 °C.

Test microorganisms

The antimicrobial activity of MSEO was tested against the most important food-borne pathogenic bacteria such as *Bacillus cereus* (ATCC 11778), *Listeria monocytogenes* (ATCC 19118), *Salmonella enteric* subsp. *enteric* serovar Typhimurium (*S.* Typhimurium) (ATCC14028), *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538). All strains were purchased as lyophilized cultures from the Iranian Research Organization for Science and Technology (Tehran, Iran). The lyophilized bacteria were cultured twice in Brain heart infusion (BHI) broth (Merck, Darmstadt, Germany) at 35 °C for 18 h. The bacterial cultures were streaked on BHI agar (Merck, Darmstadt, Germany) slants and incubated at 35 °C for 18 h. The bacterial cultures were stored in a refrigerator at 4 °C and subcultured monthly.

Preparation of bacterial inocula

To prepare bacterial inocula, the cells were transferred from working cultures to BHI broth. The cultures were incubated at 35 °C for 18 h. Secondary subcultures were also prepared and incubated at the same condition. The absorbance of bacterial cultures was adjusted to an optical density (OD) of 0.1 at the wavelength of 600 nm using a UV/VIS spectrophotometer (Unico 2100, Dayton, NJ, USA). Serial dilutions (tenfold) of these cultures were prepared, and bacterial counts were determined by duplicate plating on BHI agar after incubation at 35 °C for 24 h.

Detection of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against foodborne pathogenic bacteria

The antimicrobial effect of EO and its nanoemulsion was investigated by detection of their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against pathogenic food-borne bacteria. MIC and MBC of EO and its nanoemulsion were determined by the broth dilution method. Briefly, serial dilutions of EO (0.5–10 µL/mL) were prepared in tubes of BHI broth. Each bacterial suspension was inoculated into the tubes with a final concentration of 1×10^5 CFU/mL. Tubes of positive control (bacterial suspension in BHI broth) and negative control (BHI broth containing Tween 80) were also prepared. Finally, all tubes were incubated at 35 °C for 24 h. The MIC value was defined as the lowest concentration of EO that prevented the visible growth of bacteria after incubation. For detection of MBC, 100 µL from each negative test tube was subcultured on the surface of plate count agar (PCA) plates and incubated at 35 °C for 24 h. The lowest concentration of EO that results in no visible colonies on PCA plates was defined as MBC.

Evaluation of the growth behavior of bacteria

The antimicrobial properties of MSEO and its nanoemulsion were also studied by analysis of their effect on the behavior of pathogenic bacteria in broth culture during incubation at 35 °C for 72 h. Briefly, the tubes (10 ml) of BHI broth containing sub-MIC levels (25, 50 and 75% of MIC) of pure EO and nanoemulsion were prepared. The selected bacteria were inoculated in the tubes at the final dose of 1×10^5 CFU/ml. The tubes were incubated at 35 °C with aeration. The bacterial growth in the culture medium was analyzed after 0, 18, 24, 48, and 72 h of incubation. For this purpose, serial dilutions of BHI cultures were prepared using 0.1% peptone water at the selected times. One hundred (100) µL of the prepared dilutions were cultured on the surface of nutrient agar plates and incubated at 35 °C for 24 h. The counts of bacteria were calculated as $\log_{10}$ CFU/mL [32].

Investigation of cellular damage using scanning electron microscopy (SEM)

To compare the activity levels of EO and its nanoemulsion against bacterial cell membranes, the study was carried out based on the method described by Zhang et al. [33]. All tested microorganisms (each approximately 10^8 CFU/ml), were treated (in the logarithmic growth phase) with each MIC value of pure EO and its nanoemulsion. After incubation at room temperature for 3 h, bacterial cultures were centrifuged at 5000×*g* for 10 min. The cells were separated and washed twice with phosphate buffer solution (PBS) (0.1 M, pH 7.0). Then, bacterial cells were suspended in PBS solution containing 2.5% glutaraldehyde and incubated for 2 h at − 4 °C to be fixed. After incubation of samples at room temperature for 3 h, cells were separated by centrifugation. They were washed twice again with PBS (0.1 M, pH 7.0). The suspensions were centrifuged, and the cells were suspended into the solutions containing various concentrations of ethanol in water (30%, 50%, 70%, 80%, 90% and 100%) for 10 min each. After dehydration, bacterial cells were fixed on an SEM stub and coated with gold by sputtering under vacuum condition. The cells were observed by scanning electron microscope (MIRA3 FEG-SEM, Tescan, Czechia).

Evaluation of antioxidant activity

The radical scavenging capacity of the EO and its nanoemulsion were determined by DPPH (2,2-Diphenyl-1-picrylhydrazyl) [34]. Briefly, a solution of DPPH (0.004%) was prepared in 95% methanol. Different concentrations of the EO and nanoemulsion were prepared in methanol solution (5 ml) of DPPH. Methanol was used as the blank solution (methanol). Test tubes were vortexed and incubated at room temperature in a dark place. After a period of 30 min, their absorbance was read against the blank at 517 nm using a UV/Vis spectrophotometer (Unico 2100, China). Radical scavenging activity (RSA%) of samples was calculated by the following formula:

$$\text{Radical scavenging activity (RSA\%)} = (A_C - A_S)/A_C \times 100$$

where, A_C is the absorbance of control sample (DPPH radical in methanol except the test compound), A_S is the absorbance of test sample (DPPH radical in methanol mixed with the test compound). The concentration of EO needed for 50% inhibition (IC_{50}) of DPPH oxidation was calculated from the linear regression equation prepared from inhibition percentage against EO concentration. A lower IC_{50} value correlated with greater radical scavenging activity. The same experiments were performed on butylated hydroxytoluene (BHT) as a positive control.

Statistical analysis

All experiments were performed in triplicates and the results were presented as the mean ± standard deviation. SPSS software version 21 was used to analyze the data. Microbial counts in different treatment groups were compared by one-way analysis of variance (ANOVA). Homogeneity of variances was evaluated by Levene's test. Tukey's multiple comparisons test was used to determine the significant differences in mean values.The difference was considered significant when the p-value < 0.05.

Results and discussion

Chemical composition of the essential oil

Since the diversity of chemical compounds of EOs has significant effects on their antioxidant and antimicrobial properties, identification of the chemical compounds of an EO is very important. In the present study, the EO extracted from the aerial parts of *M. spicata* was viscous and yellowish oil with a strong characteristic odor. The composition of MSEO was analyzed by GC–MS. According to the results, 18 components were identified, representing 99.89% of the EO. Qualitative analytical results were presented in Fig. 1. The major components of the EO were carvone (78.76%) and limonene (11.50%), respectively. The results of this study about the main components of the EO were in good agreement with findings of previous studies [35, 36, 46]. However, there were slight differences in the percentage of EO components with other studies. These differences may be due to several factors such as differences in the extraction methods, weather conditions, soil composition, geographical conditions, plant ecotype, phenophase and genotype as well as agronomic conditions such as plant age, harvest time and crop density [10, 46].

