Essential oils microemulsions prepared with high-frequency ultrasound: physical properties and antimicrobial activity

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Abstract Essential oils (EOs) have demonstrated antimicrobial activity against bacteria due to the effects of their major components. The direct application of EOs may present a rapid volatilization of its components and can decrease their effectiveness. Encapsulation by means of emulsification can provide protection to lipid compounds on a microscale. The aim of this study was to characterize microemulsions of cinnamon essential oil (CEO), oregano essential oil (OEO), and rosemary essential oil (REO) prepared by high-frequency ultrasound and evaluate their antimicrobial activities against *Escherichia coli* and *Listeria monocytogenes*. The microemulsions (oil-in-water, O/W) of EOs were prepared using high-frequency ultrasound, applying a wave amplitude of 84 μm for 15 min (REO and CEO) or 30 min (OEO). The antimicrobial activity was determined by inoculating 10^8 CFU/mL of bacteria. Nonsurvival of the bacteria was confirmed by plate count in tryptic soy agar, determining the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The microemulsions exhibited droplet size diameters of 1.98 to 5.46 μm , showing high encapsulation efficiencies (79.91–81.97%) and low separation rates (2.50–6.67%). The MIC and MBC for the microemulsions for both bacteria were 20–75% less than values obtained for the non-encapsulated EOs. This study demonstrates that high-frequency ultrasound is a suitable technique for obtaining stable microemulsions to

deliver natural antimicrobials that can be applied to control bacteria of high relevance in food safety.

Keywords Essential oils · Encapsulation · Antimicrobial activity · Microemulsions · High-frequency ultrasound

Introduction

Recent studies have demonstrated that some essential oils (EOs), such as those of garlic, cinnamon, thyme, oregano, clove, basil, coriander, citrus peel, eucalyptus, ginger, rosemary, and peppermint, among others, have antimicrobial activity against bacteria, yeast, and molds. The hydrophilic or hydrophobic character of the EOs is related to their antimicrobial activity, the proportion of the main components in each of them (terpenoids, phenolics, and aldehyde compounds), and the type of microorganism that they might inhibit or inactivate (Fisher and Phillips 2008). The chemical structure of the components of EOs affects their mode of action as antimicrobials; altering the lipids of the microbial cytoplasmic membrane produces loss of its impermeability to protons and ions of higher size, thus disturbing electron transport, denaturation of proteins, dehydration, and cellular lysis. Therefore, several authors (Yeon-Ji et al. 2015; Dolça et al. 2015) have suggested the addition of EOs in food to act as natural preservatives. However, EOs have an important impact on food taste, due to their distinctive flavor, affecting the acceptability of the products to which these are added. The direct application of EOs may present rapid volatilization of their components. In order to avoid these disadvantages, encapsulation emerges as an interesting technology to mask the taste and prevent the volatilization of the EO components. Different encapsulation techniques have been widely applied in the

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pharmaceutical, cosmetic, chemical, and food industries, to extend the functionality of the bioactive compounds encapsulated in an extensive variety of materials conforming to the capsule matrix (Char et al. 2015; Niu et al. 2016).

Microencapsulation in food science has been continuously researched, studying a wide variety of encapsulated materials, while seeking high resistance to processing conditions and stability of quality characteristics during the storage of the food products. There are various encapsulation techniques, such as simple coacervation, complex coacervation, spray drying, freeze-drying, top-spray fluidized bed coating, interfacial polymerization, molecular inclusion, and emulsification (Velázquez-Contreras et al. 2014). The most common methods for the encapsulation of EOs are spray drying and complex coacervation (Alvarenga Botrel et al. 2012); however, an interesting alternative to encapsulating them is emulsification, since this technique has high retention of volatile compounds, increases the solubility of EOs and bioavailability of EOs, and limits their oxidation during storage (Mahfoudhi et al. 2016). Different types of emulsions have been shown to provide a controlled release of the encapsulated compounds that have antimicrobial activity against different bacteria, yeast, and molds. Ghosh et al. (2012) performed the encapsulation of mustard EO in microemulsions by a phase inversion method, to evaluate its antimicrobial activity against *Escherichia coli* in model systems. Liang et al. (2012) evaluated the antimicrobial activity of peppermint EO emulsions against *Listeria monocytogenes* and *Staphylococcus aureus*. The authors concluded that this technique is efficient in protecting the EO without affecting its antimicrobial activity. Donsì et al. (2012) studied the encapsulation of carvacrol, limonene, and cinnamaldehyde in nanoemulsions prepared by high-pressure homogenization and tested the antimicrobial activity of these nanoemulsions against *E. coli*, *Lactobacillus delbrueckii*, and *Saccharomyces cerevisiae*. Their results showed that the solubility of the lipidic compounds is enhanced when these are added as nanoemulsions in the medium, promoting their antimicrobial activity.

To date, microencapsulation by means of emulsification has been studied through applying different techniques of preparation, such as high-pressure homogenization, microfluidization, and high-frequency ultrasound homogenization. This last method requires a lower amount of emulsifiers compared to other techniques and is more accessible (Cardoso-Ugarte et al. 2016).

The aim of this study was to characterize the microemulsions of cinnamon, oregano, and rosemary EOs prepared by high-frequency ultrasound and evaluate their antimicrobial activity against *E. coli* and *L. monocytogenes*.

