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Antimicrobial Activity and Physicochemical Characterization of Oregano, Thyme and Clove Leave Essential Oils, Nonencapsulated and Nanoencapsulated, Using Emulsification

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Abstract

Background and objective: Functional properties of essential oils are attributed to their components, many of which exhibit antimicrobial activity against pathogenic and spoilage microorganisms in a wide variety of foods. However, essential oils are unstable compounds; therefore, they can be encapsulated for a better protection and increase of functionality. In this work, antimicrobial activities of oregano, thyme and clove leave essential oils (non-encapsulated and nanoencapsulated) were assessed against *Escherichia coli* ATCC 29922, *Salmonella typhimurium* ATCC 14028 and *Staphylococcus aureus* ATCC 25923 using emulsification.

Material and methods: The essential oils were characterized based on their physicochemical properties. Nanoemulsions were prepared, using 5% (w w^{-1}) of essential oils, and then characterized based on their physical properties, stability and encapsulation efficiency. The microdilution antimicrobial assay was carried out to assess minimum inhibitory concentration and minimum bactericidal concentration of the essential oils and their nanoemulsions. Data from physical properties of the essential oils and physical properties, stability and encapsulation efficiency of the nanoemulsions were statistically analyzed.

Results and conclusion: Antimicrobial activity of the essential oils showed decreases in minimum inhibitory concentration by 27-60% for the nanoencapsulated oils, compared to nonencapsulated oils. Nanoencapsulated and nonencapsulated oregano essential oils exhibited the lowest minimum inhibitory concentration and minimum bactericidal concentration values. Based on the results, nanoencapslulated essential oils may further be used in various foods to avoid microbial contaminations.

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1. Introduction

Essential oils (EOs) are extracted from plants, including a mixture of several biosynthesized chemical substances [1]. There is an increasing interest in food industries for EOs because they are produced from natural sources, use of them is safe and they are friendly to environment and exhibit antioxidant and antimicrobial properties. Microbial controls using EOs have become one of the most important research areas with the aim of replacing synthetic preservatives [2]. The antimicrobial properties of EOs are based on their components; of which, phenolic compounds are one of the most important groups. Studies have assessed the antimicrobial activity of components in EOs, including thymol, carvacrol and eugenol, against pathogenic and deteriorative microorganisms that are common in foods [3-5]. The action mechanisms of the antimicrobial compounds depend on the type of microorganisms to be inhibited or inactivated. These compounds can cause cellular damages or intervene in metabolic processes of the microorganisms [6]. Several studies have demonstrated that various terpenic compounds in EOs include synergistic effects as antimicro-

bials since they demonstrate interactive antibacterial effects on biochemical processes of the target bacteria [7]. However, EOs are chemically unstable when exposed to certain environmental conditions such as light, moisture, oxygen and elevated temperatures; all of which can cause the loss of their antimicrobial and antioxidant properties [8]. An alternative to protect EO properties against degradation and interaction with other food components is encapsulation. The encapsulation process involves formation of a multicomponent structure in form of composite particles; usually including two substances of core material and the encapsulating agent [9]. Using a diffusion process, oil can pass through the materials of capsule and be released in a controlled manner at required sites. Various encapsulation techniques such as emulsification, spray drying, coaxial electrospray system, freeze drying, coacervation, in-situ polymerization, extrusion, supercritical fluid technology and fluidized bed coating have been described. Study on this subject is broad since antimicrobial activity of EOs, encapsulated with different encapsulating agents or encapsulation techniques, includes various effects on pathogenic and deteriorative microorganisms in foods. The most common technique for EO encapsulation is spray drying [2]. In recent years, studies have been carried out to assess antimicrobial effects of oregano, coriander, cinnamon and garlic encapsulated EOs in model systems [6,10,11] against various microorganisms of interest in foods. Examples of these microorganisms include *Micrococcus luteus*, *Bacillus* (*B.*) *subtilis*, *B. cereus*, *Rhodorula glutinis*, *Candida utilis*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Penicillium glaucum*, *Geotrichum candidum*, *Alternaria alternate*, *Escherichia* (*E.*) *coli*, *Salmonella* (*S.*) *typhimurium* and *Staphylococcus (Staph.) aureus* [2]. Food industries constantly investigate efficient and cost-effective intervention strategies to control growth of common microorganisms to ensure microbial quality and safety of foods [12].

