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β -Cyclodextrin inclusion complexes containing clove (*Eugenia caryophyllata*) and Mexican oregano (*Lippia berlandieri*) essential oils: Preparation, physicochemical and antimicrobial characterization

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ABSTRACT

Mexican oregano and clove essential oils are widely used as antimicrobial, insecticidal, antifungal and anti-oxidant compounds. To reduce their volatility and their hydrophobicity, they were encapsulated in β -cyclodextrin (β -CD) complexes by co-precipitation method, and the physicochemical and microbiological properties of these inclusion complexes were characterized. Different essential oil (EO) to β -CD weight ratios were tested (4:96, 8:92, 12:88, and 16:84 w/w) and encapsulation efficiency and rate were determined. GC-MS and GC-FID analysis were also performed to determine the main oil constituents, which were eugenol and carvacrol in clove and Mexican oregano essential oils respectively and to quantify their ratio in inclusion complexes. ¹H NMR spectroscopy confirmed essential oil inclusion. The 4:96 ratio (clove essential oil: β -CD) gave the highest eugenol content and greatest microencapsulation efficiency; and the 8:92 and 12:88 ratios (Mexican oregano essential oil: β -CD) the highest carvacrol content. Complexes antimicrobial activity was tested against two food-related microorganisms: *Listeria monocytogenes* ATCC 19114 and *Escherichia coli* ATCC 25922 by means of agar disk diffusion assay. The results demonstrated the inclusion of the majority of biologically active clove and Mexican oregano essential oil molecules, providing increased stability by reducing their volatility and preserving their biological properties.

1. Introduction

Synthetic antimicrobials have long been used as safe preservatives to control a variety of microbial risks. Nevertheless, these compounds are not entirely satisfactory for consumers seeking natural and healthy foods, and the replacement of synthetic products with more natural alternatives has received increasing interest in recent years. The essential oils obtained from spices are among these alternatives, as their active components (including terpenes, aldehydes, acids, alcohols, phenols, esters and ketones) are well known for their antimicrobial activity (Ortuño, 2006; Hu, Gerhard, Upadhyaya, Venkitanarayanan, & Luo, 2016). Nevertheless, they have limitations such as their volatility, oxidation, and easy degradation by light and temperature. EO of Mexican oregano (*Lippia berlandieri*) and clove (*Eugenia caryophyllata*) are some of the most effective antimicrobials, particularly against strains of *E. coli*; their main components are carvacrol, thymol, and eugenol respectively and their precursors. The properties of these EO, and particularly their antimicrobial

and antioxidant activities have been extensively studied (Burt, 2004; Arana-Sánchez et al., 2010; Guarda, Rubilar, Miltz, & Galotto, 2011), and some recent works deal with their encapsulation to improve their stability.

Indeed, microencapsulation is extensively used in pharmaceutical, food and cosmetic industries due to the advantages offered by this technology to protect active ingredients through the creation of a physical barrier between the nucleus and the shell materials against adverse reactions (lipid oxidation, degradation, evaporation, nutritional deterioration) and external medium (pH, humidity, light, temperature) (Xiao, Liu, Zhu, Zhou, & Niu, 2014). Molecular inclusion in cyclodextrin complexes has been demonstrated as a relevant method, particularly for volatile and hydrophobic molecules (Cevallos, Buera, & Elizalde, 2010).

Cyclodextrins are toroidal shape oligosaccharides, produced enzymatically from starch, with hydrophobic cavities and hydrophilic exterior, which allows a wide range of compounds to be encapsulated

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(Almenar, Auras, Rubino, & Harte, 2007; Del Valle, 2004; Vyas et al., 2008). During the formation of an inclusion complex, water molecules are displaced to the outside of the lipophilic cavity, on account of the presence of new lipophilic guest molecules that induces a new equilibrium. The extended use of cyclodextrins takes place in the pharmaceutical, cosmetics, food, chemical and several other industries and in the USA they belong to the GRAS (generally recognized as safe) list of FDA (Food and Drugs Administration) (Vyas et al., 2008; de Carvalho & Pinto, 2012). In particular, β -CD permits the encapsulation of mono and sesquiterpenes, main compounds of various EO (Astray, Gonzalez-Barreiro, Mejuto, Rial-Otero, & Simal-Gándara, 2009).