Material and methods

Rosemary essential oil (REO) and oregano essential oil (OEO) were purchased from Laboratorios Hersol (Mexico), and cinnamon essential oil (CEO) was purchased from TECNAAL (Mexico). For the preparation of oil-in-water (O/W) emulsions, inulin (Fructagave PR95, Mexico) was used as a stabilizer and Tween 80 (Sigma-Aldrich, USA) was used as an emulsifying agent. The microorganism strains used in this study were *E. coli* ATCC 25922 and *L. monocytogenes* Scott A, obtained from the collection of the Food Microbiology Laboratory of Universidad de las Americas Puebla.

Microemulsions preparation

The continuous phase of the O/W microemulsions was formed by an aqueous solution of 5% (w/w) inulin and 3% (w/w) of Tween 80. Different concentrations of the oil phase were used to formulate the microemulsions, in order to add the same concentration of encapsulated EO depending on the minimum inhibitory concentration (MIC) or the minimum bactericidal concentration (MBC) of the non-encapsulated EO in the system: 10% (w/w) for CEO, 30% (w/w) for REO, and 45% (w/w) for OEO. Different percentages of each EO were used in order to contain at least the MIC or MBC of the non-encapsulated EO in one milliliter of microemulsion. The microemulsions were prepared using an ultrasonic homogenizer (Cole Parmer, CP 505, USA), with a wave amplitude of 84 μm for 15 min for REO and CEO and for 30 min for OEO. The homogenization time was determined by the concentration of EO in each system. Previous experiments demonstrated that these ultrasonication times were sufficient to achieve good homogenization.

Essential oil characterization

Chemical composition

The chemical compositions of the EOs were determined by gas chromatography coupled to mass spectrometry (GC–MS, 6850 N, Agilent Technologies, USA). Each spectrogram was compared with the National Institute of Standard Technology library (NIST, USA), identifying the different compounds present in the EOs.

Physical properties

The refractive index determination for the EOs was made using a digital refractometer (ATAGO 1T, Japan). Their viscosities were measured using a Cannon Fenske

Viscometer, and their densities were determined following AOAC method 962.37 (1995), using glass pycnometers.

Microemulsion characterization

Particle size

Particle size distributions of the microemulsion droplets were measured by laser diffraction using a particle analyzer (Bluewave, Microtrac, USA). The particle size data reported were given as the median particle diameter (D_{50}).

Micrographs

Microemulsions were directly observed under an optical microscope (Primo Star, Carl Zeiss, Germany), and images of the microemulsions were captured using a microscope camera (AxioCam ERc5s, Zeiss, Germany).

Color

A tristimulus colorimeter (CR-400, Konica Minolta, Japan) was used to measure the color parameters of the EO microemulsions, using the Hunter scale (L, a, b). The hue value was calculated as $\text{Hue} = \tan^{-1} (b/a)$. The result of the hue value in radians was then converted to degrees ($^{\circ}$).

pH

Each microemulsion pH was measured at 20 ± 5 °C using a potentiometer (PC45, Conductronic, Mexico), previously calibrated with buffers at pH 4 and 7. To observe pH stability in the microemulsions, the pH was measured at five different times (0, 7, 14, 21, and 28 days) for each formulation.

Emulsion stability

Immediately after the microemulsion preparation, aliquots of 30 mL of the system (H_0) were transferred to graduated conical falcon tubes, sealed, and stored at room temperature for 28 days at 25 ± 1 °C. The volume of the cream upper phase (H_1) was measured every day. Stability was determined by calculating the separation phase as the ratio of H_1/H_0 , expressed in percentage.

Encapsulation efficiency

The encapsulation efficiency (EE) was determined according to the method described by Yeon et al. (2015) with some modifications. An ultraviolet–visible (UV/VIS) spectrophotometer (model 6850, Jenway, UK) was used to determine the maximum peaks found in the spectrograms

of the EOs dissolved in *n*-hexane, registered from 190 to 500 nm. The maximum peaks found for the EOs were at 310 nm for CEO, 255 nm for OEO, and 225 nm for REO. Calibration curves were obtained for each EO dissolved in *n*-hexane, prepared at standard concentrations (0.5–5 μL). To determine the EE, the extraction of the free EO in the microemulsion (FEOM) proceeded by pouring 100 μL of the microemulsion into 9000 μL of hexane in a tube and then shaking it for 15 s at 60 rpm. Subsequently, the absorbance was set to a specific wavelength for each EO. The EE was calculated with the following equation (Eq. 1):

$$EE\% = \frac{EOM - FEOM}{EOM} \times 100 \quad (1)$$

where *EOM* represents the theoretical EO content of the microemulsion formulation and *FEOM* is the free amount of the EO measured in the microemulsion.

Antimicrobial activity

Preparation of the inoculum

The inoculum was prepared by inoculating each bacteria type (*E. coli* or *L. monocytogenes*) separately in tryptic soy broth (TSB, Merck Laboratories, Germany) and then incubating this at 35 °C for 24 h. From each broth, the bacteria were cultivated in tryptic soy agar (TSA, Laboratories Merck, Germany), incubated at 35 °C for 24 h, and then refrigerated at 4 °C until use, but for no longer than 3 weeks. To reactivate the bacteria, two loopfuls from the TSA were taken to inoculate 10 mL of TSB, which was later incubated at 35 ± 1 °C for 24 h, to achieve approximately 1×10^8 colony-forming units (CFU)/mL, which corresponds to the beginning of the stationary phase of their growth curve.