Encapsulation of EOs has been demonstrated as a good alternative to increase the oil antimicrobial activity and preservation time during storage. However, a few studies have been reported up to date on encapsulation of EOs and emulsification techniques [13,14]. In particular, many studies have focused on EO compounds as antimicrobials, which are generally extracted and purified before the encapsulation processes [15,16]. However, encapsulation of EOs may be less expensive than the encapsulation of specific compounds for practical uses. Various EOs must be studied to develop their uses in distinctive foods with sensory compatibility. Regarding nanoencapsulated EOs, studies have published on *Zataria multiflora* and oregano EOs [17,18]. However, there are a little information on clove leave EO because the herbal behavior is different from the clove flower EO. Nowadays, effects of various proportions of stabilizing and emulsifying agents and EOs are described, as well as other homogenization methods. These together can completely change the physical properties, stability and encapsulation efficiency (EE) of the emulsions [17,18-20].

Therefore, the aim of this study was to characterize and compare antimicrobial activities of oregano, thyme and clove leave EOs, nonencapsulated or nanoencapsulated, against EE, *E. Coli* and *S. aureus* using emulsification. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were calculated for nonencapsulated and nanoencapsulated EOs against the bacteria in model systems using microdilution.

2. Materials and methods

2.1. The essential oils

Oregano essential oil (OEO), thyme essential oil (TEO) and clove leave essential oil (CLEO) were purchased from Laboratories Hersol S.A. de C.V., Mexico.

2.1.1 Chemical characterization of the essential oils

The EOs were analyzed using Agilent Technologies 6850N Gas Chromatographer (Palo Alto, CA, USA), equipped with Agilent 5975 C Mass-Spectrometer Detector (Palo Alto, CA, USA). The flow rate of the carrier gas (helium) was set at 1.1 ml min^{-1}. Derivatives were separated using HP 5 Fused Silica Column (Agilent, Palo Alto, CA, USA; 30-m length, 250 mm and 0.25-µm film thickness). The injector was set at 300°C and operated in a split mode of 10:1. The column oven was set at 60°C for 2 min and then ramped to 250°C at a rate of 10°C min^{-1}. The EO constituents were identified by comparing current mass spectra to those in National Institute of Standards and Technology Mass Spectral Database [21].

2.1.2 Physical properties of the essential oils

The EOs were characterized based on their color parameters (L, a, b) in Hunter scale using colorimeter (Model CR400, Konica Minolta, Japan) and refractive index using digital refractometer (PAL-BX / RI, ATAGO, Japan) according to A.O.A.C. 920.141 [22]. The EO viscosity was calculated using Cannon-Fenske Viscometer (Model 350, Thomas Scientific, USA) and the density was calculated using glass pycnometer according to A.O.A.C. 920.134 [23,24]. These experiments were carried out in triplicate.

2.2. Preparation and characterization of nanoemulsions

Nanoemulsions of 5% (w w^{-1}) EOs (dispersed phase) were prepared at 10% (w w^{-1}) of agave inulin as stabilizing (Fructagave PR95, Agaviotica, Mexico) and 3% (w w^{-1}) of polysorbate 80 as emulsifying agents (Tween 80, Sigma-Aldrich, USA) for the continuous phase. Nanoemulsions were homogenized using ultrasonic device (Ultrasonic Processor CP 505, Cole Parmer, USA) at 84 µm of amplitude for 15 min [25].

2.2.1 Physical properties of nanoemulsions

Nanoemulsions were characterized based on their color parameters (L, a, b) in Hunter scale using colorimeter (Model CR400, Konica Minolta, Japan) and pH using potentiometer (PC45, Conductronic, Mexico) according to A.O.A.C. 981.12 [26]. Both equipments were calibrated. The EO viscosity was calculated using Cannon-Fenske Viscometer (350, Thomas Scientific, USA) [23] and density using glass pycnometer [24]. These calculations were carried out in triplicate.