Recently, studies on oregano and clove EO encapsulation using β -CD have been reported (Arana-Sánchez et al., 2010; Guarda et al., 2011; Wang et al., 2011; Santos, Kamimura, Hill, & Gomes, 2015; Gong et al., 2016), demonstrating the inclusion of major component molecules (thymol, eugenol or carvacrol) in the β -CD cavities, the antimicrobial and antioxidant properties of the formed microparticles, and evaluating the active compounds release kinetics. This proves the continuing importance of studies about encapsulation of these molecules.

In this context, the aim of this work was to prepare inclusion complexes of these EO in β -CD by precipitation method, to characterize them and to confirm their antimicrobial activity. This antimicrobial product, which is easy to handle, with an improved stability and water solubility, could then be used as a natural food preservative, especially in the area of food-packaging.

2. Materials & methods

2.1. Materials

Clove (*Eugenia caryophyllata*) was purchased in Commercial Cardona, Chihuahua, Mexico, and clove EO was obtained by hydrodistillation. Essential oil of Mexican oregano (*Lippia berlandieri*) was acquired from CIRENA (Research Center for Natural Resources, Salta, Mexico). GC standards were purchased from Fluka Chemical Corp. β -cyclodextrin (Cavamax W7 food grade) was kindly provided by Wacker Biochem, (Mexico), Trypticase Soy Agar (TSA) and Mueller-Hinton agar by Bioxon (Mexico). All other reagents used were analytical grade.

2.2. Extraction of clove essential oil

A modified Schilcher apparatus was utilized in the hydrodistillation process. The flow rate was 10 to 15 mL/min. Cooling water was kept at 19 °C to prevent the higher molecular weight components from being stuck in the cooler. 200 g of clove was added to 4 L of water and introduced in a boiling flask. The system was heated to 100 °C for 5 h.

2.3. Preparation of EO: β -CD inclusion complexes

The precipitation method (Bhandari, D'Arc, & Thi Bich, 1998) was used to prepare inclusion complexes of clove essential oil: β -CD and Mexican oregano essential oil: β -CD. By magnetic stirring at 55 °C a sample of 10 g of β -CD was dissolved in 100 mL of an ethanol:water (1:2) mixture. The amounts of each essential oil ratio needed to obtain essential oils: β -CD weight ratios of 0:100, 4:96, 8:92, 12:88, and 16:84, respectively, were previously dissolved in ethanol (10% w/v) and then slowly added to the warm β -CD solution, maintaining stirring and warming. The final solution was covered and stirred for a further 4 h and kept overnight at 4 °C. The obtained complexes were recovered by filtration and then dried in a convection oven at 50 °C for 24 h. Subsequently, they were placed in a desiccator for 24 h. The samples were weighed to equilibrium, and stored at 25 °C. All the ratios were prepared in triplicate.

Process efficiency (PE), entrapment efficiency (EE) and drug loading (DL) were calculated using Eqs. (1)–(3).

$$PE = \frac{\text{Total weight of obtained inclusion complexes (mg)}}{\text{initial } \beta\text{CD weight (mg)} + \text{initial EO weight (mg)}} \times 100 \quad (1)$$

$$EE = \frac{\text{weight of entrapped EO (mg)}}{\text{initial EO weight (mg)}} \times 100 \quad (2)$$

$$DL = \frac{\text{weight of entrapped EO (mg)}}{\text{weight of obtained inclusion complexes (mg)}} \times 100 \quad (3)$$

Weight of entrapped EO was determined by GC-FID analysis.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis conditions

Identification of volatile components of clove and Mexican EO was carried out by gas chromatography coupled to a mass spectrometer (GC-MS) Perkin-Elmer Auto Systems XL. The capillary column selected was PE-5 (stationary phase methylphenyl silicone 5%), with dimensions of 60 m in length and 0.25 mm diameter nonpolar. The operating conditions for each of the two oils were as follows. Column temperature was raised from 55 to 120 °C at a rate of 8 °C/min, and held there for 1 min, and then the temperature was raised from 120 to 200 °C at a rate of 8 °C/min, and held at this final temperature for 9 min. Carrier gas was helium at a flow rate of 1 mL/min, and identification of the compounds was based on comparison of their mass spectra with the Saturn library and NIST 98 library data from GC-MS system.