MIC and MBC of the essential oils and microemulsions

Model systems were prepared by adding different amounts (20 μL up to 1000 μL) of non-encapsulated or encapsulated REO, OEO, or CEO and completing the volume to 10 mL with TSB; 0.5 mL of Tween 80 was added for a better distribution of the EOs in the TSB. Subsequently, 0.1 mL of the reactivated bacteria were inoculated into 10 mL of the model system, hand mixed vigorously, and then incubated at 35 ± 1 °C for 24 h. To confirm bacterial inhibition, a plate count in TSA was performed. The MIC was defined as the minimum concentration of EO that did not allow the growth of *E. coli* or *L. monocytogenes* after 24 h of incubation at 35 ± 1 °C in the inoculated TSA. Finally, the model systems in TSB were left in incubation for 24 h more, and the same procedure for plate counting in TSA was carried out. The MBC was defined as the

minimum concentration of EO that did not allow bacteria growth after 48 h of incubation in the inoculated TSA.

Statistical analysis

The physical properties of EOs and the microemulsions and the EES were analyzed by analysis of variance and Tukey's paired comparison test, using a significant level of $\alpha = 0.05$. The statistical software used was Minitab V.17 (Minitab, Inc., State College, Pa., USA).

Results

The chemical compositions of the studied EOs are shown in Table 1. For REO, the major components identified were 1R- α -pinene (25.87%), eucalyptol (21.95%), and camphor (23.08%). For OEO, the major components identified in this study were β -cimene (27.23%), linalool (29.51%), and carvacrol (27.34%). For CEO, the main component was cinnamaldehyde (50.64%).

The results for the density, viscosity, and refractive index of the studied EOs encapsulated in microemulsions are shown in Table 2. These three parameters of the studied EOs showed significant differences ($p < 0.05$) among them.

Table 1 Major components in evaluated rosemary, oregano and cinnamon essential oils

Essential oil	Components	Peak area (%)
Rosemary	1R- α -pinene	25.70
	Eucalyptol	21.95
	Camphor	23.08
	Camphene	6.43
	β -pinene	5.50
	α -terpineol	3.75
	Bornyl acetate	2.35
	Caryophyllene	2.39
Oregano	β -cymene	27.23
	Linalool	29.51
	Carvacrol	27.34
	γ -terpinene	4.67
	Caryophyllene	4.73
Cinnamon	Cinnamaldehyde	50.64
	Caryophyllene	16.54
	Caryophyllene oxide	12.15
	α -caryophyllene	5.47
	γ -cadinene	3.82

The densities of the microemulsions were similar to those of the non-encapsulated EOs (Tables 2, 3). The pH values of the microemulsions were in the range of 3.80 to 4.77; however, the pH of the emulsion prepared with CEO was significantly higher ($p < 0.05$) than the values obtained for REO and OEO, which may be attributed to its chemical composition. The pH values of the microemulsions remained stable, with no changes in the pH values determined 28 days after microemulsion preparation. From the color parameters of the studied microemulsions, it can be observed that the lowest luminosity (L value) was obtained for OEO ($p < 0.05$) (Table 3). In terms of the hue values of the EO microemulsions, all presented angles towards the yellow-green color.

Table 4 shows the values of the droplet size of the studied EO microemulsions, and it is worth noting that the diameter distribution was unimodal. The results obtained from the droplet size (D_{50}) determined for the microemulsions were 2.08 ± 0.13 , 3.30 ± 0.06 , and 5.30 ± 0.65 μm for the microemulsions of CEO, REO, and OEO, respectively. The microemulsions were shown to be stable in terms of high EEs and low percentages of phase separation. The percentages of phase separation were in the range of 2.50–6.67%. The EE obtained with REO (81.97%) was significantly higher ($p < 0.05$) than values obtained for OEO (80.99%) and CEO (79.91%). The particle size diameters of the EOs dispersed in the microemulsions were also corroborated by micrographs taken with an optical microscope (Fig. 1).

The antimicrobial activity of the non-encapsulated and encapsulated EOs can be observed by means of the reduction percentages of the MIC or the MBC for each type of bacteria. A reduction of MIC and MBC of 75% and 20% was obtained with CEO for *E. coli*; for *L. monocytogenes*, the reduction of MIC and MBC was 60% and 20%, respectively. Evaluating the REO antimicrobial activity, the MIC was 70% less for both bacteria when it was encapsulated, and the MBC was reduced by 20% for *L. monocytogenes* and 60% for *E. coli*. In the case of OEO, the reduction in the MIC and MBC was 75% for *L. monocytogenes* and 50% for *E. coli* when applying the encapsulated oil. It can be observed that *E. coli* is less resistant to the inhibitory and bactericidal effect of non-encapsulated CEO than *L. monocytogenes* but showed similar resistance to non-encapsulated REO or OEO. The microemulsions were demonstrated to be more effective than the non-encapsulated EOs against *L. monocytogenes* and *E. coli* (Table 5).

Table 2 Physical parameters of rosemary, oregano, and cinnamon essential oils

Parameter ¹	Rosemary	Oregano	Cinnamon
Density (g/cm ³)	0.907 ± 0.001 ^c	0.946 ± 0.009 ^b	1.024 ± 0.003 ^a
Viscosity (P)	0.179 ± 0.001 ^c	0.302 ± 0.001 ^b	0.657 ± 0.006 ^a
Refractive index	1.470 ± 0.039 ^c	1.507 ± 0.011 ^b	1.595 ± 0.001 ^a

¹Average results are reported from triplicate measurements. Different superscripts letters in rows show significant differences ($p < 0.05$) among essential oils after data analysis by Tukey test

Table 3 Physical parameters of oil/water microemulsions of rosemary essential oil (30%), oregano essential oil (45%), and cinnamon essential oil (10%)