2.2.2 Encapsulation efficiency (EE)

The EE of EOs was calculated using UV/VIS spectrophotometer (Cary 100, Varian, USA) according to protocols from literatures with modifications [27]. The maximum peak was calculated in spectrograms of the EOs dissolved in n-hexane (J. T. Baker, USA) at a wavelength range of 190-500 nm. The maximum peak found for OEO, TEO and CLEO included 271, 274 and 281 nm, respectively. The EE was calculated through a calibration curve achieved from five EO standard solutions diluted with n-hexane and prepared at various concentrations (0.5-5 µl ml^{-1}). To calculate the EE, extraction of the free essential oil from nanoemulsion was carried out by dissolving 700 µl of nanoemulsions in 9.3 ml of n-hexane with constant stirring (60 rpm) for 15 s. These assessments were carried out in triplicate. Then, absorbance was set on a specific wavelength according to the EO. The EE was calculated as a percentage using the following equation:

$$EE\ \% = \frac{EOM - FEOM}{EOM} \times 100 \quad \text{eq. 1}$$

Where, EOM was EO content of the nanoemulsion formulation fixed at 5% (ml_{EO} $(ml_{nanoemulsion})^{-1}$) and free essential oil from nanoemulsion was free EO from nanoemulsion (ml_{EO} $(ml_{nanoemulsion})^{-1}$).

2.2.3 Stability of nanoemulsions

Stability of the nanoemulsions was assessed using visual inspection and describing the first signs of development of creaming. For this assessment, 25 ml of each nanoemulsion were transferred into a graduated tube (by triplicate) and stored in a dark place at 23±1.0°C for 28 days. Then, nanoemulsions were inspected and creaming was recorded after 7, 14, 21 and 28 days of storage according to Hebishy et al. with minor modifications [28]. Granulometric distribution of the EO droplets dispersed in nanoemulsions was assessed in triplicate using dynamic light scattering particle analyzer (Nanotrac Wave II, Microtrac, USA).

2.3 Antimicrobial activity of O/W essential oils and nanoemulsions

2.3.1 Microbial culture preparation

Strain cultures used in this study included *E. coli* ATCC 29922, *S. typhimurium* ATCC 14028 and *Staph. aureus* ATCC 25923 provided by the Food Microbiology Laboratory of the Universidad de las Americas Puebla, Mexico. The bacterial strains were cultivated on tryptone soy agar (BD BIOXON, Mexico) and incubated at 37±1°C for 24 h. These were then stored at 4°C for a maximum of three weeks. At the end of storage, the bacterial strains were reactivated and cultivated. Suspensions were prepared using two loopfulls of the bacterial cultures to inoculate tubes containing 10 ml of tryptone soy broth (BD BIOXON, Mexico). Tubes were incubated at 37±1°C for 24 h.

2.3.2 Calculation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of EOs and nanoemulsions was evaluated using microdilution with mild modifications [29]. Briefly, serial dilutions of EOs and nanoemulsions were prepared and transferred into the wells of microplates. From a stock solution of 25 µl ml^{-1} of EOs, 1-40 µl were added into the wells to achieve EO concentrations of 0.1-4 µl ml^{-1}. Nanoemulsions were prepared at 5% (w w^{-1}) of EOs, adding 0.5-10 µl of the nanoemulsions into the wells to achieve concentrations of 0.1-2 µl ml^{-1} of EOs using tryptone soy broth. Then, 2.5 µl of the bacterial suspensions at 10^8 CFU ml^{-1} concentrations were added to each well to achieve a final concentration of 10^6 CFU ml^{-1}. Microplates were incubated at 37±1°C for 18 h. After the incubation, 10 µl of resazurin (Sigma Aldrich, Germany) at a concentration of 0.015% were added to each well to assess the microbial growth. After incubation at 37±1°C for 2 h, the MIC value was calculated as the concentration of EOs or nanoemulsions that did not change from blue resazurin to pink resorufin. Active microbial growths were detected by the reduction of blue resazurin to pink resorufin. The MBC of EOs and nanoemulsions was calculated using microdilution with mild modifications [29]. Briefly, 5 µl of solutions from negative wells were spread on tryptone soy agar plates. The MBC value corresponded to the lowest concentration of EOs or nanoemulsions with negative subcultures after incubation at 37±1°C for 24 h. These calculations were carried out in triplicate.

2.4 Statistical analysis

Data were statistically analyzed using Minitab Software v.17 (LEAD Technologies, Sate College, PA, USA) and ANOVA and Tukey tests with a confidence level of 95%.