2.5. Gas chromatography equipped with flame ionization detector (GC-FID) analysis conditions

Quantification was determined using a Varian 3900 chromatograph equipped with a FID detector (Varian) and a Varian CP-SII8CB capillary column (30 m \times 0.25 mm, film thickness 0.25 μ m) and one meter of precolumn. Column temperature was raised from 55 to 65 °C at a rate of 1 °C/min, and held there for 5 min, then the temperature was raised from 65 to 200 °C at a rate of 7 °C/min, and held there for 1 min, finally the temperature was raised from 200 °C to 290 °C at 10 °C/min and held there for 5 min. Carrier gas was helium at a flow rate of 1 mL/min, injector and detector temperatures were set at 220 and 290 °C, respectively, and 1 μ L of the extract was injected automatically. EO extraction from β -CD complexes was carried out by sonication (Ayala-Zavala et al., 2008). Each EO was dissolved separately in dichloromethane (500 mg/L). On the other hand, 20 mg of samples were dissolved in 2 mL of deionized water and 4 mL of dichloromethane was added. The mixture was sonicated for 30 min and the organic phase recovered. After repeating five times, the total volume of the organic phase was used for quantification of molecules. To quantify the major constituents of essential oils, standard calibration curves with carvone as internal standard were used.

2.6. Moisture determination

Moisture determination was carried out by drying a sample (3–4 g) of pure β -CD, and essential oil complexes in a vacuum oven (Shel Lab 1410, Oregon, USA) at 70 °C for 24 h and at < 6.7 kPa pressure. The percentage of moisture content was calculated by weight difference between the initial and the final samples.

2.7. ^1H NMR spectroscopy

^1H NMR spectra of both EO: β -CD complexes and free β -CD were performed with a Bruker FOURIER 300 spectrometer (Bruker Instruments Inc. USA.) operating at 300 MHz. Solutions were prepared using D₂O.

2.8. Moisture sorption-desorption isotherms

The sorption-desorption isotherms of pure β -CD and complexes of

both EO were determined by Dynamic Vapor Sorption (DVS), using a DVS Advantage automated vapor sorption analyzer (Surface Measurement Systems Ltd., London, U.K.), exposing the samples to different values of relative humidity (0, 15, 30, 45, 60 and 90%) at 24.8 °C, and following the methodology described by [Argyropoulos, Alex, Kohler and Müller \(2012\)](#).

2.9. Scanning electron microscopy (SEM)

The morphology of the complexes and β -CD was assessed by LEO435VP scanning electron microscope (LEO Electron microscopy Ltd., Cambridge, UK) operated at 8 kV. The samples were placed on conductive double-faced adhesive tape and sputter-coated with silver.

2.10. Antimicrobial activity

2.10.1. Microbial inoculum preparation

Two microorganisms were used due to their relationship with food: the Gram-positive *L. monocytogenes* ATCC 19114 and the Gram-negative *E. coli* ATCC 25922. These strains were obtained from the culture collection of the Faculty of Chemical Sciences (Autonomous University of Chihuahua, Mexico) and maintained on slants of Trypticase Soy Agar at 4 °C. The preparation of the inoculum consisted of a saline suspension from selected colonies isolated of TSA plates after 24 h incubation. Then, turbidity of the suspension was adjusted to the concentration of 1×10^8 CFU/mL using the 0.5 McFarland nephelometer standard.

2.10.2. Agar disk diffusion assay

Samples antimicrobial activity was performed by the agar disk diffusion method ([Gortzi, Lala, Chinou, & Tsaknis, 2007](#)). Treatments consisted of dissolving 0.5, 1 and 2 g of samples in 10 mL of ethyl alcohol, respectively, to obtain solutions with concentrations of 50, 100 and 200 mg/mL. These treatments were compared with the quantity equivalent of free EO contained in inclusion complexes. As controls, discs with ethyl alcohol and β -CD were used.

Subsequently, sterile blank filter discs of 5 mm diameter were dispensed over the surface of Mueller-Hinton agar petri dishes (previously seeded by spreading with the inoculum of each bacterium). Then, 4 μ L of each treatment were added. This was performed in triplicate. The plates were inverted and placed in an incubator at 35 °C over a period of 15 min after applying the disks. After 24 h of incubation, each plate was examined and the resulting inhibition zones were measured.

