Journal of Medicinal Plants and By-products (2019) 2: 115-123

Original Article

Antiradical, Antibacterial and Oxidative Stability of Cinnamon Leaf Oil Encapsulated in β-cyclodextrin

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Article History: Received: 03 October 2018 /Accepted in revised form: 08 June 2019

Abstract

Safety and quality of food have become a challenge for the food industry looking to replace synthetic preservatives with natural agents. In this context, cinnamon leaf essential oil (CLO) showed to be potent antioxidant and antimicrobial agent; however, its active compounds are highly reactive volatiles and grant strong odors and flavors when used as food additive. With this in mind, the objective of this study was to evaluate the antiradical and antimicrobial activity, and oxidative stability of CLO encapsulated within β-cyclodextrin (β-CD). Radical scavenging activity of encapsulated CLO measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and trolox equivalents antioxidant capacity (TEAC) methods showed values of 67.3 mol (Trolox equivalent) TE/g and 177.2 mol TE/g of sample, respectively. In addition, the total phenol and flavonoid contents were 48.7 mg (eugenol equivalents) EE/g of capsules and 8.6 mg CE/g of capsules, respectively. The antibacterial activity of the encapsulated oil against *Escherichia coli*, *Salmonella enterica* subsp. *enterica* serovar Choleraesuis, *Listeria monocytogenes* and *Staphylococcus aureus* showed a minimum inhibitory concentration of 5.5 mg/mL for all the tested bacteria. β-CD protected the bioactive properties of the essential oil exposed to ozone, compared to the free oil. Based on these results, the encapsulation of CLO in β-CD can be considered as a viable method for stabilizing its bioactive compounds.

Keywords: Encapsulation, Natural products, *Cinnamomum zeylanicum*, Eugenol

Introduction

Consumers are demanding high quality, more organic food, with longer lifetimes, with the absence of synthetic additives [1]. However, the microbiological quality of foods is particularly critical given their exposure during production, processing and storage, which can cause contamination by spoilage and pathogenic bacteria, fungi and yeast [2]. Additionally, oxidation caused by free radicals promotes the loss of quality of food products in many levels, producing off-flavor compounds, color changes, and affecting the nutritional quality [3]. It is known that chemical additives are highly effective in controlling postharvest diseases in several fruits and vegetables [4]. However, due to health problems and environmental risks mainly, these additives are not the "solution" that consumers would be willing to accept [5]. Therefore, it is necessary alternative methods to control deterioration and prolong shelf life.

Cinnamon leaf (*Cinnamomum zeylanicum* Blume) can be used to extract a very active antimicrobial and antioxidant essential oil with potential uses as food additive [6]. The main component of cinnamon leaf essential oil (CLO) is eugenol accounting for 80% of the total composition [5]. To test the antioxidant capacity, Melgarejo *et al*., [7]

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applied CLO vapors to grapes observing an increment in antioxidant activity and flavonoid content. Du *et al*., [8] applied edible films containing essential oils of cinnamon, clove, and allspice on apples inoculated with *Escherichia coli*, *Salmonella enterica*, or *Listeria monocytogenes*, where cinnamon had the highest activity against the tested bacteria. However, negative sensorial changes were observed [9] as well as the volatility of the oils compounds complicating their addition in their liquid form [10]. In one study, applying chitosan coatings and oregano essential oil (2.5 and 5 µL/mL) to grapes produced an antifungal effect; however, it was not sensorially acceptable [11]. As it can be observed, the application of essential oils in their free liquid form presents disadvantages as volatility and reactivity of the active components; as an alternative to solve these problems, this study proposed the encapsulation.

Recent studies showed that encapsulation is one of the most effective methods to mask the strongest odors and flavors of essential oils when are applied in food systems. Some authors such as Ayala-Zavala *et al*. [12] reported an effective encapsulation of CLO in β-CD using the precipitation method with a total volatile load of 12.76%. On the other hand, Hill *et al*. [13] reported an entrapment efficiency of 41.72% of cinnamon bark extract successfully encapsulated in β-CD using a freeze-drying method. In addition, the encapsulation could protect the active compounds against oxidation, heat degradation, evaporation, and UV irradiation, thus providing a viable approach to its use as a food preservative [14]. However, studies about their stability and preservation of CLO properties have not been proposed. Considering the stated problematic, the present study evaluated the effect of the β-CD encapsulation on the antiradical, antibacterial and oxidative stability of CLO during ozone exposure.

Material and Methods

Reagents

CLO used was of food grade and acquired by Sigma-Aldrich Co. and β-CD was kindly provided by Wacker Biochem, (Mexico). All other reagents used were of analytical grade.