Parameter ¹	Rosemary	Oregano	Cinnamon
Viscosity (P)	0.189 ± 0.006 ^a	1.084 ± 0.020 ^b	0.109 ± 0.001 ^c
Density (g/cm ³)	0.98 ± 0.01 ^b	0.99 ± 0.01 ^{ab}	1.02 ± 0.01 ^a
pH	3.80 ± 0.35 ^b	4.20 ± 0.07 ^b	4.77 ± 0.01 ^a
<i>Color parameters</i>			
L	87.68 ± 0.22 ^a	83.78 ± 0.13 ^b	85.44 ± 0.35 ^c
a	− 0.81 ± 0.13 ^a	− 0.99 ± 0.02 ^b	− 1.84 ± 0.01 ^c
b	2.04 ± 0.04 ^c	3.96 ± 0.06 ^a	2.98 ± 0.01 ^b
Hue (°)	111.6 ± 0.1 ^b	104.1 ± 0.2 ^c	121.6 ± 0.1 ^a

¹Average results are reported from triplicate measurements. Different superscripts letters in rows show significant differences ($p < 0.05$) among essential oils after data analysis by Tukey test

Table 4 Particle size (D_{50}), phase separation and encapsulation efficiency of oil/water microemulsions of rosemary essential oil (30%), oregano essential oil (45%), and cinnamon essential oil (10%)

Microemulsion	Rosemary	Oregano	Cinnamon
D_{50} (μm)	3.71 ^b	5.46 ^a	1.98 ^c
% Phase separation (after 28 days)	6.67 ± 0.01 ^a	2.50 ± 0.01 ^c	3.33 ± 0.01 ^b
% Encapsulation efficiency	81.97 ± 0.01 ^a	80.99 ± 0.01 ^b	79.91 ± 0.01 ^c

Average results are reported from triplicate measurements. Different superscripts letters in rows show significant differences ($p < 0.05$) among essential oils after data analysis by Tukey test

Fig. 1 Micrographs of O/W microemulsions formulated with different essential oils: **a** cinnamon (10/90), **b** rosemary (30/70) and **c** oregano (45/55)

Table 5 Antimicrobial activity reported as minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) of non-encapsulated and encapsulated rosemary, oregano and cinnamon essential oils in microemulsions (% v/v)

Essential oils	<i>Listeria monocytogenes</i>		<i>Escherichia coli</i>	
	MIC	MBC	MIC	MBC
<i>Rosemary</i>				
Non-encapsulated	5.0	7.5	5.0	7.5
Encapsulated	1.5	6.0	1.5	3.0
<i>Oregano</i>				
Non-encapsulated	9.0	9.0	9.0	9.0
Encapsulated	2.25	2.25	4.5	4.5
<i>Cinnamon</i>				
Non-encapsulated	1.2	1.2	1.0	1.0
Encapsulated	0.5	1.0	0.2	0.8

Discussion

Non-encapsulated essential oil and microemulsion characterization

The major components (Table 1) of REO were 1R- α -pinene, eucalyptol, and camphor; this result was similar to the composition reported by Jalali-Heravi et al. (2011). Giarratana et al. (2016) evaluated the chemical composition of REO, finding values very similar to those determined in this study; the reported components were α -pinene (23.98%), eucalyptol (18.76%), and camphor (22.62%). Other studies have attributed REO's antimicrobial activity to the components α -pinene, terpinene, camphor, bornyl acetate, caryophyllene, carvacrol, and isocarnosol, some of which were found as major components in the analysis of chemical composition (Genena et al. 2008; Martac and Podea 2012). In the case of OEO, the major components identified were β -cymene, linalool, and carvacrol, which agrees with the results of Gilling et al. (2014), who found that carvacrol is one of the major components of OEO. Another component reported by Pirigharnaei et al. (2011), in addition to carvacrol at 29.85%, was γ -terpinene at 9.56%. The amount of carvacrol in this study was 27.34%; the difference in the values can be attributed to diverse aspects like geographical location of the harvest site, climate, and other environmental conditions (Toncer et al. 2009). On the other hand, the analysis showed cinnamaldehyde was the main component present in CEO at 50.64%, which agrees with other studies that reported that cinnamaldehyde is the primary component recognized to have antibacterial activity, with a reported value of 68.95% (Rajeswara et al. 2007; Dal Pozzo et al. 2012).