Properties of nanoemulsion

The prepared nanoemulsion was a homogeneous fluid with milky-white color and typical odor of the EO. It had no sign of instability, such as creaming, sedimentation, or phase separation.

The diameter of droplets is an important characteristic of a nanoemulsion, affecting its fate in in vivo conditions [5]. Therefore, the determination of the diameter of EO droplets in a nanoemulsion is necessary. In the present study, the mean droplet size of MSEO nanoemulsion was 51.46 nm (Fig. 2A). Similar findings were also reported by Noori et al. [10] for *Zingiber officinale* (ginger) oil nanoemulsion (57.4 nm) prepared by Tween 80 and water by ultrasonic emulsification method. In another study, Ozogul

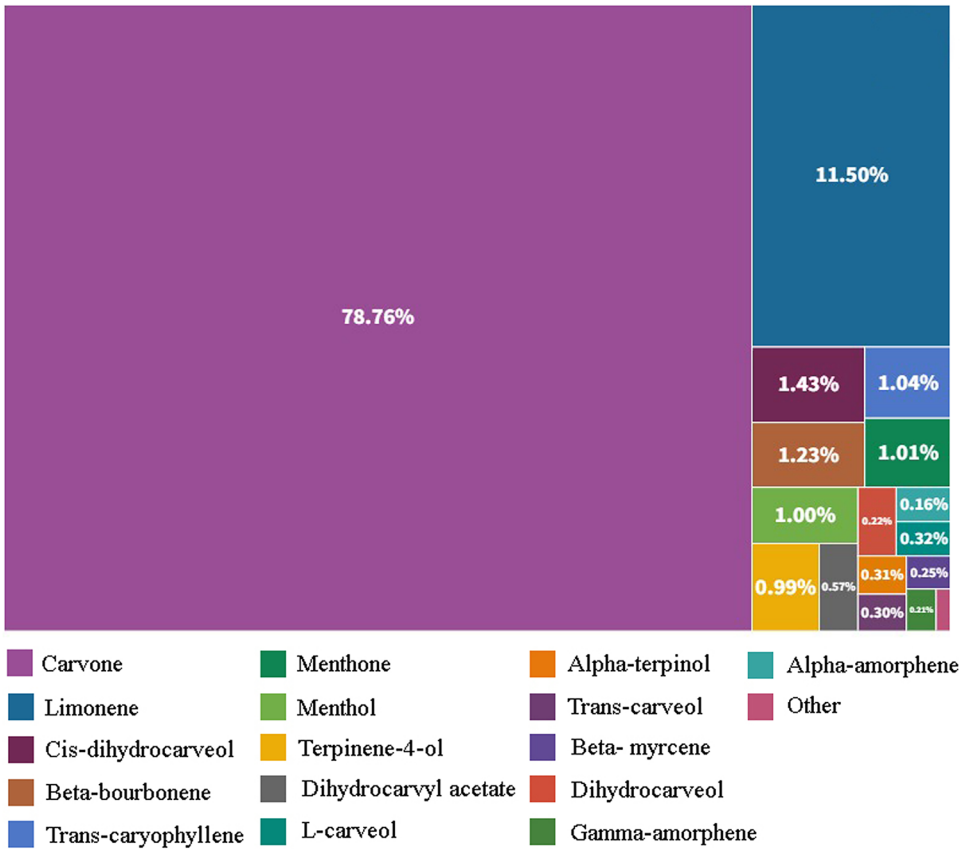

Fig. 1 Chemical composition of *Mentha spicata* L. essential oil determined by GC-MS analysis

et al. [37] reported the mean diameter of 59.48 nm for sage EO nanoemulsion droplets produced using Tween 80 and ultrasonic homogenization. However, a higher particle size was reported by Bhargava et al. [30] for oregano oil nanoemulsion (148 nm).

Since, Tween 80 has higher hydrophilic-lipophilic balance (HLB-15) than other polysorbates, it is considered as a favorable surfactant for the preparation of oil-in-water emulsions [38]. This surfactant is a small molecule that can rapidly adsorb on the surface of oil droplets, resulting in the production of emulsion droplets with minimal diameters as well as the improvement of formulation stability [39–41].

The polydispersity index is another important factor that affects the properties of nanoemulsions. PDI value is defined as the particle size distribution of the EO droplets [5]. A small PDI (values lower than 0.3) indicates the narrow distribution and uniformity of particle size in an aqueous system [5, 41]. In the current study, the PDI value was determined as 0.296 for the developed nanoemulsion. The low PDI of EO nanoemulsion confirmed the effect of the ultrasonic homogenization method in the production of homogenous and uniformly distributed nanoemulsion. Emulsions prepared by an ultrasonic homogenizer, usually contain fewer poly-dispersed droplets and have more stability than those produced by other mechanical devices [42, 43]. Similar PDI value (0.27) was reported by Salvia-Trujillo et al. [44] for the sage EO nanoemulsion. Bhargava et al. [30] reported that the PDI value of the nanoemulsion containing oregano EO was 0.3.

Zeta potential is commonly used for determination of the surface charge at the interface area of the droplets. The electrical charges of surfactants surrounded the oil droplets mainly affect this index. The nature of surfactants can be cationic, anionic, or nonionic [10]. EO nanoemulsions formulated in the present study contained Tween 80 as a non-ionic surfactant. Therefore, it would be expected that the electrical charge of EO particles to be close to zero. However, a Zeta potential of − 13.2 mV was determined for the nanoemulsion (Fig. 2B). Da Silva Gündel et al. [41] also found a negative charge in the nanoemulsion containing *Cymbopogon flexuosus* oil and Tween 80. Similar findings have also been reported by Noori et al. [10] about the nanoemulsion containing *Zingiber officinale* EO. Severe mechanical stresses such as the ultrasonication process can led to the release of free carboxyl and hydroxyl groups from EO which are responsible for the negative charge of EO nanoemulsion. So, these groups move toward the interface of oil droplets, where they can bind with water molecules. Chemical groups such as deprotonated alcohols (R–O–) and carboxylic acids (R–COO–) may contribute to the negative charge on the