Encapsulation Process

CLO capsules were prepared with a ratio of 16:84 (essential oil:β-CD) using the precipitation method, which was chosen based on previous studies that demonstrate that this conditions trapped major of the oil compounds and showed an effective encapsulation evidenced by infrared analysis [12]. Capsules were prepared according to the precipitation method, where a portion of 50 g of β-CD was dissolved in 500 mL of ethanol:water (1:2) at 55 °C with stirring. Subsequently, 9.54 g of CLO dissolved in ethanol (10%) was slowly added to the aqueous β-CD solution. During the essential oil addition, the β-CD solution was constantly stirred and maintained at 55 °C. Afterward, the solution was removed from heating and stirred for 4 h at room temperature. The final solution was stored for 24 h at 4 °C, forming a precipitate which was recovered by filtration and dried in a convection oven at 50 °C for 24 h. The capsules were removed from the oven and stored in desiccators at 25 °C until used.

Antioxidant Properties of CLO Capsules

CLO capsules (1 g) were homogenized with 15 mL of 80% methanol in a homogenizer (Ultra-Turrax T25) for 3 min. Then, the sample was sonicated for 30 min at 10 °C and subsequently centrifuged at 1200 x*g* for 15 min at 4 °C in a refrigerated centrifuge (Beckman Coulter). The supernatant was filtered and the residue was suspended in 10 mL of 80% methanol, sonicated, centrifuged and filtered (this was done three times in total). The supernatant was adjusted to a volume of 40 mL with 80% methanol and subsequently, the total phenol and flavonoid contentsas well as their antiradical activity were evaluated, using free CLO as the positive control.

The total phenolic content was determined according to Folin-Ciocalteu method [15] with some modifications. Seventy five µL of Folin-Ciocalteau reagent [1:10], 15 µL of capsule extract or free CLO and 60 µL of 7.5% Na_2CO_3 were added in a microplate (COSTAR 96 half media) by triplicate, allowed to stand in the dark for 30 min and absorbance was measured at 748 nm in a microplate reader (FLUOstar Omega, BMG Labtech). A calibration curve of eugenol was prepared and results were expressed as mg equivalents of eugenol per gram of sample (mg EE/g of sample).

Total flavonoids were measured by the method described by Zhishen and col. [16] with some modifications. In 2 mL vials, 100 µL of capsule extract or free CLO were mixed with 430 µL of

mixture A (1.8 mL of 5% $NaNO_2$ in 24 mL of distilled water) and allowed to stand for 5 min. Subsequently, 30 µL of 10% $AlCl_3$ were added and allowed to stand for 1 min. Finally, 440 µL was added to the mixture B (12 mL of 1M NaOH with 14.4 mL of distilled water). From this reaction, 150 µL were taken by triplicate, placed into a microplate (COSTAR 96 half media) and measured at 496 nm on a microplate reader. A calibration curve of catechin was prepared and the results were expressed as mg of catechin equivalents per gram of sample (mg CE/g of sample).

The capacity of the extracts to inactivate the 2, 2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical was calculated according to the method proposed by González-Aguilar *et al*. [17]. DPPH radical was prepared to dilute 1.97 mg of DPPH radical with 50 mL of pure methanol and adjusted to 0.7 absorbance at 518 nm. For the reaction, 10 µL of thesample extract or free CLO were placed on a microplate and then 140 µL of adjusted DPPH radical was added. The microplate was allowed to stand in darkness for 30 min and the absorbance was read in a microplate reader at 518 nm. The percent of radical inhibition were obtained using the following formula: *% Inhibition= [(initial absorbance-final absorbance)/initial absorbance]* x *100*; where: *initial absorbance* is the absorbance of the adjusted radical and *final absorbance* is the absorbance of radical with the sample. The percentage inhibition was related with a standard curve of Trolox, and the results were expressed as µmol of Trolox equivalents per gram of sample (µmol TE/g of sample).

The ability of the CLO capsules extracts and free CLO to inactivate the free radical $ABTS^{\bullet+}$ (2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) was also evaluated. The radical cation $ABTS^{\bullet+}$ was prepared according to the method described by Re *et al*. [18], weighed 19.3 mg and dissolved in 5 mL of distilled water. On the other hand, 37.8 mg of $K_2S_2O_8$ were dissolved in 1 mL of distilled water and 88 µL of this solution were added to five mL of $ABTS^{\bullet+}$ radical solution. This mixture was allowed to stand in darkness at room temperature for 16 h. After wards, the radical was adjusted to 0.7 absorbance by adding pure ethanol, reading at 754 nm. Subsequently, 5 µL of sample and 245 µL of the radical were added to a microplate by triplicate and allowed to stand in darkness for 5 min and the absorbance was read in a microplate reader at 754 nm. The percent of radical inhibition were obtained using the following formula: *% Inhibition= [(initial absorbance-final absorbance)/initial absorbance]* x *100*; where *initial absorbance* is the absorbance of the adjusted radical and *final absorbance* is the absorbance of radical with the sample. The percentage inhibition was related with a standard curve of Trolox, and the results were expressed as µmol of Trolox equivalents per gram of sample (µmol TE/g of sample).