3. Results and discussion

3.1. Characterization of the essential oils

3.1.1 Chemical characterization

Differences in the chemical characterization of EOs is usually common due to the EO composition, which depends on various geographical and environmental factors [30]. Therefore, it was important to identify the major components of EOs used as antimicrobials in this study. The major components identified in the EOs are shown in Table

1. The OEO contains more than 30 compounds, including carvacrol that is usually one of the major components of OEO. In this study, carvacrol (60.76%) was detected as a major component of the OEO. Carvacrol is a phenolic compound and mainly responsible for the OEO anti-microbial activity [31]. Similarly, the antimicrobial activity of TEO is mainly attributed to carvacrol and thymol [32]. In the current study, the major component of TEO was thymol (30.76%). Clove EOs exhibit antimicrobial activity princip-ally due to the eugenol content of the oil [33]. In this study, one of the major components found in CLEO was eugenol (25.72%).

Table 1. Chemical characterization of the essential oils

Essential oil	Major component	Area (%)
OEO	Carvacrol	60.76
	Syn carvacrol	15.21
	Syn m-cymene	6.92
	Syn δ-terpinen	5.64
	Caryophyllene	3.53
	Syn linalol	2.52
TEO	Thymol	30.76
	m-cymene	21.77
	1r-α-pinene	9.35
	Syn-linalol	6.16
	Syn δ-terpinen	6.14
	δ-terpinen	4.64
	Caryophyllene oxide	2.22
	Caryiophyllene	2.09
CLEO	α-caryophyllene	32.16
	Eugenol	25.72
	Syn δ-cadiene	12.43
	4-((1E)-3-hidroxy-1-propenyl)-2-methoxyphenol	8.82
	α-farnesene	4.11
	Caryophyllene oxide	3.37
	Diepicedrene-1-oxide	3.28

OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil. Analyses were carried out in triplicate.

3.1.2 Physical properties of the essential oils

Every EO includes different properties, which are linked to the type of source plants, as well as other factors. It is possible to find variations in EO properties from similar plant genera [30]. Therefore, physical properties of the EOs were differentiated before the nanoencapsulation process to show how their properties were affected. Due to properties such as density and viscosity of the dispersed phase, EO properties are better to characterized to allow formulation of stable emulsions. Physical properties of the EOs are presented in Table 2. Color is a physical property of the EOs, which includes a large variation depending on the type of EOs. In this study, color parameters for OEO, TEO and CLEO were recorded based on the Hunter scale (Table 2). Moreover, high luminosity and tendency toward yellow and green were recorded for the EOs. A refractive index value of 1.4774 has been reported for OEO by other researchers, which is close to values from the current study (Table 2). The refractive index of TEO (1.499) was also similar to that of other studies [34,35]. In other studies, the refractive index for CLEO ranged 1.5330-1.5350, which was similar to that of the present study [36]. Viscosity is a characteristically physical property of EOs and varies depending on the type of EOs. In general, values for the OEO, TEO and CLEO from this study (Table 2) were similar to values from other studies [37]. These included 0.066±0.006 P for clove flower and 0.041±0.001 P for cinnamon EOs. In the current study, the density value of OEO (Table 2) was similar to that 0.92 g $(cm^3)^{-1}$ in literatures [38]. Another study [39] reported a density value of 0.925 g $(cm^3)^{-1}$ for TEO, which was similar to that the present study did. In the present study, the CLEO presented the highest value of density [1.046-1.053 g $(cm^3)^{-1}$], as shown by other studies [36].

Table 2. Physical properties of the essential oils

Property	OEO	TEO	CLEO
Color			
L	80.490±0.310^b	81.440±0.100^a	81.600±0.200^a
a	2.230±0.030^b	1.060±0.020^c	3.920±0.030^a
b	8.370±0.050^b	3.920±0.030^c	16.430±0.030^a
Refractive index	1.510±0.001^b	1.502±0.006^b	1.535±0.004^a
Viscosity (P)	0.095±0.004^a	0.048±0.003^c	0.079±0.004^b
Density (kg L^{-1})	0.947±0.041^a	0.920±0.071^a	1.042±0.020^a

OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil. Different letters in the same row show significant differences ($P \leq 0.05$). Measurements were carried out in triplicate. Color parameters L, a, b are in Hunter scale.

3.2 Characterization of the nanoemulsions

Emulsions include different properties, according to the materials used in their preparation [40]. Therefore, it is necessary to characterize physical properties of the emulsions to show correlations with their stability and assess their use as antimicrobials in model systems. The physical properties and stability of the O/W nanoemulsions of EOs are shown in Table 3.