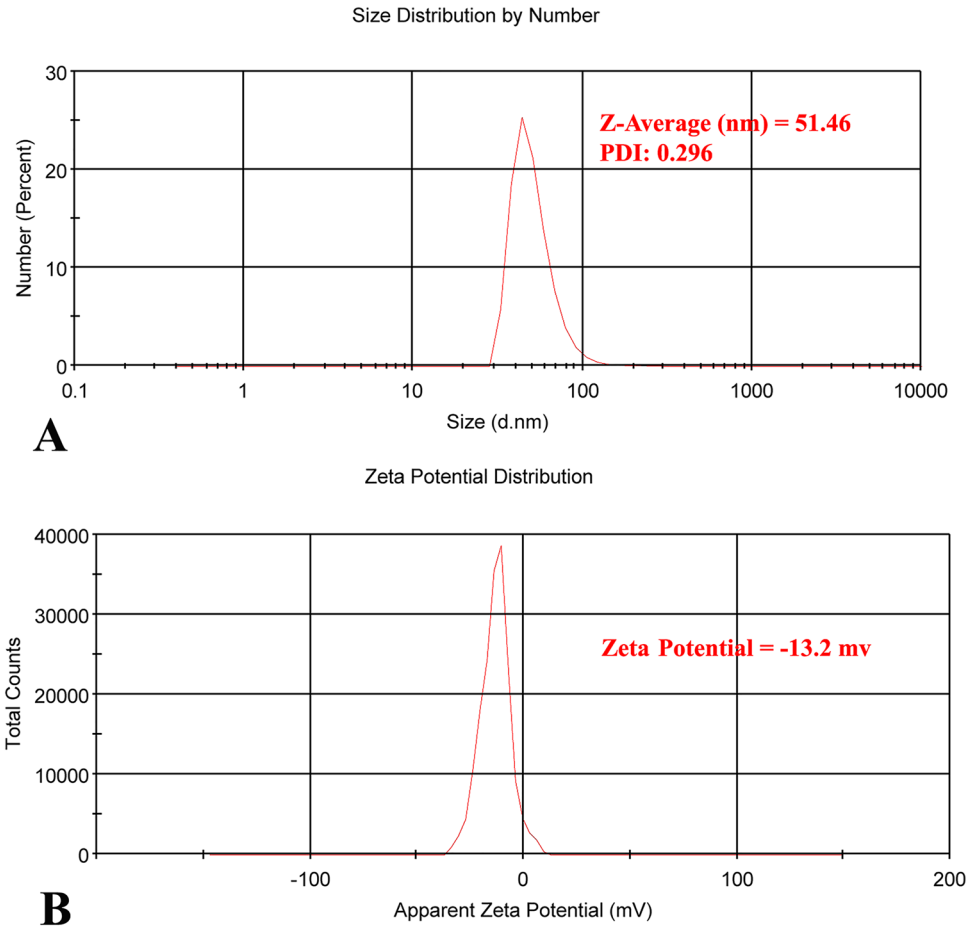

Fig. 2 The droplet size distribution (**A**) and Zeta potential (**B**) of nanoemulsion containing *Mentha spicata* L. essential oil prepared by the ultrasonic homogenization method

surface of oil droplets after the ultrasonic homogenization process.

The stability of particles in a suspension can be evaluated by determination of Zeta potential. If the particles in a suspension had highly negative or positive charges, they will repel each other which prevents their aggregation. When the value of Zeta potential is near zero, interactions between particles are facilitated due to the absence of surface charges, resulting in aggregation or flocculation of particles. A suspension with a Zeta potential value of higher than 30 mV is generally recognized as stable [45].

MIC and MBC of pure EO and its nanoemulsion against bacteria

The antimicrobial properties of MSEO have been reported on a wide range of microorganisms in previous studies [46, 47]. The MIC and MBC values of MSEO and its nanoemulsion on five foodborne pathogenic microorganisms were given in Table 1. The pure EO and its nanoemulsion exhibited a good antimicrobial effect against all tested bacterial strains. Tween 80 did not show bactericidal or inhibitory activity against the bacteria tested.

Among the foodborne pathogenic bacteria, *B. cereus* was the most susceptible bacteria against the bacteriostatic effect

Table 1 MIC and MBC of pure *Mentha spicata* L. essential oil and its nanoemulsion against the selected foodborne pathogenic bacteria

	Pure essential oil		Nanoemulsion	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Escherichia coli	6.0	7.0	2.0	3.0
Salmonella Typhimurium	7.0	8.0	2.0	3.0
Listeria monocytogenes	5.0	6.0	1.0	2.0
Bacillus cereus	4.0	6.0	2.0	2.0
Staphylococcus aureus	5.0	7.0	2.0	2.0