Antibacterial Activity of the CLO Capsules

The antibacterial activity of encapsulated and free CLO was tested against *Escherichia coli* 0157:H7 (ATCC 43890), *Salmonellaenterica* subsp. *enterica* serovarCholeraesuis (ATCC 14028), *Listeria monocytogenes* (ATCC 7644) and *Staphylococcus aureus* (ATCC 65384). Each inoculum was transferred (~20 µL) to tubes containing 10 mL of trypticase soy broth and incubated at 37 °C for 24 h. After this, inoculums were adjusted to a concentration of $1x10^6$ colony forming units per mL (CFU/mL) [19]. Subsequently, 5 µL of inoculum and 295 µL of tryptic soy broth containing different concentrations of the capsules were placed on a microplate by triplicate. The plate was placed inside a microplate reader (FLUOstar Omega, BMG Labtech), defining the incubator at 37 °C. The absorbance was set to read mode at 600 nm with orbital shaking for 30 s before each reading. This was programmed to carry 48 individual measurements for a period of 24 h [20]. The minimal inhibitory concentration (MIC) and time sensibility curves were recorded. Experimental growth data for each bacterial strain were fitted to the Baranyi function [21] by using a complementary tool for Microsoft Excel (D-model, J. Baranyi, Institute of Food Research, Norwich, UK). Kinetic parameters such as phase lag time (h), specific growth rate (µmax) and the maximum growth in the stationary phase (Ymax), for each growth curve was calculated from the values generated by the Baranyi function. The coefficient of determination (R^2) which indicates the goodness of the fit was also calculated.

Oxidative Stability of CLO Capsules During Exposure to Ozone

Aqueous mixtures of free oil and capsules were prepared and bubbled with ozone gas to evaluate the protective effect of encapsulation. CLO and CLO capsules were mixed with distilled water at concentration of 0.1 mg/mL, respectively. Each mixture was treated by bubbling with ozone [22],

by using an ozone generator reactor mark (G11-G24 Ozone Generator). Operation settings of this generator were as follow: fed stream oxygen of 5 psi, ozone gas came directly by a hose (5 mm diameter) with a flow of 20 L/min, which was bubbled directly into each sample. The mixtures were subjected to 5, 7.5, 10, 12.5 and 15 min taking a control for both aqueous mixtures. Thereafter, the antioxidant capacity and eugenol content for each sample was determined as described before, this experiment was replicated 3 times, and results are the mean of this replicates.

Molecular Docking of Eugenol β-cyclodextrin

Chemical structure of β-cyclodextrin (PubChem ID: 444041) was used as capsule model with eugenol (PubChem ID: 3314) to simulate molecular interactions in the complex. The docking analysis was carried out using AutoDoc Vina application to obtain affinity energy (kcal mol−1) and root-mean-square deviations (RMSD) between β-cyclodextrin and eugenol using UCSF Chimera version 11.2 software to identify amino acids involved with the obtained model interactions [30].

Statistical Analysis

A completely randomized design was performed to evaluate the effect of the encapsulation on the antiradical, antibacterial activity and oxidative stability of the CLO and CLO capsules exposed to ozone. An analysis of variance (ANOVA) was used to estimate significant differences (P≤0.05) between treatments and Tukey-Kramer test was used for means comparison, using NCSS (2007) software.

Results and Discussion

Total Phenols, Flavonoids and Antiradical Activity of the Encapsulated Oil

Fig. 1A and B shows the content of total phenols and flavonoids present in the free and β-CD encapsulated oil. Significant differences between treatments (P≤0.05) on phenolic compounds were found. Free CLO showed the highest content of total phenols and flavonoids with values of 802.21 mg EE/g and 166.34 mg CE/g, respectively. Even when the encapsulated oil presented lower values, it is important to highlight the presence of these bioactive compounds, giving results of 48.77 mg EE/g and 8.65 mg CE/g for total phenols and flavonoids, respectively.