2.11. Statistical analysis

Data collected was analyzed by one-way ANOVA with a confidence level of 0.95 and Tukey-Kramer multiple mean comparisons. Data was analyzed using the MINITAB Release16 statistical package (Minitab Inc., USA).

3. Results and discussion

3.1. Composition and quantification of the essential oils

Gas chromatography (GC-MS) analysis was used to determine the EO composition, and the quantification of their main volatile compounds was performed by GC-FID analysis. Eugenol was the major volatile detected in the clove EO, with eugenyl acetate and β -caryophyllene in lower amounts. The main molecule detected in Mexican oregano EO was carvacrol, in addition to minor constituents such as p-cymene, β -caryophyllene and thymol. Both eugenol and carvacrol exhibit high antimicrobial and antioxidant activities and are approved food additives by the U.S. Food and Drug Administration ([Santos et al., 2015](#); [Gong et al., 2016](#)).

The contents of eugenol and carvacrol in clove and oregano OE respectively, were used as a model for evaluating the efficiency in the

Table 1

Total volatiles amount, process efficiency, entrapment efficiency and drug loading of inclusion complexes at various EO: β -CD ratios.

Proportion Essential oil: β -cyclodextrin (w/w%)	Process Efficiency (w/w%) ^a	Entrapment Efficiency (w/w%) ^a	Drug Loading (w/w%) ^a
Mexican Oregano: β -Cyclodextrin			
4:96	89.22 \pm 0.99 ^b	57.26 \pm 2.75 ^c	2.29 \pm 0.11 ^d
8:92	93.69 \pm 0.77 ^a	77.57 \pm 3.01 ^a	6.20 \pm 0.24 ^c
12:88	93.10 \pm 1.24 ^a	65.55 \pm 2.06 ^b	7.86 \pm 0.24 ^a
16:84	88.71 \pm 0.33 ^b	46.99 \pm 0.63 ^d	7.51 \pm 0.10 ^b
Clove: β -Cyclodextrin			
4:96	88.10 \pm 1.11 ^c	63.63 \pm 8.60 ^a	2.54 \pm 0.34 ^c
8:92	94.98 \pm 0.92 ^a	50.62 \pm 1.81 ^b	4.05 \pm 0.14 ^b
12:88	92.17 \pm 0.38 ^b	38.01 \pm 1.76 ^c	4.56 \pm 0.21 ^a
16:84	88.67 \pm 0.42 ^c	30.12 \pm 1.34 ^d	4.82 \pm 0.21 ^a

^{a,b,c}Values with different superscript letters in the same column (each essential oil) were significantly different $P < 0.05$ (Tukey-Kramer multiple mean comparisons).

^{*} Values given are averages of three replicate samples.

inclusion process ([Table 2](#)). Overall, the most efficient ratios, combining the highest entrapment efficiency ([Table 1](#)) and a high level of eugenol (clove EO) or carvacrol (Mexican oregano EO) ([Table 2](#)) were 4:96 for the clove EO and 8:92 for the Mexican EO. These ratios were used to characterize the inclusion complexes by ¹H NMR spectroscopy, SEM and moisture sorption isotherms.

3.2. Characterization of EO: β -CD inclusion complexes

Process efficiency (PE), entrapment efficiency (EE) and drug loading (DL) of inclusion complexes are given in [Table 1](#). PE (%) was significantly higher ($P < 0.05$) for the 12:88 and 8:92 ratios compared to the 4:96 and 16:84 for both EO: β -CD complexes. On the other hand, it is important to emphasize that increasing the amount of essential oil reduced the EE, probably because of the saturation of the matrix, as suggested by [Ayala-Zavala et al. \(2008\)](#). EE values showed that optimum EO: β -CD ratios at 8:92 for Mexican oregano EO (71%) and 4:96 for clove EO (61%). These values are on the same range and even slightly higher than those obtained by [Abarca, Rodriguez, Guarda, Galotto, and Bruna \(2016\)](#), with the same method of inclusion.