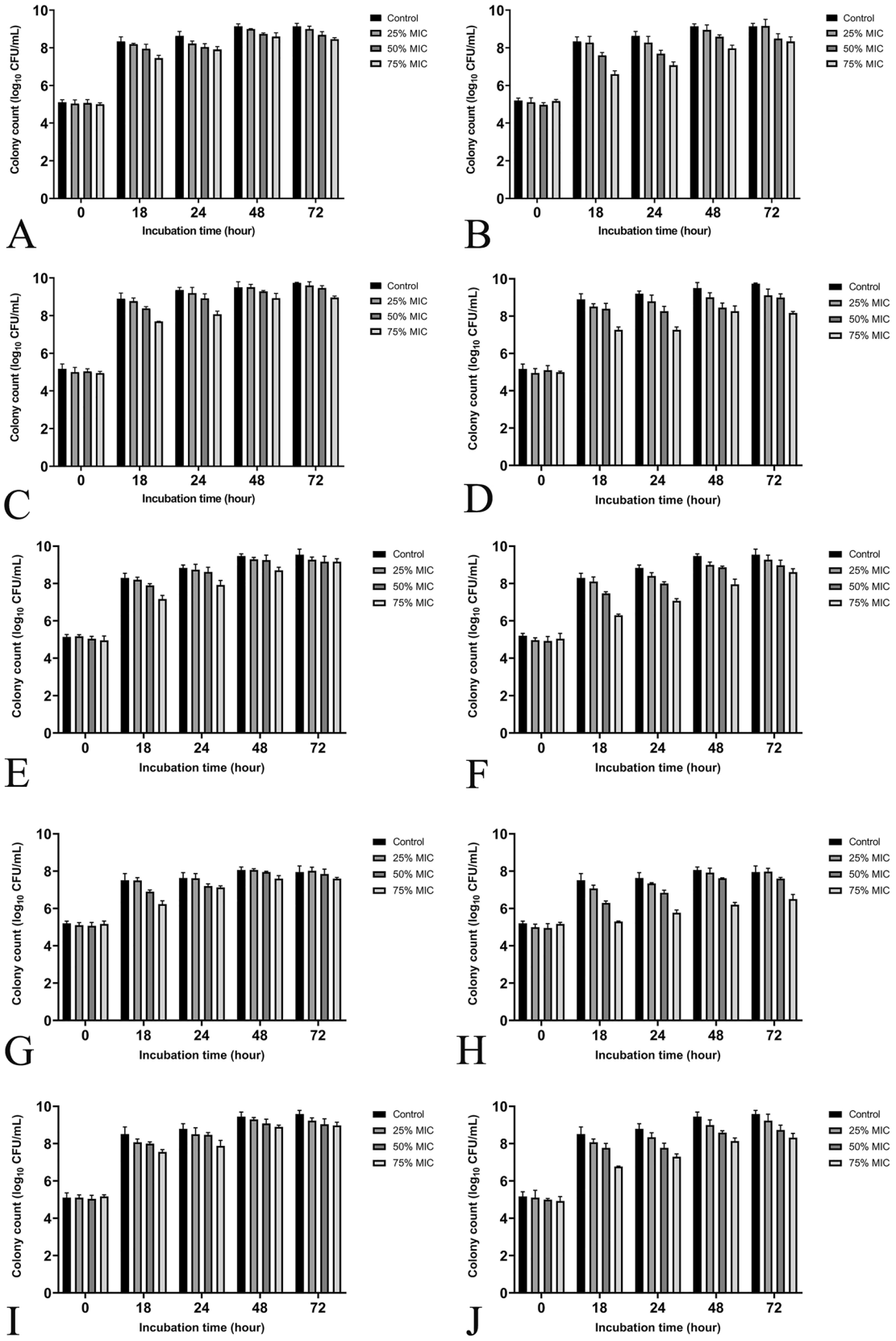

Fig. 3 Colony counting of *Escherichia coli* (**A**, **B**), *Salmonella* Typhimurium (**C**, **D**), *Listeria monocytogenes* (**E**, **F**), *Bacillus cereus* (**G**, **H**) and *Staphylococcus aureus* (**I**, **J**): Treated with sub-MIC concentrations of pure essential oil of *Mentha spicata* L. (**A**, **C**, **E**, **G** and **I**), Treated with sub-MIC concentrations of nanoemulsion containing essential oil of *Mentha spicata* L. (**B**, **D**, **F**, **H** and **J**)

of MSEO with an MIC value of 4 mg/mL. The EO had a bactericidal effect on both *B. cereus* and *L. monocytogenes* with a MBC value of 6 mg/ml. The pure EO had the lowest antimicrobial effect on S. Typhimurium with MIC of 7 mg/ml and MBC of 8 mg/ml. The encapsulation of EO into the nanoemulsion system significantly enhanced ($p < 0.05$) its antimicrobial activity against all tested foodborne bacteria, where the MIC and MBC values of oil nanoemulsion were lower than pure oil.

After the combination of nanoemulsions with the microorganism, some portions of the interior contents of nanoemulsions discharge resulting in microbial cell lysis[2]. Low molecular surfactants such as Tween are usually related to the enhanced antimicrobial effect of EO [22, 48]. However, this activity is variable depending on the type of bacterial strain [49]. In this study, among the tested bacteria, *L. monocytogenes* displayed the highest sensitivity to the nanoemulsion form of EO with the MIC value of 1 mg/mL. After incorporation of EO into nanoemulsion, the MBC value for *E. coli* and *S*. Typhimurium was decreased to 3 mg/mL. The bacteriostatic and bactericidal concentrations of EO nanoemulsion against *B. cereus* and *S. aureus* were 2 mg/mL. These results were in agreement with the findings of previous studies about the EOs nanoemulsions. Moghimi et al. [50] showed that the antimicrobial activity of *Thymus daenensis* EO enhanced after incorporation in nanoemulsion. Sundararajan et al. [51] reported that *Ocimum basilicum* L. EO nanoemulsion has lower MIC values than the free oil against all the tested pathogens. Similar results were also reported in the studies about the antibacterial activity of D-limonene [33] and oregano oil nanoemulsions [30].

The encapsulation of EO in nanoemulsion systems can improve their antimicrobial activity by the following mechanisms: (1) The reduction of the size of EO droplets in nanoemulsion facilitates penetration of antimicrobial compounds and their passive transport through the outer cell membrane of bacteria [43]; (2) the small size of EO nanoemulsion provides a larger specific surface area to interact with the microbial cell [43, 53]; (3) The fusion of nanoemulsion droplets with the phospholipid bilayer of microbial cell membrane can improve the targeted release of EO at the target sites [43, 49]; (4) The sustained release of EO from the nanoemulsion droplets prolongs its activity [54]; (5) The EO components are protected against volatilization or oil degradation [41].

According to the result shown in Table 1, it can also be concluded that the antimicrobial activity of EO and its nanoemulsion against Gram-positive bacteria (*B. cereus, S. aureus* and *L. monocytogenes*) was more than Gram-negative bacteria (*E. coli* and *S*. Typhimurium). The main reason is associated with the structural differences in the bacterial membrane. Gram-negative bacteria have a thin layer of peptidoglycan in their cell wall that is surrounded by an outer membrane mainly of lipopolysaccharide. This membrane has low permeability for bioactive compounds and reactive oxygen species (ROS) [55]. In confirmation of our results, Shahbazi [46] reported that the Gram-positive food-borne pathogenic bacteria were more susceptible to MSEO than Gram-negative bacteria. Also, Noori et al. [10] found that the antimicrobial effect of *Zingiber officinale* EO nanoemulsion on Gram-positive bacteria was higher than Gram-negative bacteria.

Growth behavior of bacteria

In Fig. 3, the growth curves of pathogenic foodborne bacteria treated with sub-inhibitory concentrations of pure EO (Fig. 3A, C, E, G and I) and its nanoemulsion (Fig. 3B, D, F, H and J) (25, 50 and 75% of MIC) in broth culture at 35 °C for 72 h were presented. The results showed that the effect of EO on the growth of the studied bacteria followed a dose-dependent manner. Increasing the concentration of EO led to an increase inits inhibitory effect on bacterial growth, as reported in previous studies [15, 56]. It was found that 75% MIC of pure EO and its nanoemulsion had the highest inhibitory effect on the growth curve of bacteria. As shown in Fig. 3, the antimicrobial effect of the pure EO was decreased during the storage period and it has not significant inhibitory effect ($p > 0.05$) on the growth of bacteria after 48 h compared to control sample. The decrease in the inhibitory effect of EO could be related to the physicochemical instability of phenolic compounds in EO and their degradation by environmental factors such as light, oxygen, pH, temperature and moisture [57]. Comparison of the results over storage time showed that the inhibitory effect in the cultures containing EO nanoemulsion was higher than those containing pure EO. For example, using 75% of MIC of pure EO, the count of *S*. Typhimurium (Fig. 3C) was decreased by 0.78 log CFU/mL after 72 h of storage. However, this value about the nanoemulsion was 1.57 log CFU/mL (Fig. 3D). *B. cereus* had a high sensitivity to the sub-inhibitory concentrations of oil nanoemulsion. After 72 h of incubation with 75% of MIC of nanoemulsion, the colony count of this bacterium treated was increased by 1.33 log CFU/mL (Fig. 3H). This value about the cells treated with the free form of oil was 2.43 log CFU/mL (Fig. 3G). These results demonstrate that the incorporation of EOs into nanoemulsion is an effective method for the protection of their active ingredients and improvement of their stability.