3.2.1 Physical properties of the nanoemulsions

In this study, color parameters were recorded for OEO, TEO and CLEO nanoemulsions based on the Hunter scale. High values of luminosity and tendency toward yellow and green were recorded (Table 3). In fact, characterization of color parameters was necessary to show if it was possible to carry out MIC assays without any interference using the color change test. The pH is an important parameter since a significant change in its value may suggest chemical changes of the components in emulsions, which affect its stability [40]. According to other studies, food emulsions

generally include a pH range of 2.5-7.5, similar to the current pH ranges of OEO, TEO and CLEO nanoemulsions (4.64-5.04) [41]. The pH of OEO nanoemulsion was reported as the highest pH, which could affect its stability since it was the most unstable nanoemulsion (Table 4). In stability of the emulsions, it was shown that a more viscous continuous phase could make the creaming process slower [42]. In this study, a nanoemulsion viscosity of 0.018 P was recorded. Generally, viscosity of emulsions is linked to the viscosity of continuous phase [41]. However, no significant differences ($P>0.05$) were recorded for the viscosity of OEO, TEO and CLEO nanoemulsions in this study (Table 3) possibly due to their low EO contents (5%). Density is an important parameter for the stability of emulsions [43]. If the continuous and dispersed phases include different densities, the great pressure on dispersed phase droplets drives a separation process called creaming [42]. In the current study, density values of the OEO, TEO and CLEO nanoemulsions were similar (Table 3) with an average value of 1.033 g $(cm^3)^{-1}$. However, no significant differences ($P>0.05$) were seen in density values of the OEO, TEO and CLEO nanoemulsions; possibly due to their low EO contents (5%).

3.2.2 Encapsulation efficiency (EE)

Calculation of EE is important because this parameter demonstrates retention of the oil in the capsule [44]. In this study, EE was calculated in a range of 76.2-96.7% (Table 3); of which, the CLEO nanoemulsion presented the highest values of EE. Smaller particle sizes together with greater EE values could explain the greater stability of CLEO and TEO nanoemulsions, compared to that of OEO nanoemulsion.

Table 3. Physical property and stability of O/W nanoemulsions of the essential oils

Property	OEO	TEO	CLEO
Color			
L	80.530±0.390^b	81.750±0.280^a	81.820±0.300^a
a	0.750±0.010^b	0.710±0.010^c	0.780±0.010^a
b	1.130±0.010^a	0.550±0.010^c	1.010±0.010^b
pH	5.040±0.010^a	4.730±0.040^b	4.640±0.010^c
Viscosity (P)	0.018±0.002^a	0.018±0.002^a	0.018±0.003^a
Density (kg l^{-1})	1.034±0.021^a	1.032±0.010$_a$	1.034±0.032^a
D_{10} (µm)	174.600±0.058^a	97.700±0.045^b	90.800±0.057^c
D_{50} (µm)	232.500±0.058^a	130.800±0.045^b	116.400±0.057^c
D_{90} (µm)	343.000±0.058^a	237.800±0.045^c	301.000±0.057^b
EE (%)	76.243±0.001^c	92.182±0.001^b	96.709±0.001^a

OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil; EE: encapsulation efficiency. Color parameters L, a, b are in Hunter scale. Different letters in the same row show significant differences ($P\leq0.05$). Measurements were carried out in triplicate.

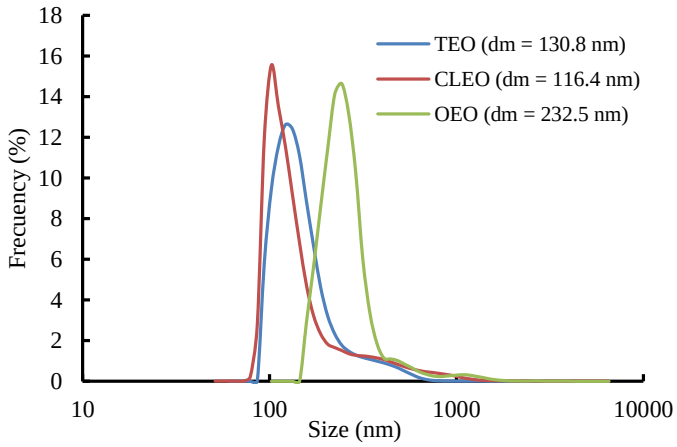
Table 4. Stability of essential oils nanoemulsions during storage at 23°C