For both essential oils, the maximal DL value was reached for the 12:88 ratio, as lower values were obtained for the 16:84 ratio suggesting that the maximum essential oil inclusion was reached ([Del Toro-Sánchez et al., 2010](#)). Mexican oregano EO gave higher EE, allowing a

Table 2

Recovery of carvacrol and eugenol at various EO: β -CD ratios.

Proportion Essential oil: β -cyclodextrins (w/w%)	Relative major volatile of all volatiles ^a (w/w%)	Ratio of major volatile in inclusion complexes (w/w%) ^a
Mexican oregano: β -cyclodextrin		
4:96	66.13 \pm 5.39 ^c	1.51 \pm 0.07 ^d
8:92	71.22 \pm 0.80 ^{ab}	4.42 \pm 0.18 ^c
12:88	73.63 \pm 0.93 ^a	5.79 \pm 0.14 ^a
16:84	68.78 \pm 0.72 ^{bc}	5.17 \pm 0.05 ^b
Clove: β -cyclodextrin		
4:96	97.61 \pm 0.61 ^a	2.49 \pm 0.35 ^c
8:92	97.94 \pm 0.35 ^a	3.97 \pm 0.14 ^b
12:88	97.27 \pm 0.28 ^a	4.44 \pm 0.20 ^a
16:84	96.32 \pm 0.88 ^b	4.64 \pm 0.24 ^a

^{a,b,c}Values with different superscript letters in the same column (each essential oil) were significantly different $P < 0.05$ (Tukey-Kramer multiple mean comparisons).

^{*} Values given are averages of three replicate samples.

maximum DL around 7%, concordant with the values obtained by Kamimura, Santos, Hill and Gomes (2014) who obtained carvacrol DL around 7–8% in hydroxypropyl-beta-cyclodextrin.

The difference in DL observed between the two EO (7% for Mexican oregano EO and less than 5% for clove EO) could be attributed mainly to the structural differences between the two major components of the EO: carvacrol for Mexican oregano EO and eugenol for clove EO. To a greater degree than their molecular weight, the difference on their structural conformation, and particularly the more linear structure of eugenol, could involve less favourable interactions with β -CD as described by Lawtrakul, Inthajak, and Toochinda (2014).

3.3. ^1H NMR spectroscopy

The NMR technique was successfully used to study the structural properties of β -CD complexes, particularly in solution. Chemical-shift changes in the ^1H NMR spectra were used to confirm the complex formation process, the most significant variations usually being observed for the H-atoms located within the β -CD cavity. The induced shift ($\Delta\delta$) refers to the difference in chemical shifts in the presence or absence of the other reagents, calculated using the following equation:

$$\Delta\delta = \delta(\text{complex}) - \delta(\text{free}) \quad (4)$$

The positive and negative signs showed downfield and upfield shifts, respectively.

The ^1H chemical shifts of free and complexed β -CD prepared with clove EO (4:96) and Mexican oregano EO (8:92) are shown in Table 3. H-3 and H-5 protons, situated inside the cavity, and the H-6 proton, were shifted upfield, with values of $\Delta\delta = -0.03$, -0.04 and -0.02 respectively (clove EO: β -CD complexes), and values of $\Delta\delta = -0.03$, -0.8 and -0.05 for inclusion complexes of Mexican oregano EO, due to the insertion of the aromatic ring of EO molecules (mainly eugenol and carvacrol) inside the β -CD lipophilic cavity, and in accordance with the literature (Locci, Lai, Piras, Marongiu, & Lai, 2004; Terekhova, Kumeev, & Alper, 2007; Gong et al., 2016).

On the other hand, in the clove EO: β -CD complexes, protons located on the exterior of the cavity (H-1 and H-4) were comparatively unaffected, as was H-2. Concerning Mexican oregano EO: β -CD complexes, chemical shifts of H-1 and H-4 protons were equal but not pronounced, and the H-2 proton was unaffected. The chemical shift results for free and complexed β -CD protons demonstrated that guest molecules of both EO were included inside the cavity through hydrophobic interactions, confirming the formation of inclusion compounds between volatile molecules of clove Mexican oregano EO with the β -CD molecule.