The physical properties of the EOs were analyzed to evaluate whether these affected the properties of the microemulsions. The density values for the EOs evaluated varied between 0.907 and 1.024 g/cm³. CEO demonstrated the highest value for density; in addition, this value is in accordance with that reported by Florido et al. (2014), who gave a density of 1.027–1.055 g/cm³. Albado-Plaus et al. (2001) reported a specific density for OEO of 0.9234 g/cm³, which is similar to that obtained in this study (0.946 $\pm$ 0.009 g/cm³). According to Genena et al. (2008), REO has a density of 0.894–0.913 g/cm³ and the value obtained in this study was 0.907 g/cm³. The densities of the microemulsions were similar to those of the non-encapsulated EOs (Table 2 and Table 3). Lugovska and Sidor (2014) reported density values within 1.03–1.10 g/cm³ for O/W emulsions of citrus oil, with the same type of microemulsions formulated in this study. It can be observed that EOs usually have low viscosity values like the results obtained in this study (0.179–0.657 P). Siddiqui and Ahmad (2013) performed a study on viscosity of different oils, reporting the following data: almond oil, 0.438 $\pm$ 0.018 P; clove oil, 0.066 $\pm$ 0.006 P; eucalyptus oil, 0.337 $\pm$ 0.033 P; walnut oil, 0.296 $\pm$ 0.002 P; cinnamon oil, 0.041 $\pm$ 0.001 P; lemon grass oil, 0.321 $\pm$ 0.005 P; garlic oil, 0.092 $\pm$ 0.004 P; black cumin seed oil, 0.308 $\pm$ 0.009 P; and linseed oil, 0.254 $\pm$ 0.005 P. Among the physical properties of the microemulsions, viscosity is one of the most important, because of the possibility of droplet coalescence (Nasery et al. 2016). The viscosities, densities, and pH values obtained in this study are within the ranges of 0.189–1.084 P, 0.98–1.02 g/cm³, and 3.80–4.77, respectively (Table 3). The microemulsion of OEO presented the highest viscosity (1.084 P), despite OEO not being the EO with highest viscosity; this result was attributed to the high concentration of this EO in the microemulsion, at 45%. The opposite result was obtained with the microemulsion of CEO with a viscosity of 0.109 P, which was formulated with 10% of EO, despite being the most viscous oil. The refractive index represents another important physical characteristic of EOs and usually ranges from 1.450 to 1.590. The values obtained for REO (1.468), OEO (1.490), and CEO (1.540–1.640) agree with the values reported in the literature (Rodríguez-Rojo et al. 2012). Microemulsions are systems that resemble “milk”; therefore, the values obtained for the color parameter *Lh* (luminosity) were > 83 , with a tendency to be white (Table 3). The lowest and highest *Lh* values were obtained for OEO (83.78) and CEO (85.44). The microemulsions evaluated presented positive values for the *bh* parameter (2.04–3.96), which indicated a yellow contribution to color, and negative values for the *ah* parameter (– 0.81 to – 1.84), which indicated a green contribution to the color, results that agree with their appearance. In terms

of hue, the degrees were within the range 104.1°–121.6°, which is consistent with the ones found by Taylor et al. (2017). The luminosity, the *ah* and *bh* color parameters, and the hue were significantly different ($p < 0.05$) among the three microemulsions.

Particle size distribution is a very important indicator for the quality, stability, uniformity, and dispersibility of the microemulsions; using high-frequency ultrasound provides enough energy to generate intense disruptive forces and minimize the droplet sizes (Ghosh et al. 2013). The small particle sizes of the obtained microemulsions applying high-frequency ultrasound are small enough (1.98–5.46 μm) to be related to their good stability during storage, as reported by Cardoso-Ugarte et al. (2017). Zhang et al. (2017) reported an average particle size of 8.69 μm in clove/cinnamon nanoemulsions, which is similar to the values obtained in this study; the differences may be due to the type of EO and its concentration in the microemulsion formulations. Also, the particle size is strongly related to the EEs; the ultrasonic homogenization technique used demonstrated high values of EE (79.91%–81.97%) and low phase separation (2.50%–6.67%) in the microemulsions, promoting high stability during storage (Table 4). Niu et al. (2016) obtained similar results studying microemulsions with three different oil phase compositions and pH values, reporting EEs between 80 and 92%; they mentioned that systems with such EEs have good stability. Aslan and Dogan (2018) evaluated the influence of the use of ultrasonic homogenizers on the stability of emulsions and specified that when emulsions are formulated with mechanical homogenizers there is phase separation from the first day, but this is not the case when formulating emulsions with ultrasound. The percentage of separation after 28 days indicates the stability of the microemulsions (Table 4). The phase separation causes ruptures of the membrane due to gravity and colloidal and mechanical forces and, for this reason, expresses the sensitivity to fusion. Trujillo-Cayado et al. (2015) studied the stability properties of microemulsions (O/W) of cocoa oil in different proportions by high-pressure homogenization. The authors reported values of separation phases in a range of 6–8% and particle sizes of 1–10 μm after 33 days.

Antimicrobial activity

The resistance of Gram-negative bacteria to the components of EOs is associated with a protective role of the protein membrane layer or cell wall of lipopolysaccharides, which limits the rate of diffusion of the hydrophobic compounds through the lipopolysaccharide layer. Zhang et al. (2017) confirmed this, in reporting that nanoemulsions of a blend of clove and cinnamon EOs showed higher antimicrobial activity against four tested microorganisms,

E. coli, *Bacillus subtilis*, *Salmonella* Typhimurium, and *Staphylococcus aureus*, than when using the EOs mixture directly.

Both the MIC and MBC of non-encapsulated CEO was 1.2% (v/v) for *L. monocytogenes* and 1.0% for *E. coli*. The antimicrobial activity of CEO is attributed to its major component cinnamaldehyde, and its mode of action is in the bonding of a carbonyl group in its chemical structure to the cellular proteins present, avoiding the action of the amino acid decarboxylase enzymes. The encapsulated CEO presented different MIC and MBC values for the studied bacteria: for *E. coli*, the values were 0.2% and 0.8%, while for *L. monocytogenes* these were 0.05% and 1.0%, respectively, demonstrating that the encapsulated CEO is more effective than the non-encapsulated CEO (Table 5).

On the other hand, REO presented an MBC value higher than the MIC for both types of bacteria when applied, whether encapsulated or not. A similar behavior has been reported for the MIC and MBC values of EOs of oregano, cinnamon, basil, and rosemary against bacteria such as *S. aureus*, *L. monocytogenes*, *Bacillus cereus*, *Yersinia enterocolitica*, *E. coli*, and *S. Typhimurium*, obtaining higher values obtained for the MBC (Mello et al. 2012). Camphor is one of the main antimicrobial components in REO, and its antimicrobial mechanism of action involves its lipophilic character, since it easily crosses the cytoplasmic membrane of the microorganism cells, causing damage to the wall and leakage of macromolecules. In addition, the antimicrobial activity of this EO is also due to the presence of other components such as phenols, aldehydes, and alcohols, which have demonstrated inhibition of different bacterial strains such as *S. aureus*, *E. coli*, *Salmonella enteritidis*, *B. subtilis*, and *Enterococcus faecalis* (Yañez and Cuadro 2012).