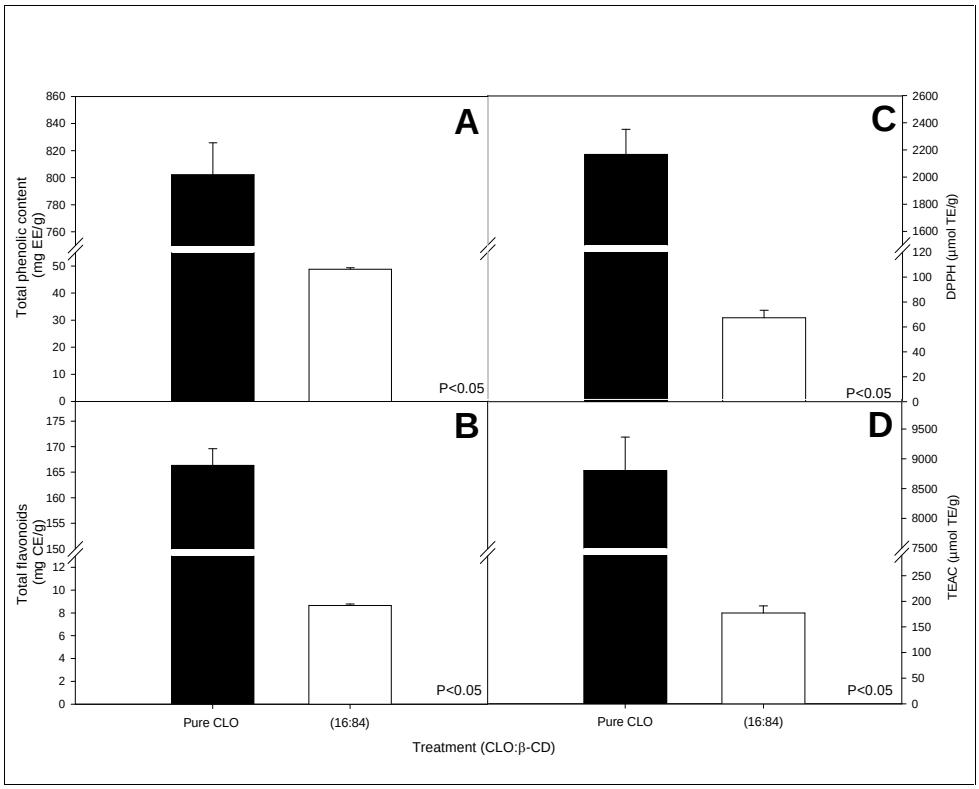

Fig. 1 Content of total phenols, flavonoids and antioxidant capacity (DPPH and TEAC) of free and encapsulated CLO.