3.4. Moisture sorption isotherms

The sorption and desorption isotherms of β -CD and clove EO: β -CD complexes (4:96 weight ratio) and Mexican oregano EO: β -CD complexes (8:92 weight ratio) at 24.8 °C are shown in Fig. 1. Moisture sorption of β -CD molecules showed an increase in water absorption

beginning at 45% RH up to 60% RH which is the highest moisture absorption and which stayed constant until 90% RH (about 0.17 mg of water absorbed per mg of β -CD). These readings agreed with reports of water content of β -CD of about 14–15% from other research (Ayala-Zavala et al., 2008; Del Toro-Sánchez et al., 2010). Moreover, sorption isotherms of inclusion complexes from both EO showed lower water absorption than the free β -CD, which was more marked in the case of Mexican oregano EO: β -CD complexes, confirming that increasing the relative humidity also increases water absorption. The samples attained 0.11 and 0.12 mg of water absorbed per mg of both EO: β -CD complexes, respectively, at 90% RH, which was less than for the β -CD molecule. The isotherms correspond to type II and, as reported by Suihko et al. (2001), are characteristic of hydrophilic polymers having both surface adsorption and also absorption in the solid phase. The decrease of the water absorption capacity of β -CD in the inclusion complexes can be explained by interactions with guest molecules, resulting in lower values of moisture sorption in the complexes formed (Fig. 2).

3.5. Morphology

Analyses of both inclusion complexes and β -CD surface morphologies were made by SEM. Fig. 3 shows free β -CD with various sizes of rectangular shaped crystals. Furthermore small particles on the surface of the crystals can also be seen, a fact mentioned by other authors (Songkro et al., 2012). As can be seen from the micrographs, the morphology of β -CD had completely changed after forming inclusion complexes with EO of clove (Fig. 3b) and Mexican oregano (Fig. 3c). The samples appeared as rhomboid shaped crystals, and for Mexican oregano EO: β -CD complexes, small aggregates of irregular size amorphous pieces were present. The complexes showed no visible fractures, cracks or pores, indicating good protection and adequate protection of EO.

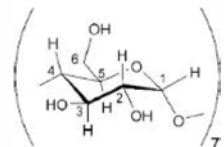
3.6. Antimicrobial activity

Zones of inhibition (in millimeters) obtained from the different amounts of free EO and corresponding complexes are presented in Table 4 and Fig. 3. Concerning free EO, both Mexican oregano and clove EO demonstrated their antimicrobial activities against *L. monocytogenes* ATCC 19114 and *E. coli* ATCC 25922. According to the statistical analysis, no significant difference was observed as a function of the EO amount. Mexican oregano EO seemed to be more efficient than clove EO, with larger inhibition zones, for both microorganisms.

This antimicrobial activity was preserved with the inclusion of these EO in β -CD, as confirmed by the results given in Table 4, for 0.5 g and 1 g of complexes, with similar values than those of free EO. Lower effectiveness of treatment of 2 g of samples against two microorganisms was observed, and could be attributed to a too high amount of complexes in a small volume, involving a supersaturation of the solution preventing the complete dissolution of the complexes and thus the release of the EO.

Table 3

^1H NMR chemical shifts (δ) of the free β -CD and EO: β -CD complexes in D_2O . $\Delta\delta$ represents the chemical shift differences (ppm) between the two states (free or complexed β -CD).

	H-atom	δ /ppm Free β -CD	Clove EO: β -CD		Mexican oregano EO: β -CD	
			δ (ppm)	$\Delta\delta$ (complexed-free) (ppm)	δ (ppm)	$\Delta\delta$ (complexed-free) (ppm)
	H-1	5.04	5.03	-0.01	5.02	-0.02
	H-2	3.55	3.55	0	3.55	0
	H-3	3.93	3.89	-0.04	3.88	-0.05
	H-4	6.62	3.61	-0.01	3.60	-0.02
	H-5	3.82	3.76	-0.06	3.7	-0.12
	H-6	3.85	3.82	-0.03	3.79	-0.06