The findings of previous studies have confirmed the results of the present study. Da silva Gundal et al. [41] in a study about the effect of *Cymbopogon flexuosus* oil on the microbial death curve of *Candida albicans, Pseudomonas aeruginosa* and *Cryptococcus grubii* confirmed that the activity of EO is increased after incorporation in nanoemulsion.

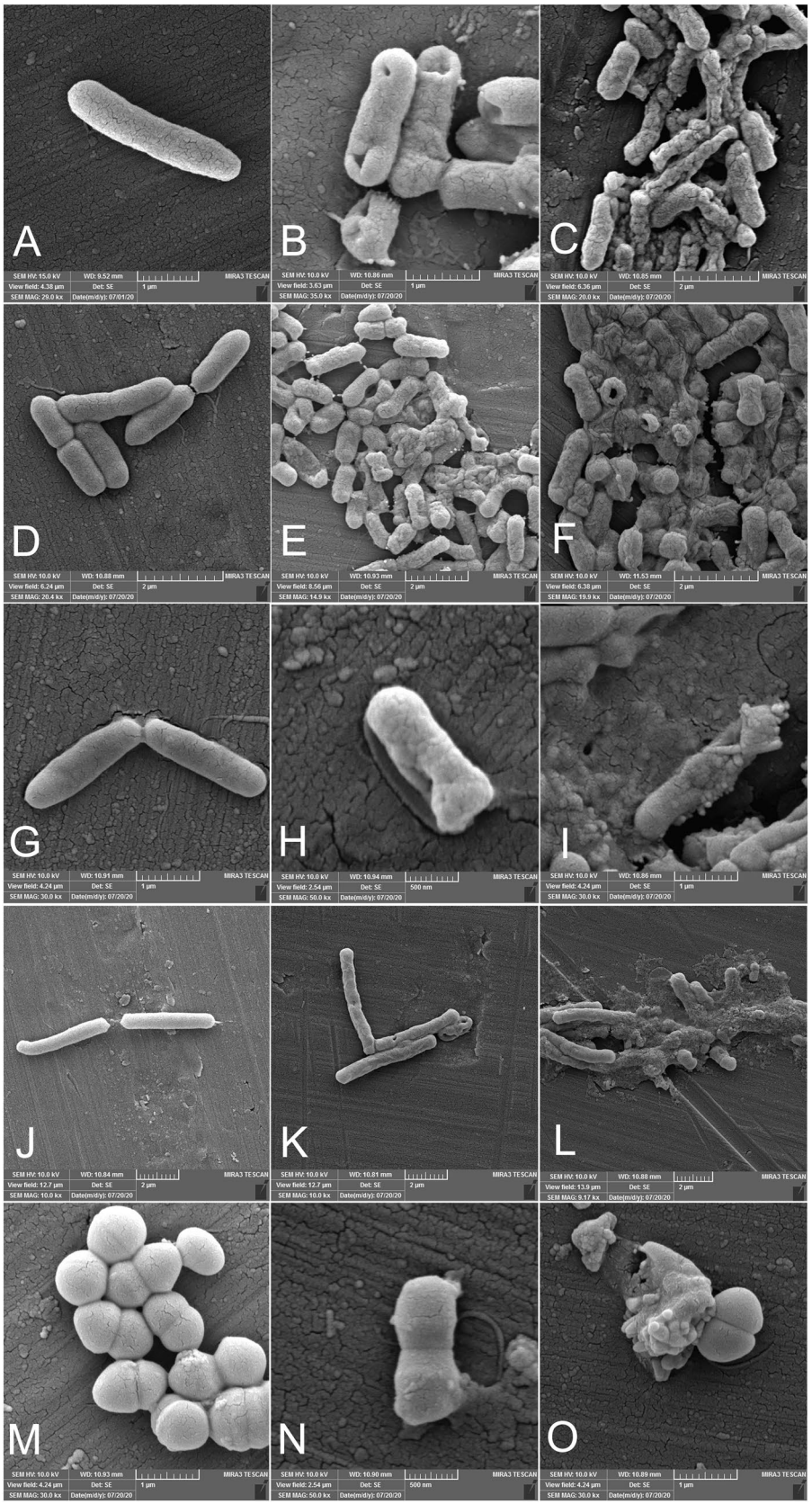

Fig. 4 Scanning electron micrographs of *Escherichia coli* (**A**–**C**), *Salmonella* Typhimurium (**D**–**F**), *Listeria monocytogenes* (**G**–**I**), *Bacillus cereus* (**J**–**L**) and *Staphylococcus aureus* (**M**–**O**) cells: Untreated (**A**, **D**, **G**, **J**, **M**), treated with free essential oil of *Mentha spicata* L. (**B**, **E**, **H**, **K** and **N**), treated with nanoemulsion containing essential oil of *Mentha spicata* L. (**C**, **F**, **I**, **L** and **O**)

Table 2 DPPH scavenging activity of *Mentha spicata* L. essential oil and its nanoemulsion

Sample	IC_{50}[a] (µg/mL)
Pure essential oil	2580 ± 150.2[b]
Essential oil nanoemulsion	2140 ± 125.5
BHT[c]	19.90 ± 2.35