Dolça et al. (2015) evaluated the action of REO microencapsulated by coextrusion/gelling against *E. coli*. They obtained a 91.05%–99.41% bacterial count reduction (CFU/mL) with the dried microcapsules. The process they evaluated is more complicated than microencapsulation by microemulsion, since it consists of eight steps and more time (3 h) for the formation of the microcapsules.

Raeisi et al. (2016) studied the microbial quality of chicken meat incorporating CEO and REO, individually and in combination, against *L. monocytogenes*. The concentration used to evaluate individual or combined EOs was 0.5%. The initial count of bacteria (0 days) was 3.2 log CFU/g, and this increased after 15 days of storage up to 8.9 log CFU/g. When the EOs were used in combination, a stronger effect was observed in preserving the microbial quality (6.3 log CFU/g). Compared to our study, the CEO and REO encapsulated in microemulsions individually were more effective because after 24 h of storage, with a

higher initial inoculum (8 log CFU/mL), the bacterial population was completely inhibited.

The non-encapsulated OEO presented the same MIC and MBC values for *L. monocytogenes* and *E. coli* (9%). In this study, carvacrol is one of the major components of OEO, and it has a hydroxyl group on the phenolic ring; this component has demonstrated the ability to alter the outer cell membrane, causing leakage of protons and potassium ions and inhibition of the ATP synthesis of the microorganism (Fisher and Phillips 2008; Semeniuc et al. 2017). Dorman and Deans (2000) confirmed this antimicrobial mechanism of carvacrol when studying the inhibition of different pathogenic bacteria. The encapsulated OEO presented a different antimicrobial effect for the studied bacteria: For *L. monocytogenes*, the MIC and the MBC were 2.25%, and for *E. coli*, they were 4.5%. Therefore, the encapsulated OEO was demonstrated to be more efficient for bacteria inhibition, in comparison with the use of non-encapsulated EO, with *E. coli* shown to be less resistant than *L. monocytogenes*. The microencapsulation technique has been demonstrated to enhance the antimicrobial effect of EOs and provides prolonged activity over time, as demonstrated also by Teodoro et al. (2014).

Arana-Sánchez et al. (2010) microencapsulated three Mexican OEOs by spray drying; their antimicrobial activity was demonstrated by determining the MBC against 10^8 CFU/mL of *E. coli*. The study observed that microencapsulated OEOs decreased the MBC up to four-fold compared with the non-microencapsulated EOs. Despite the good results against bacteria, the study confirmed that using spray drying as a microencapsulation technique changed the composition of the EOs; the EEs varied from 53 to 81%.

Furthermore, Speranza et al. (2015) reported that the droplet size of the oils influenced the antimicrobial activity of emulsions. They observed that when the specific surface area of the emulsions was larger, the antimicrobial activity was more effective against the microorganisms studied. Additionally, the dispersed droplets increased the interfacial area, and thus, the interactions in the medium were higher (Nuñez et al. 2000). Therefore, the small droplet size of the microemulsions obtained with the ultrasonic homogenizer is demonstrated to promote the antimicrobial activity of the EOs against the evaluated bacteria.

Conclusion

The microemulsions are showed to be stable, reporting small droplet diameters, with high EEs (> 79%) and low separation phases (< 7%). In addition, microemulsions yielded MIC and MBC for both bacteria that were 20–75% lower than those obtained with the non-encapsulated EOs.

Using high-frequency ultrasound for emulsification was demonstrated to be effective for the delivery of EOs, which can be considered as natural antimicrobials. It is suggested that EO microemulsions be used in the food industry for different products and applications against bacteria of special safety concern. The use of EOs with antimicrobial activity encapsulated in microemulsions may be less expensive than the direct application of the extracted and purified active compounds from EOs, due to the reduced quantity needed to inhibit or inactivate bacteria.

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Compliance with ethical standards

Conflict of interest The Authors' declares that they have no conflict of interest.

References

- AOAC (1995) Official methods of analysis. Association of Analytical Chemist, Washington
- Albado-Plaus E, Saez-Flores G, Grabiell-Ataucusi S (2001) Composicion quimica y actividad antibacteriana del aceite esencial del *Origanum vulgare* (orégano). Rev Med Hered 12(1):16–19. <https://doi.org/10.20453/rmh.v12i1.660>
- Aslan D, Dogan M (2018) The influence of ultrasound on the stability of dairy-based, emulsifier-free emulsions: rheological and morphological aspect. Eur Food Res Technol 244:409–421. <https://doi.org/10.1007/s00217-017-2966-3>
- Alvarenga Botrel D, Vilela Borges S, de Barros V, Fernandes R, Dantas Viana A, Gomes M, da Costa J, Reginaldo Marques G (2012) Evaluation of spray drying conditions on properties of microencapsulated oregano essential oil. Int J Food Sci Technol 47(11):2289–2296. <https://doi.org/10.1111/j.1365-2621.2012.03100.x>
- Arana-Sánchez A, Estarrón-Espinosa M, Obledo-Vázquez EN, Padilla-Camberos E, Silva-Vázquez R, Lugo-Cervantes E (2010) Antimicrobial and antioxidant activities of Mexican oregano essential oils (*Lippia graveolens* H. B. K.) with different composition when microencapsulated in β -cyclodextrin. Lett Appl Microbiol 50(6):585–590. <https://doi.org/10.1111/j.1472-765x.2010.02837.x>
- Cardoso-Ugarte GA, López-Malo A, Jiménez-Munguía MT (2016) Application of nanoemulsion technology for encapsulation and release of lipophilic bioactive compounds in food. In: Grumezescu AM (ed) Emulsions, 1st edn. Elsevier, Amsterdam, pp 227–256
- Cardoso-Ugarte GA, Ramírez-Corona N, López-Malo A, Palou E, San Martín-González MF, Jiménez-Munguía MT (2017) Modeling phase separation and droplet size of W/O emulsions with oregano essential oil as a function of its formulation and homogenization conditions. J Dispers Sci Technol 39(7):1065–1073. <https://doi.org/10.1080/01932691.2017.1382370>