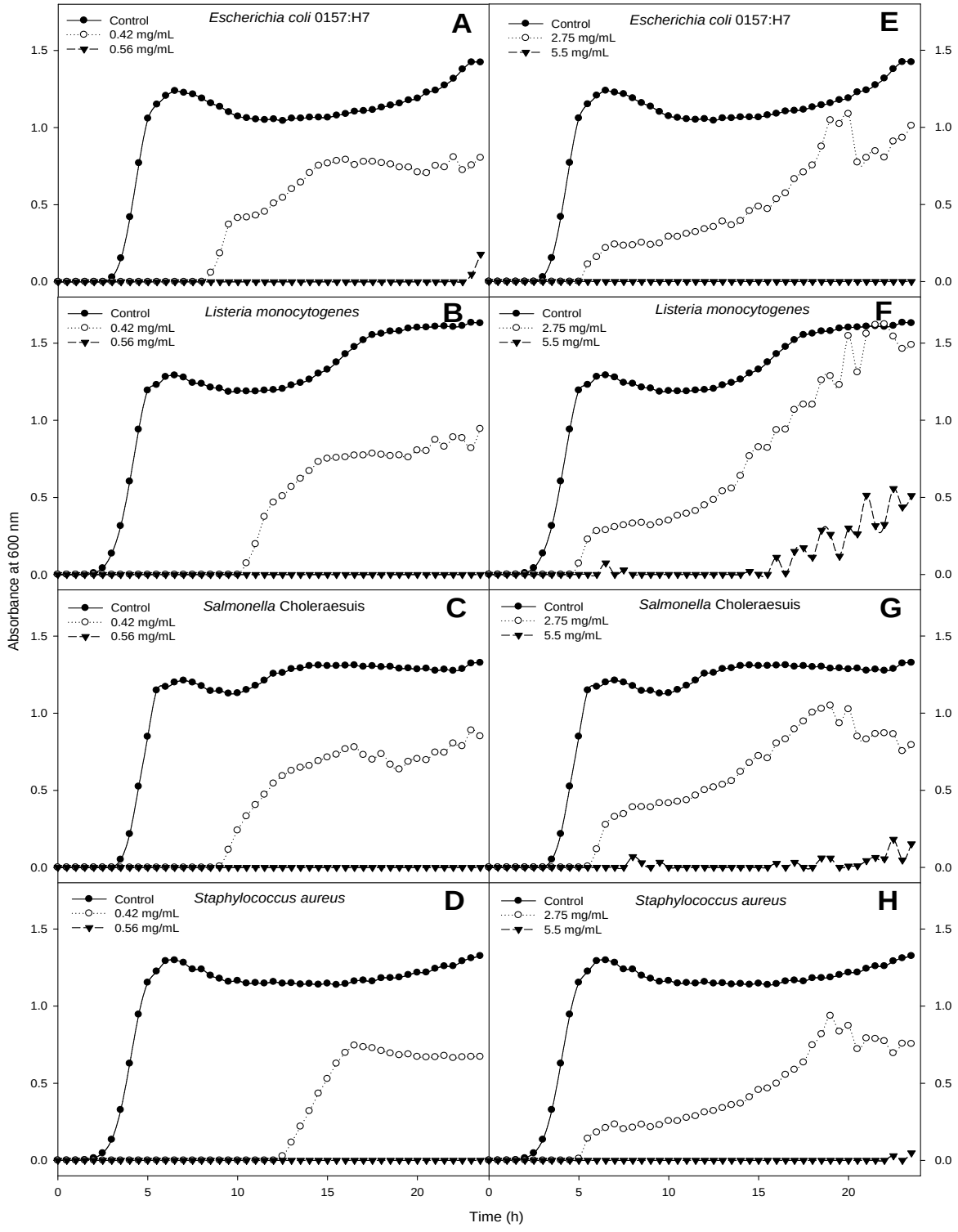

Fig. 2 Effect of free and encapsulated CLO concentration on growth parameters of Escherichia coli 0157: H7, Listeria monocytogenes, Salmonella Choleraesuis and Staphylococcus aureus.

The composition (GC-MS) of trapped volatiles in this system was already published by our research group, finding: cinnamaldehyde, eugenol, copaene and β-caryophyllene; as well as hydrogen bonding interactions were found among CLO components and β-CD [12]. In addition, the antiradical activity of the free and encapsulated CLO against DPPH and ABTS radicals is shown in Fig. 1C and D. It

may be noted that a significant effect was found ($P \leq 0.05$) between the two treatments. Free CLO showed the highest antiradical capacity against DPPH and TEAC radicals with 2166.38 µmol TE/g and 8802.57 µmol TE/g, respectively. Meanwhile, the radical scavenging activity of the encapsulated oil by DPPH and TEAC were 67.36 µmol TE/g and 177.19 µmol TE/g, respectively.

Brewer [23] reported that spices and herbs including cinnamon containing high concentrations of phenolic compounds which are assumed as responsible for its high antioxidant capacity. Some studies about the encapsulation of CLO in β-CD using ratios of 4:96, 8:92, 12:88 and 16:84, showed that 16:84 ratio contains the higher content of phenolic compounds [24]. Gas chromatography studies have shown that eugenol is the major constituent of CLO, constituting about 78% of total volatiles and the 5% after microencapsulation process [12]. These values are similar to those obtained in this study where the percentage of eugenol in free and encapsulated CLO represented the 80.22 and 4.87%, respectively. On the other hand, Tajkarimi [25] reported that the major components of the essential oils can constitute up to 85% of the total. Similarly, Bhandari *et al*. [26] conducted a study of encapsulation of lemon essential oil in β-CD and they reported that the product was similar to the original lemon oil in the proportions of major aromatic volatiles. However, molecular inclusion depends on the physicochemical properties of the compounds to be trapped, occurring a competitive inclusion among the oil constituents.

Antibacterial Capacity of the CLO Free and Encapsulated

The MIC of free and encapsulated CLO for all tested bacteria were 0.5 and 5.5 mg/mL, respectively. Comparing the MIC values for free and encapsulated CLO, and taking into account the eugenol content previously reported for these systems [12], that are 78.3 and 5% of eugenol for free and encapsulated CLO, respectively. Meaning that 0.5 mg/mL of free CLO were needed it to inhibit the inoculum of the tested bacteria, containing 0.39 mg of eugenol/mL. On the other hand, encapsulated CLO showed a MIC value of 5 mg/mL containing 0.27 mg of eugenol/mL. Therefore, the antibacterial activity of CLO was improved after encapsulation with β-CD; needing 0.12 mg/mL more of free CLO than the encapsulated to achieve the same effect. In order to see the effect of the oil on the bacteria growth parameters, we used a concentration below the MIC, which was 2.75 mg/mL, corresponding to 1/2 MIC. Free and encapsulated CLO were capable of affecting the growth parameters of all tested bacteria (Fig. 2), reflected in an extension of the λ and a reduction of the μ_{max} and Y_{max}.