[a]Concentration providing 50% inhibition

[b]Data shown are the means ± standard deviation

[c]Butylated hydroxytoluene

Cell membrane integrity

Generally, the activity of EOs against the cellular cytoplasmic membrane has a key role in their antimicrobial effect [33]. The lipophilic compounds of EOs cause a loss of membrane integrity in the cell membrane which further disturbs the equilibrium of inorganic ions and pH homeostasis in bacterial cells [23, 51]. Finally, the lysis of cell membrane by EOs results in cell materials release and death [20]. In the present study, to further uncover the mechanism of action of EO and its nanoemulsion, their effect was visualized on the foodborne bacteria with the SEM microscope after incubation of bacteria by MIC values of EO and its nanoemulsions for 3 h. The images showed different degrees of deformation and distortion in the bacterial cells (Fig. 4B, C, E, F, H, I, K, L, N and O). In treated samples with EO, treated cells showed evidence of shrinkage in the cell membrane (Fig. 4B, E, H, K and N). Their shapes were Irregular and the integrity of the cells was distorted. Also, the EO increased permeability in the bacterial cell membrane and the release of the cell constituents was observed (Fig. 4E and N). After treatment with nanoemulsion, the bacterial cells were remarkably disintegrated. They showed a broad range of structural abnormalities and cellular fragments and debris can be observed. It was observed that the levels of cell membrane damages by nano-encapsulated oil were higher than free oil (Fig. 4C, F, I, L and O). The untreated bacterial cells had an intact and smooth surface (Fig. 4A, D, G, J and M). Previous studies also confirmed the results of the present study. Krishnamoorthy et al. [58] reported significant morphological changes in *E. coli* cells after treatment with EO nanoemulsion. Moghimi et al. [50] found shrinkage in the surfaces and destruction of cells in the treated *E. coli* cells with *Thymus daenensis* EO nanoemulsion. Also, Sugumaret al. [59] observed leakage of cytoplasmic contents, irregular shape and distorted integrity in the treated cells of *S. aureus* with Eucalyptus oil nanoemulsion.

Antioxidant activity

The DPPH was known as a model of lipophilic radical and it is frequently used as a stable free radical to investigate the antioxidant activity of natural compounds. The absorbance of DPPH radical at the wavelength of 517 nm decreases by reduction with an antioxidant [23]. The antioxidant properties of MSEO have been reported in previous studies [60, 61]. As shown in Table 2, the DPPH radical scavenging activities of EO and its nanoemulsion were analyzed and compared with BHT. The highest scavenging activity was observed by BHT (IC_{50} of 19.90 ± 2.35 µg/mL). Pure EO showed the lowest radical scavenging activity with IC_{50} of 2580 ± 150.2 µg/mL. The antioxidant activity of EO was significantly ($p < 0.05$) increased by the incorporation of oil into nanoemulsions and the IC_{50} value of nanoemulsion was decreased to 2140 ± 125.5 µg/mL (17.05 ± 0.03%). This result was in agreement with the previous studies. Balasubramani et al. [23] also reported that the IC_{50} value for DPPH radical scavenging activity of *Vitex negundo* L. oil nanoemulsion was higher than pure oil. A similar effect was found by Lou et al. [52] on the DPPH radical-scavenging ability of nano-emulsified *Citrus medica* L. EO. They reported that the ability of EO nanoemulsion and its pure form to inhibit free radicals was 72.4% and 44.3%, respectively. Sundararajan et al. [51] reported that the IC_{50} values for the DPPH scavenging activity of *Ocimum basilicum* oil and its nanoemulsion were 13.21 µg/ml and 10.47 µg/ml, respectively. The increased antioxidant activity of EO after formulation in nanoemulsion may be associated with the increase of specific surface of EO and thus efficient and fast adsorption of free radicals. Furthermore, the incorporation of EOs into nanoemulsions can decrease the degradation of their components and increase their solubility in aqueous systems [10].

Conclusion

In the present study, a food-grade nanoemulsion of MSEO was successfully produced by the ultrasonic emulsification method. The EO nanoemulsion was produced with homogenous droplets and minimal particle size. The antimicrobial properties of MSEO were improved against food-borne pathogenic bacteria by its incorporation into the nanoemulsion system. Among the tested bacteria, the highest activity of nanoemulsion was observed against *L. monocytogenes* with the MIC value of 1 mg/mL. The antimicrobial activity of MSEO and its nanoemulsion against Gram-positive bacteria was higher than it was found for Gram-negative bacteria. The levels of cell membrane damage by EO nanoemulsion were remarkably higher than free oil. Also, the inhibitory effect on the growth curve of bacteria in the cultures

containing nano-encapsulated oil was higher than in those containing free EO during the storage period. Moreover, the radical scavenging activity of MSEO was improved after encapsulation into nanoemulsion. The findings of this study could support the use of plant EOs nanoemulsions, as novel natural preservatives, in the food industry for improving the safety and shelf life of food products. However, more research is needed in the future about the antimicrobial and antioxidant effects in food models as well as the safety of MSEO nanoemulsions.