Nanoemulsions	Observation period (days)			
	7	14	21	28
OEO	4-7 drops of oil were observed on the surface, without a total phase separation.	8-10 drops of oil were observed on the surface, without a total phase separation.	12-14 dispersed drops of oil were observed on the surface, without a total phase separation.	14-16 dispersed drops of oil were observed on the surface, without a total phase separation.
TEO	No visible changes	No visible changes	No visible changes	No visible changes
CLEO	No visible changes	No visible changes	No visible changes	No visible changes

OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil
Measurements were carried out in triplicate.

3.2.3 Stability of nanoemulsions

In this study, the most stable nanoemulsions belonged to TEO and CLEO with no visible changes during an observation period of 28 days (Table 4). The OEO nanoemulsion showed first signs of creaming with the presence of lipid droplets on the surface of nanoemulsion on Day 7. At the end of observation period, the nanoemulsion exhibited 14-16 dispersed oil drops on its surface with no total phase separation. Droplet size plays a critical role in determining long-term stability of the emulsions [45]. A very small droplet size of 116.4-232.5 nm (D_{50}) was reported for the nanoemulsions (Figure 1); of which, the smallest size belonged to CLEO nanoemulsion. The best description for an emulsion is through its droplet size distribution, which statistically provides records of the dispersed phase fragmentation [42]. Decreased average droplet sizes can prevent or minimize creaming [46]. In this study, the greater stability of CLEO nanoemulsion might be seen because it included the lowest droplet size values (Figure 1, Table 3).

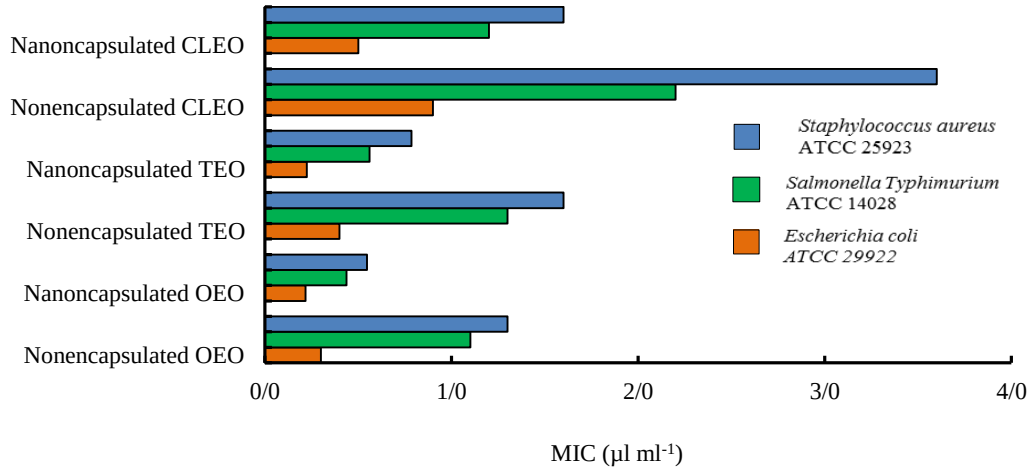


OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil; dm: medium diameter. Analyses were carried out in triplicate.

Figure 1. Nanoemulsion droplet sizes

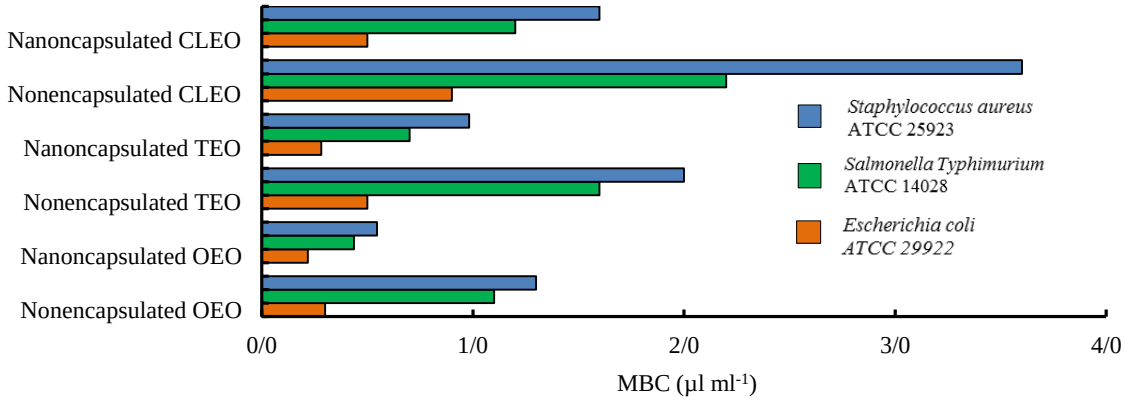
3.3 Antimicrobial activity of O/W essential oils and nanoemulsions