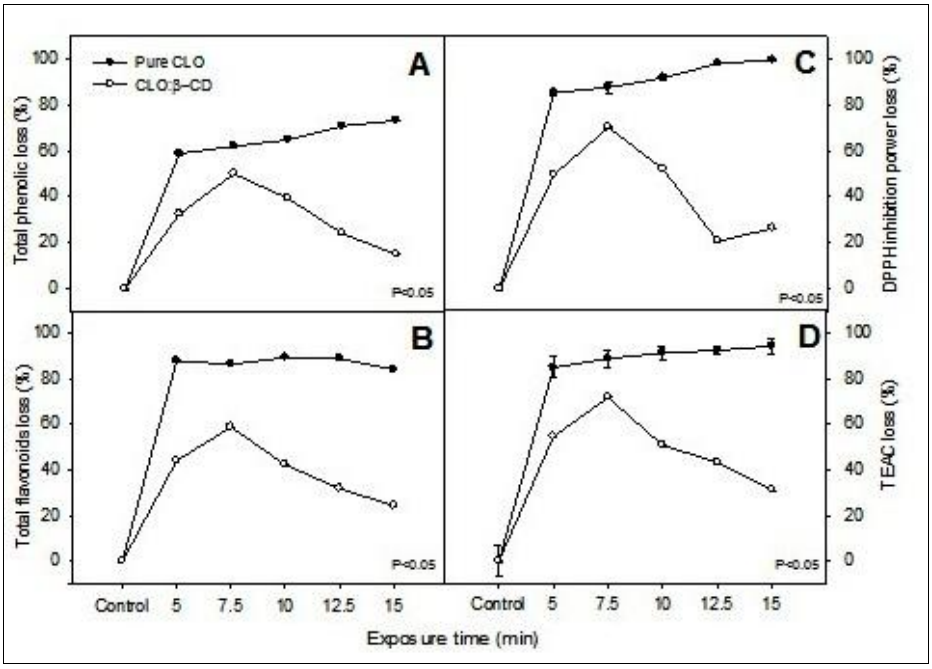

Fig. 3 Loss of total phenols, flavonoids and antioxidant capacity (DPPH and TEAC), of CLO free and encapsulated within β-CD and exposed to ozone during 15 min

For *E.coli*, it was observed an extension of the lag phase (24 vs 13.22 h), decrement of µmax (0 vs. 0.11 h^{-1}) and Ymax (0 vs. 91) were observed, respectively. *L. monocytogenes* presented an extended phase lag (15.59 vs 9.82 h), decreasing µmax (0.06 vs 0.1 h^{-1}) and Ymax (0.4 vs 0.9) when treated with the higher concentration. Similarly, *S.* Choleraesuis exposed to the higher concentration of capsules oil presented a length phase lag (21.10 vs 6.43 h), decreased µmax (0.04 vs 0.07 h^{-1}) and Ymax (0.15 vs 0.90). In the case of *S.aureus* phase lag (22.9 vs 12.38 h), a decrease of µmax (0.01 vs 0.09 h^{-1}) and Ymax (0.09 vs. 0.79), respectively. These results demonstrated the antibacterial activity of the CLO capsules.

Studies have shown that phenolic compounds found in essential oils like eugenol, carvacrol and thymol, are primarily responsible for the bactericidal and bacteriostatic properties, which may be attributed to the hydroxyl groups (-OH) that are highly reactive. The antimicrobial activity of eugenol was much potent than other natural compounds [27]. It has been shown that the antimicrobial effects of the essential oils cause structural and functional damage to bacterial cells [25]. Given the complex mixture of constituents in the essential oils is difficult to attribute antimicrobial mode of action to a specific mechanism. However it is known that damage occurs on the cell wall, cytoplasmic membrane, causing protein denaturation, coagulation of cytoplasm, inactivation of essential enzymes and functional alteration of the genetic material [23], thereby producing destruction of the cell [28].

Lag phase is considered the time that takes for a bacterium to adapt to new environments, and when affected, this facilitates inactivation or inhibition of maximum growth, as observed in treatment with CLO free or encapsulated, which were able to extent lag phase considering controls without EO. This indicates that addition of CLO affected the ability of bacteria to adapt to the environment; this may be affecting the cell membrane functionality, either by inhibiting absorption of nutrients, affecting some essential enzymes or membrane proteins essential for cellular multiplication or diffusion mechanisms within the cell. Therefore, deeper studies are needed to understand the mechanisms by which CLO exert their antibacterial effect.

Stability of CLO Encapsulated within β-CD when eExposed to Ozone

Figure 3 shows the loss of phenolic compounds(A), flavonoids (B), and antioxidant capacity (C and D) of free and encapsulated CLO to ozone during 15 min. Significant differences on the content of phenolic compounds was observed ($P \leq 0.05$) between the two oil treatments. CLO encapsulated within β-CD presented less variation in the content of phenolic after 15 min of treatment, losing around 15% of its content compared to 77% loss in free CLO. A similar effect was observed with respect to the loss of antioxidant capacity as measured by the methods of DPPH and TEAC, losing 25% of activity compared to 100% of loss found in free CLO ($P \leq 0.05$). These results demonstrated the protective stability of the CLO encapsulated within cyclodextrin.