References

1. M.A. Hussain, C.O. Dawson, Economic impact of food safety outbreaks on food businesses. Foods **2**, 585–589 (2013)
2. Y. Ozogul, E.K. Boğa, I. Akyol, M. Durmus, Y. Ucar, J.M. Regenstein, A.R. Köşker, Antimicrobial activity of thyme essential oil nanoemulsions on spoilage bacteria of fish and food-borne pathogens. Food Biosci. **36**, 100635 (2020)
3. M.H. Moosavy, S. Esmaeili, A.M. Mortazavian, E. Mostafavi, B. Habibi-Asl, H. Hosseini, S.A. Khatibi, Behaviour of *Listeria monocytogenes* in Lighvan cheese following artificial contamination during making, ripening and storage in different conditions. Int. J. Dairy Technol. **70**, 365–371 (2017)
4. S.A. Khatibi, A. Misaghi, M.H. Moosavy, A. Akhondzadeh Basti, S. Mohamadian, A. Khanjari, Effect of nanoliposomes containing *Zataria multiflora* Boiss. essential oil on gene expression of Shiga toxin 2 in *Escherichia coli* O157: H7. J. Appl. Microbiol. **124**, 389–397 (2018)
5. H. Yazgan, Investigation of antimicrobial properties of sage essential oil and its nanoemulsion as antimicrobial agent. LWT **130**, 109669 (2020)
6. F.S. Milan, B.R.S. Maleki, M.H. Moosavy, S. Mousavi, N. Sheikhzadeh, S.A. Khatibi, Ameliorating effects of dietary *Haematococcus pluvialis* on arsenic-induced oxidative stress in rainbow trout (*Oncorhynchus mykiss*) fillet. Ecotoxicol. Environ. Saf. **207**, 111559 (2021)
7. S. Noor, Z.F. Bhat, S. Kumar, R.J. Mudiyanselage, Preservative effect of *Asparagus racemosus*: a novel additive for bioactive edible films for improved lipid oxidative stability and storage quality of meat products. Meat Sci. **139**, 207–212 (2018)
8. N. Canillac, A. Mourey, Antibacterial activity of the essential oil of *Picea excelsa* on *Listeria*, *Staphylococcus aureus* and Coliform bacteria. Food Microbiol. **18**, 261–268 (2001)
9. S.P. Anand, N. Sati, Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int. J. Pharm. Sci. Res. **4**, 2496 (2013)
10. S. Noori, F. Zeynali, H. Almasi, Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. Food Control **84**, 312–320 (2018)
11. S. Burt, Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol. **94**, 223–253 (2004)
12. S.A. Khatibi, A. Misaghi, M.H. Moosavy, G. Amoabediny, A.A. Basti, Effect of preparation methods on the properties of *Zataria multiflora* Boiss. essential oil loaded nanoliposomes: characterization of size, encapsulation efficiency and stability. Pharm. Sci. **20**, 141–148 (2014)
13. J. Gómez-Estaca, A. López de Lacey, M.C. Gómez-Guillén, M.E. López-Caballero, P. Montero, Antimicrobial activity of composite edible films based on fish gelatin and chitosan incorporated with clove essential oil. J. Aquat. Food Prod. Technol. **18**, 46–52 (2009)
14. M.H. Moosavy, P. Hassanzadeh, E. Mohammadzadeh, R. Mahmoudi, S.A. Khatibi, K. Mardani, Antioxidant and antimicrobial activities of essential oil of Lemon (*Citrus limon*) peel in vitro and in a food model. J. Food Qual. Hazards Control. **4**, 42–48 (2017)
15. S.A. Khatibi, A. Misaghi, M.H. Moosavy, A.A. Basti, M.K. Koohi, P. Khosravi, F. Haghirosadat, Encapsulation of *Zataria multiflora* Bioss. essential oil into nanoliposomes and in vitro antibacterial activity against *Escherichia coli* O157: H7. J. Food Process. Preserv. **41**, e12955 (2017)
16. F. Donsì, A. Cuomo, E. Marchese, G. Ferrari, Infusion of essential oils for food stabilization: unraveling the role of nanoemulsion-based delivery systems on mass transfer and antimicrobial activity. Innov. Food Sci. Emerg. Technol. **22**, 212–220 (2014)
17. F. Donsi, G. Ferrari, Essential oil nanoemulsions as antimicrobial agents in food. J. Biotechnol. **233**, 106–120 (2016)
18. I. Syed, P. Banerjee, P. Sarkar, Oil-in-water emulsions of geraniol and carvacrol improve the antibacterial activity of these compounds on raw goat meat surface during extended storage at 4 °C. Food Control **107**, 106757 (2020)
19. P. Sarkar, A.K. Bhunia, Y. Yao, Impact of starch-based emulsions on the antibacterial efficacies of nisin and thymol in cantaloupe juice. Food Chem. **217**, 155–162 (2017)
20. Z.H. Li, M. Cai, Y.S. Liu, P.L. Sun, Development of finger citron (*Citrus medica* L. var. *sarcodactylis*) essential oil loaded nanoemulsion and its antimicrobial activity. Food Control **94**, 317–323 (2018)
21. D.J. McClements, Edible nanoemulsions: Fabrication, properties, and functional performance. Soft Matter **7**, 2297–2316 (2011)
22. A. Prakash, R. Baskaran, N. Paramasivam, V. Vadivel, Essential oil based nanoemulsions to improve the microbial quality of minimally processed fruits and vegetables: a review. Food Res. Int. **111**, 509–523 (2018)
23. S. Balasubramani, T. Rajendhiran, A.K. Moola, R.K.B. Diana, Development of nanoemulsion from *Vitex negundo* L. essential oil and their efficacy of antioxidant, antimicrobial and larvicidal activities (*Aedes aegypti* L.). Environ. Sci. Pollut. Res. **24**, 15125–15133 (2017)
24. D.J. McClements, J. Rao, Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit. Rev. Food Sci. Nut. **51**, 285–330 (2011)
25. G. İşcan, N. Kïrïmer, M. Kürkcüoğlu, H.C. Başer, F. Demirci, Antimicrobial screening of *Mentha piperita* essential oils, J. Agric. Food Chem. **50**, 3943–3946 (2002)
26. L. Moreno, R. Bello, E. Primo-Yúfera, J. Esplugues, Pharmacological properties of the methanol extract from *Mentha suaveolens* Ehrh. Phytother. Res. **16**, 10–13 (2002)
27. G. Mahendran, S.K. Verma, L.U. Rahman, The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L): a review. J. Ethnopharmacol. **278**, 114266 (2021)
28. M. Znini, M. Bouklah, L. Majidi, S. Kharchouf, A. Aouniti, A. Bouyanzer, B. Hammouti, J. Costa, S.S. Al-Deyab, Chemical composition and inhibitory effect of *Mentha spicata* essential oil on the corrosion of steel in molar hydrochloric acid. Int. J. Electrochem. Sci. **6**, 691–704 (2011)
29. A.K. Tyagi, A. Malik, D. Gottardi, M.E. Guerzoni, Essential oil vapour and negative air ions: a novel tool for food preservation. Trends Food Sci. Technol. **26**, 99–113 (2012)