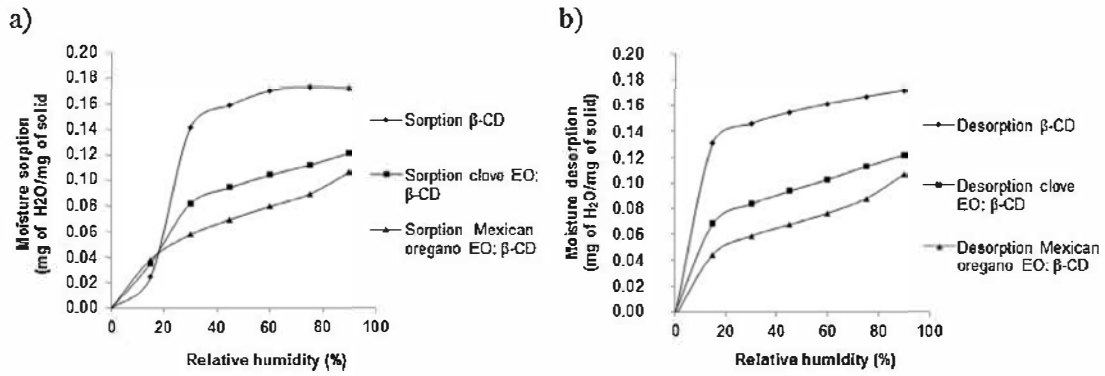


Fig. 1. Moisture sorption (a) and desorption (b) isotherms of free β -CD, clove EO: β -CD complexes (4:96) and Mexican oregano EO: β -CD complexes (8:92).

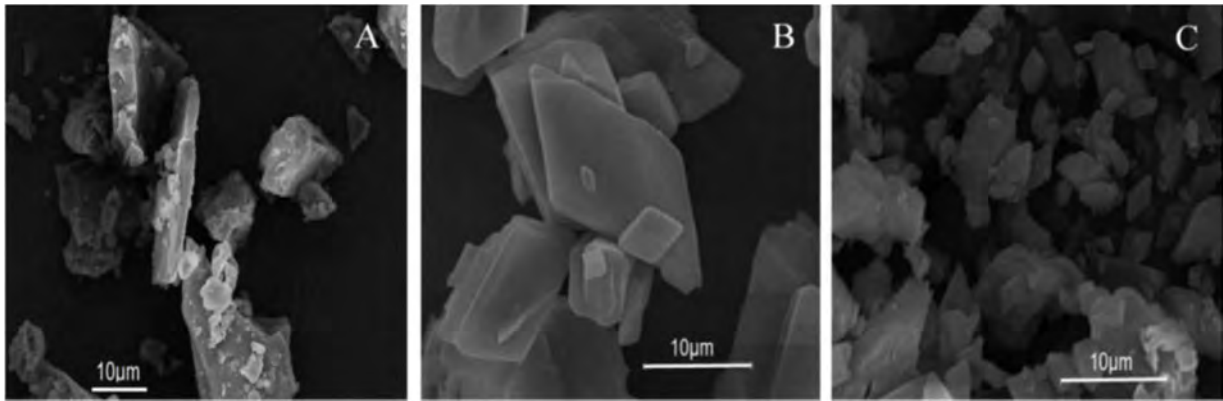


Fig. 2. Scanning electron micrographs of (A) free β -CD, magnification $\times 1500$; (B) clove EO: β -CD complexes, magnification $\times 5000$; and (C) Mexican oregano EO: β -CD complexes, magnification $\times 5000$.

These results confirm that the biological activity of both Mexican oregano and clove EO was preserved after inclusion process, with no particular alteration from β -CD. Previous works with other encapsulation processes such as spray drying (Arana-Sánchez et al., 2010), lyophilization (Wang, Lu, Lv, & Bie, 2009) or freeze-drying (Santos et al., 2015) have also proved that encapsulation did not alter the antioxidant nor the antimicrobial activities of such EO, and even

could improve them, due to the enhanced water solubility of the complexes.