- Char C, Cisternas L, Pérez F, Guerrero S (2015) Effect of the emulsification on the antimicrobial activity of carvacrol. *J Food Sci* 1:1–6. <https://doi.org/10.1080/19476337.2015.1079558>
- Dal Pozzo M, Silva E, Flores D, Hartz S, Rossatto L, Castagna A, Viegas J, Matiuzy M (2012) Antibacterial activity of essential oil of cinnamon and trans-cinnamaldehyde against *Staphylococcus* spp. Isolated from clinical mastitis of cattle and goats. *Acta Sci Vet* 40(4):1080–1084
- Dolça C, Ferrándiz M, Capablanca L, Franco E, Mira E, López F, García D (2015) Microencapsulation of rosemary essential oil by co-extrusion/gelling using alginate as a wall material. *J Encapsul Adsorpt Sci* 5(3):121–130. <https://doi.org/10.4236/jeas.2015.53010>
- Donsi F, Annunziata M, Vincensi M, Ferrari G (2012) Design of nanoemulsion based delivery systems of natural antimicrobials: effect of the emulsifier. *J Biotechnol* 159:342–350. <https://doi.org/10.1016/j.jbiotec.2011.07.001>
- Dorman HJ, Deans SG (2000) Antimicrobials agents from plants: antibacterial activity of volatile oils. *J Appl Microbiol* 88:308–316. <https://doi.org/10.1046/j.1365-2672.2000.00969.x>
- Fisher K, Phillips C (2008) Potential antimicrobial uses of essential oils in food: is citrus the answer? *Food Sci Technol* 19(3):156–164. <https://doi.org/10.1016/j.tifs.2007.11.006>
- Florido PM, Andrade IMG, Capellini MC, Carvalho FH, Aracava KK, Koshima CC, Gonçalves CB (2014) Viscosities and densities of systems involved in the deterpenation of essential oils by liquid-liquid extraction: new UNIFAC-VISCO parameters. *J Chem Thermodyn* 72:152–160. <https://doi.org/10.1016/j.jct.2013.11.026>
- Genena AK, Hense H, Smânia Junior A, de Souza SM (2008) Rosemary (*Rosmarinus officinalis*): a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. *Ciencia y Tecnología de Alimentos* 28(2):463–469. <https://doi.org/10.1590/s0101-20612008000200030>
- Ghosh V, Mukherjee A, Chandrasekaran N (2012) Mustard oil microemulsion formulation and evaluation of bactericidal. *Int J Pharm Pharm Sci* 4(4):2–5
- Ghosh V, Mukherjee A, Chandrasekaran N (2013) Ultrasonic emulsification of food-grade nanoemulsion formulation and evaluation of its bactericidal activity. *Ultrason Sonochem* 20(1):338–344. <https://doi.org/10.1016/j.ultsonch.2012.08.010>
- Giarratana F, Muscolino D, Ragonese C, Beninati C, Sciarone D, Ziino G, Mondello L, Giuffrida A, Panebianco A (2016) Antimicrobial activity of combined thyme and rosemary essential oils against *Listeria monocytogenes* in Italian mortadella packaged in modified atmosphere. *J Essent Oil Res* 28(6):467–474. <https://doi.org/10.1080/10412905.2016.1165744>
- Gilling DH, Kitajima M, Torrey JR, Bright KR (2014) Antiviral efficacy and mechanisms of action of oregano essential oil and its primary component carvacrol against murine norovirus. *J Appl Microbiol* 116:1149–1163. <https://doi.org/10.1111/jam.12453>
- Jalali-Heravi M, Moazeni RS, Sereshti H (2011) Analysis of Iranian rosemary essential oil: application of gas chromatography–mass spectrometry combined with chemometrics. *J Chromatogr A* 1218(18):2569–2576. <https://doi.org/10.1016/j.chroma.2011.02.048>
- Liang R, Xu S, Shoemaker CF, Li Y, Zhong F, Huang Q (2012) Physical and antimicrobial properties of peppermint oil nanoemulsions. *J Agric Food Chem* 60(30):7548–7555. <https://doi.org/10.1021/jf301129k>
- Lugovska O, Sidor V (2014) Influence ratio of water and oil phase of emulsions on viscosity, particle size and turbidity of products. *J Fac Food Eng Stefan cel Mare Univ Suceava Rom* 8(3):224–227
- Mahfoudhi N, Ksouri R, Hamdi S (2016) Nanoemulsions as potential delivery systems for bioactive compounds in food systems: preparation, characterization, and applications in food industry. In: Grumezescu AM (ed) *Emulsions*. Elsevier, Amsterdam, pp 365–404
- Martac IM, Podea P (2012) Determination of antimicrobial and antioxidant activities of essential oil isolated from *Rosmarinus officinalis* L. Carpathian *J Food Sci Technol* 4(2):40–45
- Mello S, Cunha A, Neudía G, Luiz F, Wernecj C (2012) Chemical composition and antimicrobial activity of essential oils from selected herbs cultivated in the South of Brazil against food spoilage and foodborne pathogens. *Ciencia Rural Santa Maria* 42(7):1300–1306. <https://doi.org/10.1590/s0103-84782012000700026>
- Nasery S, Hoseinpour S, Phung LTK, Bahadori A (2016) Prediction of the viscosity of water-in-oil emulsions. *Pet Sci Technol* 34(24):1972–1977. <https://doi.org/10.1080/10916466.2016.1233248>
- Niu F, Pan W, Su Y, Yang Y (2016) Physical and antimicrobial properties of thyme oil emulsions stabilized by ovalbumin and gum Arabic. *Food Chem* 212:138–145. <https://doi.org/10.1016/j.foodchem.2016.05.172>
- Núñez GA, Sanchez G, Gutierrez X, Silva F, Dalas C, Rivas H (2000) Rheological behavior of concentrated bitumen in water emulsions. *Langmuir* 6:6497–6502. <https://doi.org/10.1021/la990412p>
- Pirighmaei M, Zare S, Heidary R, Khara J, EmamaliSabzi R, Kheiry F (2011) The essential oils compositions of Iranian Oregano (*Origanum vulgare* L.) populations in field and provenance from Piranshahr district, West Azarbaijan province, Iran. *Avicenna J Phytomed* 1(2):106–114. <https://doi.org/10.22038/AJP.2011.129>
- Raeisi M, Tabaraei A, Hashemi M, Behnampour N (2016) Effect of sodium alginate coating incorporated with nisin, *Cinnamomum zeylanicum*, and rosemary essential oils on microbial quality of chicken meat and fate of *Listeria monocytogenes* during refrigeration. *Int J Food Microbiol* 238:139–145. <https://doi.org/10.1016/j.ijfoodmicro.2016.08.042>
- Rajeswara BR, Rajput DK, Bhattacharya AK (2007) Essential oil composition of petiole of *Cinnamomum verum* Bercht. And Presl. *J Spices Aromat Crops* 16(1):38–41
- Rodríguez-Rojó S, Varona S, Núñez M, Cocero MJ (2012) Characterization of rosemary essential oil for biodegradable emulsions. *Ind Crops Prod* 37(1):137–140. <https://doi.org/10.1016/j.indcrop.2011.11.026>
- Semeniuc CA, Pop CR, Rotar AM (2017) Antibacterial activity and interactions of plant essential oil combinations against Gram-positive and Gram-negative bacteria. *J Food Drug Anal* 25(2):403–408. <https://doi.org/10.1016/j.jfda.2016.06.002>
- Siddiqui N, Ahmad A (2013) A study on viscosity, surface tension and volume flow rate of some edible and medicinal oils. *Int J Sci* 2(6):1318–1326
- Speranza P, Badan Ribeiro AP, Cunha RL, Macedo JA, Macedo GA (2015) Influence of emulsion droplet size on antimicrobial activity of interesterified Amazonian oils. *LWT Food Sci Technol* 60(1):207–212. <https://doi.org/10.1016/j.lwt.2014.07.022>
- Taylor AB, Ioannou MS, Watanabe T, Hahn K, Chew TL (2017) Perceptually accurate display of two greyscale images as a single colour image. *J Microsc* 268(1):73–83. <https://doi.org/10.1111/jmi.12588>
- Teodoro RAR, de Barros FRV, Botrel DA, Borges SV, de Souza AU (2014) Characterization of microencapsulated rosemary essential oil and its antimicrobial effect on fresh dough. *Food Process Bioprocess Technol* 7(9):2560–2569. <https://doi.org/10.1007/s11947-014-1302-1>