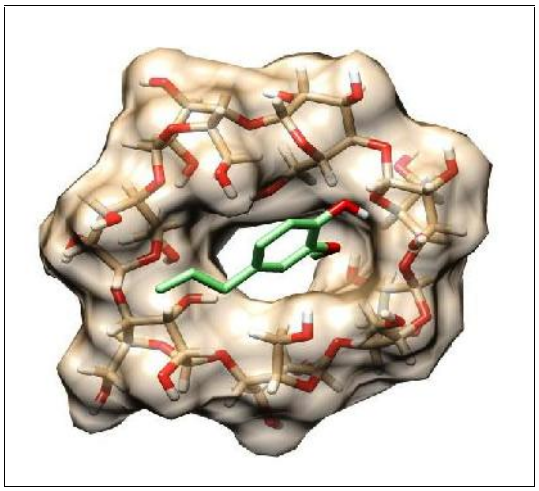

Fig. 4 Docking of Eugenol (PubChem ID: 3314) interacting with β-cyclodextrin (PubChem ID: 444041).

The microencapsulation with β-CD is reported as an effective method for the protection of active compounds against oxidation, heat degradation and evaporation [12]dueto the protection of molecules in the cavity of the β-CD. Volatility, light and oxygen lability are characteristic of the terpene constituents of CLO and these environmental factors can affect their antibacterial and antioxidant activities [29]. Exposure of essential oil constituents to changes in temperature and light also affect these compounds. Figure 4 corroborate CLO constituents like eugenol can be protected through β-CD encapsulation (score: −3.1 kcal mol^{-1}, RDSM: 1-093), offering protection against heat and UV-C [14].

Table 1 Effect of CLO encapsulated β-CD on growth parameters assessed against pathogenic bacteria.

Bacteria	Treatment	Lag (h)	µmax	Ymax
E. coli O157:H7	Control	3.10	0.64	1.15
	Free oil			
	0.42 mg/mL	6.55	0.11	0.76
	0.56 mg/mL	22.52	0.26	0
	β-CD oil			
	2.75 mg/mL	13.22	0.11	0.91
	5.5 mg/mL	24	0	0
L. monocytogenes	Control	2.40	0.50	1.3
	Free oil			
	0.42 mg/mL	9.63	0.16	0.81
	0.56 mg/mL	24	0	0
	β-CD oil			
	2.75 mg/mL	9.82	0.10	1.98
	5.5 mg/mL	15.59	0.06	0.40
S. Choleraesuis	Control	3.50	0.63	1.25
	Free oil			
	0.42 mg/mL	8.57	0.15	0.73
	0.56 mg/mL	24	0	0
	β-CD oil			
	2.75 mg/mL	6.43	0.07	0.90
	5.5 mg/mL	21.10	0.04	0.15
S. aureus	Control	2.56	0.56	1.20
	Free oil			
	0.42 mg/mL	12.33	0.22	0.69
	0.56 mg/mL	24	0	0
	β-CD oil			
	2.75 mg/mL	12.38	0.09	0.79
	5.5 mg/mL	22.9	0.01	0.90

Conclusion

CLO encapsulated in β-CD showed better antibacterial activity against the food pathogen strains, *E. coli*, *L. monocytogenes*, *S.* Choleraesuis and *S. aureus* compared with free CLO. The encapsulation offered protection to CLO components, maintaining its antioxidant and antibacterial activity when exposed to ozone. These findings suggest that CLO encapsulated in β-CD could beexploited to develop new food additives with antimicrobial and antioxidant potential with applications in the food industry.

References

1. Magkos F, Arvaniti F, Zampelas A. Organic Food: Buying More Safety or Just Peace of Mind? A Critical Review of the Literature. Crit Rev Food Sci Nutr. 2006;46:23-56.
2. Witkowska AM, Hickey DK, Alonso-Gomez M, Wilkinson MG. The microbiological quality of commercial herb and spice preparations used in the formulation of a chicken supreme ready meal and microbial survival following a simulated industrial heating process. Food Control. 2011;22:616-625.
3. Barrett DM, Beaulieu JC, Shewfelt R. Color, Flavor, Texture, and Nutritional Quality of Fresh-Cut Fruits and Vegetables: Desirable Levels, Instrumental and Sensory Measurement, and the Effects of Processing. Crit Rev Food Sci Nutr. 2010;50:369-389.
4. Ribeiro-Santos R, Andrade M, Sanches-Silva A & de Melo NR. Essential oils for food Application: Natural substances with established biological activities. Food Bioprocess Tech. 2018;11:43-71.