The antimicrobial activity of EOs depends mainly on three factors of their chemical components, type of the target microorganisms and the EO hydrophilic/hydrophobic characteristics [47,48]. Based on the results from chemical characterization, phenolic compounds with antimicrobial activity were included in OEO (60.76% of carvacrol), TEO (30.76% of thymol) and CLEO (25.72% of eugenol). Results from other studies [5] have shown that phenolic components are the major chemicals responsible for antimicrobial properties of the EOs. The chemical components of EOs act by altering cytoplasmic membrane structure of the microorganisms, inhibiting the transport of electrons or acting as potential denaturing agents of proteins, solvents and dehydrators [6]. In antimicrobial activity assessment of the three EOs, the highest activity belonged to nonencapsulated and nanoencapsulated OEO since the lowest MIC and MBC values were seen against the bacterial strains (*E. coli*, *S. typhimurium* or *Staph. aureus*), compared to tested TEO and CLEO nonencapsulated or nanoencapsulated (Figures 2 and 3). These results can be due to the high content of carvacrol (60.76%) in tested OEO. The results obtained in this study agree with other studies [48], which reported that OEO is one of the most effective EOs for antimicrobial control; its phenolic components have the ability to alter the outer cell membrane, which causes the leakage of protons and potassium ions and results in the collapse of the membrane and inhibition of ATP synthesis. In contrast, it was observed that the least effective EO was tested CLEO (nonencapsulated or nanoencapsulated), since it showed the highest values of MIC and MBC, against the bacterial strains. These results were similar to those from other studies [49], showing antimicrobial efficiency of EOs in following order of oregano > clove flower > coriander > cinnamon > thyme > mint > rosemary > mustard > cilantro > sage. Regarding the target bacterial genus, nonencapsulated and nanoencapsulated EOs exhibited excellent antimicrobial activity against *E. coli*, including the lowest MIC and MBC values. Based on the data from literatures, MIC of the OEO (*Origanum vulgare*) against *S. typhimurium* includes 0.12-3.12 µl ml^{-1} [30] and MIC of OEO (*Origanum compactum*) against *E. coli* and *Staph. aureus* includes 0.625 and 1.25 µl ml^{-1}, respectively [50]. Furthermore, MIC of TEO (*Thymus vulgaris*) against *S. typhimurium* includes 0.45-7.20 µl ml^{-1} [30] and MIC of TEO (*T. serpyllum*) against *Staph. aureus* includes 2.5 µl ml^{-1} [50]. Results from the present study are similar to those by other studies; however, composition of EOs depends on several factors including harvesting seasons and geographical sources. These can explain the differences in results by different studies [30]. Studies have documented that EO components include greater antimicrobial effects on Gram-negative bacteria [51]. In the current study, greater resistances generally belonged to *Staph. aureus* against nonencapsulated and nanoencapsulated EOs, compared to those belonged to *E. coli* and *S. typhimurium* (Figures 2 and 3). In comparison of Gram-negative bacteria in this study, a greater resistance to the EOs was detected in *S. typhimurium*, compared to that detected in *E. coli*. In this study, decreased MIC of 27-60% was seen in nanoencapsulated EOs, compared to that in nonencapsulated EOs. In characterization of the nanoencapsulated EOs, very small particle sizes of 116.4-232.5 nm (D_{50}) were detected (Table 3), which facilitated their easy diffusion into the media, as shown by other studies [52].



OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil. Analyses were carried out in triplicate, MIC: minimum inhibitory concentration.

Figure 2. Antimicrobial activity of the essential oils (nonencapsulated and nanoencapsulated) against the highlighted bacteria for minimum inhibitory concentration



OEO: oregano essential oil; TEO: thyme essential oil; CLEO: clove leave essential oil; analysis were in triplicate, MBC: minimum bactericidal concentration.