- Toncer O, Karaman S, Kizil S, Diraz E (2009) Changes in essential oil composition of Oregano (*Origanum onites* L.) due to diurnal variations at different development stages. *Not Bot Horti Agrobot Cluj-Napoca* 37(2):177–181. <https://doi.org/10.15835/nbha3723188>
- Trujillo-Cayado LA, Natera A, García MC, Muñoz J, Alfaro MC (2015) Rheological properties and physical stability of ecological emulsions stabilized by a surfactant derived from cocoa oil and high pressure homogenization. *Grasas Aceites* 66(3):1–8. <https://doi.org/10.3989/gya.1313143>
- Velázquez-Contreras C, Osorio-Revilla G, Gallardo-Velázquez T (2014) Encapsulation of orange oil in spout-fluid bed dryer with a draft tube on a bed of inert solids. *Dry Technol* 32:1718–1726. <https://doi.org/10.1080/07373937.2014.924525>
- Yañez X, Cuadro OF (2012) Composición química y actividad antibacteriana del aceite esencial de las especies *Eucalyptus globulus* y *E. camaldulensis* de tres zonas de Pamplona (Colombia). *Rev Fac Cienc Básicas* 10(1):52–61
- Yeon-Ji J, Ji-Yeon C, Yun-Joong K, Sang-Gi M, Geun-Pyo H, Mi-Jung C (2015) Physical and antimicrobial properties of trans-cinnamaldehyde nanoemulsions in water melon juice. *Food Sci Technol* 60:444–451. <https://doi.org/10.1016/j.lwt.2014.09.041>
- Zhang S, Zhang M, Fang Z, Liu Y (2017) Preparation and characterization of blended cloves/cinnamon essential oil nanoemulsions. *Food Sci Technol* 75:316–322. <https://doi.org/10.1016/j.lwt.2016.08.046>

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