30. K. Bhargava, D.S. Conti, S.R. da Rocha, Y. Zhang, Application of an oregano oil nanoemulsion to the control of foodborne bacteria on fresh lettuce. Food Microbiol. **47**, 69–73 (2015)
31. H. Yazgan, Y. Ozogul, E. Kuley, Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on foodborne pathogens and fish spoilage bacteria. Int. J. Food Microbiol. **306**, 108266 (2019)
32. R. Krishnan, V. Arumugam, S.K. Vasaviah, The MIC and MBC of silver nanoparticles against *Enterococcus faecalis*—a facultative anaerobe. J. Nanomed. Nanotechnol. **6**, 285 (2015)
33. Z. Zhang, F. Vriesekoop, Q. Yuan, H. Liang, Effects of nisin on the antimicrobial activity of D-limonene and its nanoemulsion. Food Chem. **150**, 307–312 (2014)
34. M. Gulluce, F. Sahin, M.Ü.N.E.V.V.E.R. Sokmen, H. Ozer, D. Daferera, A.T.A.L.A.Y. Sokmen, M. Sokmen, A.Y.ŞE. Polissiou, H.İC.A.B.İ Ozkan, Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. Food Chem. **103**, 1449–1456 (2007)
35. R.S. Chauhan, M.K. Kaul, A.K. Shahi, A. Kumar, G. Ram, A. Tawa, Chemical composition of essential oils in *Mentha spicata* L. accession [IIIM (J) 26] from North-West Himalayan region, India. Ind Crops Prod **29**(2–3), 654–656 (2009)
36. M. Govindarajan, R. Sivakumar, M. Rajeswari, K. Yogalakshmi, Chemical composition and larvicidal activity of essential oil from *Mentha spicata* (Linn.) against three mosquito species. Parasitol. Res. **110**(5), 2023–2032 (2012)
37. Y. Ozogul, İ Yuvka, Y. Ucar, M. Durmus, A.R. Kösker, M. Öz, F. Ozogul, Evaluation of effects of nanoemulsion based on herb essential oils (rosemary, laurel, thyme and sage) on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) fillets during ice storage. LWT **75**, 677–684 (2017)
38. V. Ghosh, A. Mukherjee, N. Chandrasekaran, Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. Ultrason. Sonochem. **20**, 338–344 (2013)
39. C. Qian, D.J. McClement, Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: factors affecting particle size. Food Hydrocoll. **25**, 1000–1008 (2011)
40. S. Sugumar, A. Mukherjee, N. Chandrasekaran, Eucalyptus oil nanoemulsion-impregnated chitosan film: antibacterial effects against a clinical pathogen, *Staphylococcus aureus*, in vitro. Int. J. Nanomed. **10**, 67 (2015)
41. S. da Silva Gündel, M.E. de Souza, P.M. Quatrin, B. Klein, R. Wagner, A. Gündel, R. de Almeida Vaucher, R.C.V. Santos, A.F. Ourique, Nanoemulsions containing *Cymbopogon flexuosus* essential oil: development, characterization, stability study and evaluation of antimicrobial and antibiofilm activities. Microb. Pathogen. **118**, 268–276 (2018)
42. W. Liu, D. Sun, C. Li, Q. Liu, J. Xu, Formation and stability of paraffin oil-in-water nano-emulsions prepared by the emulsion inversion point method. J. Colloid Interface Sci. **303**, 557–563 (2006)
43. W.C. Lu, D.W. Huang, C.C. Wang, C.H. Yeh, J.C. Tsai, Y.T. Huang, P.H. Li, Preparation, characterization, and antimicrobial activity of nanoemulsions incorporating citral essential oil. J. Food Drug Anal. **26**, 82–89 (2018)
44. L. Salvia-Trujillo, A. Rojas-Graü, R. Soliva-Fortuny, O. Martín-Belloso, Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. Food Hydrocoll. **43**, 547–556 (2015)
45. J.B. Seibert, I.V. Rodrigues, S.P. Carneiro, T.R. Amparo, J.S. Lanza, F.J.G. Frézard, G.H.B.D. Souza, O.D.H.D. Santos, Seasonality study of essential oil from leaves of *Cymbopogon densiflorus* and nanoemulsion development with antioxidant activity. Flavour Frag. J. **34**, 5–14 (2019)
46. Y. Shahbazi, Chemical composition and in vitro antibacterial activity of *Mentha spicata* essential oil against common food-borne pathogenic bacteria. J. Pathog. **2015**, 916305 (2015)
47. M. Pluchtová, T. Gervasi, Q. Benameur, V. Pellizzeri, D. Grul'ová, L. Campone, V. Sedlák, N. Cicero, Antimicrobial activity of two Mentha species essential oil and its dependence on different origin and chemical diversity, Nat. Prod. Commun. **13**, 1934578X1801300832 (2018)
48. Y. Özogul, F. Özogul, P. Kulawik, The antimicrobial effect of grapefruit peel essential oil and its nanoemulsion on fish spoilage bacteria and food-borne pathogens. LWT **136**, 110362 (2021)
49. W. Li, H. Chen, Z. He, C. Han, S. Liu, Y. Li, Influence of surfactant and oil composition on the stability and antibacterial activity of eugenol nanoemulsions. LWT-Food Sci. Technol. **62**, 39–47 (2015)
50. R. Moghimi, L. Ghaderi, H. Rafati, A. Aliahmadi, D.J. McClements, Superior antibacterial activity of nanoemulsion of Thymus daenensis essential oil against *E. coli*. Food Chem. **194**, 410–415 (2016)
51. B. Sundararajan, A.K. Moola, K. Vivek, B.R. Kumari, Formulation of nanoemulsion from leaves essential oil of *Ocimum basilicum* L. and its antibacterial, antioxidant and larvicidal activities (*Culex quinquefasciatus*). Microb. Pathog. **125**, 475–485 (2018)
52. Z. Lou, J. Chen, F. Yu, H. Wang, X. Kou, C. Ma, S. Zhu, The antioxidant, antibacterial, antibiofilm activity of essential oil from *Citrus medica* L. var. *sarcodactylis* and its nanoemulsion. LWT **80**, 371–377 (2017)
53. S. Sugumar, J. Nirmala, V. Ghosh, H. Anjali, A. Mukherjee, N. Chandrasekaran, Bio-based nanoemulsion formulation, characterization and antibacterial activity against food-borne pathogens. J. Basic Microbiol. **53**, 677–685 (2013)
54. Y. Chang, L. McLandsborough, D.J. McClements, Fabrication, stability and efficacy of dual-component antimicrobial nanoemulsions: essential oil (thyme oil) and cationic surfactant (lauric arginate). Food Chem. **172**, 298–304 (2015)
55. F. Nazzaro, F. Fratianni, L. De Martino, R. Coppola, V. De Feo, Effect ofessential oils on pathogenic bacteria. Pharmaceuticals **6**, 1451–1474 (2013)
56. M. Azizkhani, A. Misaghi, A.A. Basti, H. Gandomi, H. Hosseini, Effects of *Zataria multiflora* Boiss. essential oil on growth and gene expression of enterotoxins A, C and E in *Staphylococcus aureus* ATCC 29213. Int. J. Food Microbiol. **163**, 159–165 (2013)
57. R. Rehman, M.A. Hanif, Z. Mushtaq, A.M. Al-Sadi, Biosynthesis of essential oils in aromatic plants: a review. Food Rev. Int. **32**, 117–160 (2016)
58. R. Krishnamoorthy, J. Athinarayanan, V.S. Periasamy, A.R. Adisa, M.A. Al-Shuniaber, M.A. Gassem, A.A. Alshatwi, Antimicrobial activity of nanoemulsion on drug-resistant bacterial pathogens. Microb. Pathog. **120**, 85–96 (2018)
59. S. Sugumar, V. Ghosh, M.J. Nirmala, A. Mukherjee, N. Chandrasekaran, Ultrasonic emulsification of eucalyptus oil nanoemulsion, antibacterial activity against *Staphylococcus aureus* and wound healing activity in Wistar rats. Ultrason. Sonochem. **21**, 1044–1049 (2014)
60. M. Snoussi, E. Noumi, N. Trabelsi, G. Flamini, A. Papetti, V. De Feo, Mentha spicata essential oil: chemical composition, antioxidant and antibacterial activities against planktonic and biofilm cultures of *Vibrio* spp. strains. Molecules **20**, 14402–14424 (2015)
61. S.K. Bardaweel, B. Bakchiche, H.A. ALSalamat, M. Rezzoug, A. Gherib, G. Flamini, Chemical composition, antioxidant, antimicrobial and Antiproliferative activities of essential oil of *Mentha spicata* L. (Lamiaceae) from Algerian Saharan atlas. BMC Complemen. Altern. Med. **18**, 1–7 (2018)