Figure 3. Antimicrobial activity of the essential oils (nonencapsulated and nanoencapsulated) against the highlighted bacteria for minimum bactericidal concentration

4. Conclusion

In conclusion, the nanoencapsulation process using emulsification was effective in preserving the major constituents of pure OEO, TEO and CLEO. This result was linked to low droplet sizes and high EE of the nanoencapsulated EOs. Antimicrobial activity of the EOs depended on the EO type and solubility and the bacterial strain. Nonencapsulated and nanoencapsulated OEO showed promises in controlling growth of the bacteria in model systems, where a complete growth inhibition was observed at EO concentrations of 0.218-0.300 µl ml^{-1} for *E. coli*, 0.437-1.100 µl ml^{-1} for *S. typhimurium* and 0.546-1.300 µl ml^{-1} for *Staph. aureus*. In general, the nano-encapsulated EOs were more effective in inhibition of the bacterial strains, compared to that the nonencapsulated EOs were. Nanoemulsions, produced in this study, exhibited important antibacterial activities against the bacterial strains. However, further studies are necessary to investigate interactions of EOs with constituents in real food systems and establish models for the optimization of EO antimicrobial effects. It is also necessary to establish maximum use quantities of the EOs to ensure safety of foods with no unfavorable effects on sensory characteristics.

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6. Conflict of interest

The authors declare no conflict of interest.

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فعالیت ضدمیکروبی و ویژگی‌های فیزیکوشیمیایی اسانس روغنی برگ پونه کوهی، آویشن و میخک، ریزپوشانی نشده و نانوریزپوشانی شده به روش امولسیون سازی

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چکیده

سابقه و هدف: خواص فراسودمندی ا سانس‌های روغنی به ترکیباتشان مربوط می‌شود، بسیاری از این ترکیبات فعالیت ضدمیکروبی در برابر میکروارگانیسم‌های بیماریزا و مولد فساد در طیف گسترده ای از مواد غذایی دارند. با این حال، اسانس‌های روغنی ترکیبات ناپایداری می باشند؛ بنابراین، به منظور محافظت بهتر و افزایش عملکرد ریزپوشانی می شوند. در این تحقیق، فعالیت ضد میکروبی ا سانس روغنی برگ پونه کوهی، آویشن و میخک (ریزپو شانی نشده و نانوریزپو شانی شده) در برابر *ا شرشیا کلی* ATCC 29922، *سالمونلا تیفی موریوم* ATCC 14028 و *ا ستافیلوکوکوس اورئوس* ATCC 25923 با استفاده از امولسیون سازی بررسی شد.

مواد و روش ها: اسانس‌های روغنی بر اساس خواص فیزیکوشیمیایی طبقه بندی شدند. نانوامولسیون‌های حاوی ۵ در صد وزنی/وزنی ا سانس روغنی تهیه و سپس بر ا ساس خواص فیزیکی، پایداری و کارایی ریزپو شانی طبقه بندی شدند. اثر ضد میکروبی با روش ریز رقت سازی برای تعیین حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی ا سانس‌های روغنی و نانو امولسیون‌های آنها بررسی شد. اطلاعات به د ست آمده در مورد خواص فیزیکی ا سانس‌های روغنی و خواص فیزیکی، پایداری و کارایی ریزپوشانی نانوامولسیون ها از نظر آماری مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: فعالیت ضد میکروبی اسانس‌های روغنی با کاهش حداقل غلظت مهارکنندگی تا ۶۰-۲۷ در صد برای ا سانس‌های نانوریزپو شانی شده، در مقایسه با انواع ریزپو شانی نشده نشان داده شد. ا سانس روغنی ریزپو شانی شده و ریزپو شانی نشده پونه کوهی پایین ترین حداقل غلظت مهارکنندگی و حداقل غلظت کشندگی را نشان داد. بر ا ساس نتایج به د ست آمده، ا سانس‌های روغنی ریزپو شانی شده برای جلوگیری از آلودگی میکروبی در مواد غذایی گوناگون می توانند مورد استفاده قرار گیرند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

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واژگان کلیدی

- ترکیبات ضدمیکروبی
- اسانس‌های روغنی
- نانوریزپوشانی
- میکروارگانیسم‌های بیماری‌زا

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