4. Conclusion

Inclusion of clove and Mexican oregano EO in β -CD was successfully conducted with good entrapment efficiency of their major compounds,

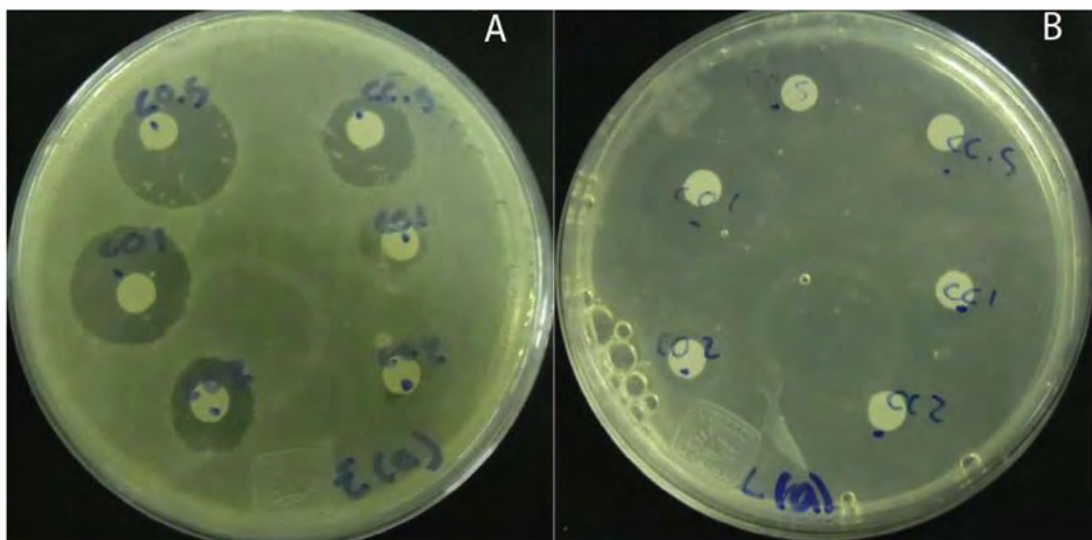


Fig. 3. Antimicrobial activities of EO: β -CD complexes (with 4:96 ratio for Clove EO: β -CD complexes and 8:92 ratio for Mexican oregano EO: β -CD complexes) against *E. coli* ATCC 25922 (A) and against *L. monocytogenes* ATCC 19114 (B).

Table 4Antimicrobial activities (measured as inhibition zone in mm) of free EO and EO:β-CD complexes against *L. monocytogenes* ATCC 19114 and *E. coli* ATCC 25922.

		Mexican oregano EO			Mexican oregano EO: β-CD (8:92)		
sample weight		0.031 g	0.062 g	0.125 g	0.5 g	1 g	2 g
<i>L. monocytogenes</i> ATCC 19114	mm of inhibition [*]	6.00 ± 2.00 ^a	7.17 ± 0.76 ^a	7.33 ± 1.15 ^a	5.00 ± 1.00 ^{ab}	5.67 ± 2.08 ^{ab}	2.00 ± 1.00 ^b
	<i>E. coli</i> ATCC 25922	4.67 ± 1.15 ^a	5.00 ± 1.00 ^a	5.67 ± 0.58 ^a	6.50 ± 3.97 ^a	4.67 ± 2.08 ^a	3.33 ± 0.58 ^a
		Clove EO			Clove EO: β-CD (4:96)		
sample weight		0.012 g	0.0245 g	0.049 g	0.5 g	1 g	2 g
<i>L. monocytogenes</i> ATCC 19114	mm of inhibition [*]	3.33 ± 0.58 ^a	4.00 ± 0.00 ^a	4.67 ± 0.58 ^a	4.00 ± 2.00 ^a	0.83 ± 0.29 ^b	0.50 ± 0.00 ^b
	<i>E. coli</i> ATCC 25922	3.00 ± 0.00 ^{ab}	3.67 ± 0.58 ^a	4.67 ± 0.58 ^a	4.33 ± 2.31 ^a	2.67 ± 1.15 ^{ab}	0.50 ± 0.00 ^b

^{a,b,c}Superscripts with the same letters in the same line were not significantly different $P > 0.5$ (Tukey-Kramer multiple mean comparisons).^{*} Values given are averages of three replicate samples.

eugenol and carvacrol respectively, as confirmed by GC–MS, GC-FID and ¹H NMR spectroscopy. Inclusion complexes of both EO were able to inhibit *L. monocytogenes* ATCC 19114 and *E. coli* ATCC 25922, demonstrating that β-CD inclusion process could an important option to increase and facilitate future applications of such EO, particularly as natural food additives, offering more stability, facilitating its handling and increasing their solubility in aqueous media.

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