5. Ayala-Zavala JF, González-Aguilar GA, Del-Toro-Sánchez L. Enhancing Safety and Aroma Appealing of Fresh-Cut Fruits and Vegetables Using the Antimicrobial and Aromatic Power of Essential Oils. J Food Sci. 2009;74:R84-R91.
6. Singh, G, Maurya S, deLampasona MP, Catalan CAN. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem Toxicol. 2007;45:1650-1661.
7. Melgarejo-Flores BG, Ortega-Ramírez LA, Silva-Espinoza BA, González-Aguilar GA, Miranda MRA, Ayala-Zavala JF.Antifungal protection and antioxidant enhancement of table grapes treated with emulsions, vapors, and coatings of cinnamon leaf oil. Postharvest Biol Tech. 2013;86:321-328.
8. Du WX, Olsen CW, Avena-Bustillos RJ, McHugh TH, Levin CE, Friedman M. Effects of Allspice, Cinnamon, and Clove Bud Essential Oils in Edible Apple Films on Physical Properties and Antimicrobial Activities. J Food Sci. 2009;74:M372-M378.
9. Antunes MDC, Cavaco AM. The use of essential oils for postharvest decay control. A review. Flavour Fragr. J. 2010;25:351-366.
10. Ponce-Cevallos PA, Buera MP, Elizalde BE. Encapsulation of cinnamon and thyme essential oils components (cinnamaldehyde and thymol) in β-cyclodextrin: Effect of interactions with water on complex stability. J Food Eng. 2010;99:70-75.
11. dos Santos NST, Athayde Aguiar AJA, de Oliveira CEV, Veríssimo de Sales C, de Melo e Silva S, Sousa da Silva R, Stamford TCM, de Souza EL. Efficacy of the application of a coating composed of chitosan and *Origanum vulgare* L. essential oil to control *Rhizopus stolonifer* and *Aspergillus niger* in grapes (*Vitis labrusca* L.). Food Microbiol. 2012;32:345-353.
12. Ayala-Zavala JF, Soto-Valdez H, González-León A, Álvarez-Parrilla E, Martín-Belloso O, González-Aguilar GA.Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in β-cyclodextrin. J Incl Phenom Macrocycl Chem. 2008;60:359-368.
13. Hill LE, Gomes C, Taylor TM.Characterization of beta-cyclodextrin inclusion complexes containing essential oils (trans-cinnamaldehyde, eugenol, cinnamon bark, and clove bud extracts) for antimicrobial delivery applications. LWT-Food Sci Technol. 2013;51:86-93.
14. Ayala-Zavala JF, Gonzalez-Aguilar GA, Alvarez-Parrilla E, De la rosa LA, Vega-Vega V. Encapsulación de antioxidantes en la industria de alimentos. In: Álvarez-Parrilla GA, De la Rosa, Ayala-Zavala, Juárez, (eds.) Antioxidantes En Alimentos y Salud. 2012;527-552. Clave editorial, México
15. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. J Enol Vitic. 1965;16:144-158.
16. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64:555-559.
17. González-Aguilar GA, Villegas-Ochoa MA, Martínez-Téllez MA, Gardea AA, Ayala-Zavala, JF. Improving Antioxidant Capacity of Fresh-Cut Mangoes Treated with UV-C. J Food Sci. 2007;72:S197-S202.
18. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26:1231-1237.
19. Vega-Vega V, Silva-Espinoza BA, Cruz-Valenzuela MR, Bernal-Mercado AT, González-Aguilar GA, Vargas-Arispuro I, Corrales-Maldonado CG, Ayala-Zavala JF. Antioxidant enrichment and antimicrobial protection of fresh-cut mango applying bioactive extracts from their seeds by-products. Food Nutr Sci. 2013;4:197.
20. Gordon R, Ryan Q, Steven M. High-Throughput Method for Dual Assessment of Antifungal Activity and Growth Kinetics Using a FLUOstar Omega. 2009. http://www.bmglabtech.com/db_assets/applications/downloads/applications/189-tea-tree-oil.pdf
21. Baranyi J, Roberts TA, McClure P. A non-autonomous differential equation to modelbacterial growth. Food Microbiol. 1993;10:43-59.
22. Gurol MD, Vatistas R. Oxidation of phenolic compounds by ozone and ozone + u.v. radiation: A comparative study. Water Research. 1987;21:895-900.
23. Brewer MS. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Compr Rev Food Sci F. 2011;10:221-247.
24. Astray G, Gonzalez-Barreiro C, Mejuto JC, Rial-Otero R, Simal-Gándara J. A review on the use of cyclodextrins in foods. Food Hydrocolloids. 2009;23:1631-1640.
25. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Control. 2010;21:1199-1218.
26. Bhandari BR, D'Arc BR, Thi Bich LL. Lemon Oil to β-Cyclodextrin Ratio Effect on the Inclusion Efficiency of β-Cyclodextrin and the Retention of Oil Volatiles in the Complex. J Agric Food Chem. 1998;46:4:1494-1499.
27. Ceylan E, Fung DYC. Antimicrobial activity of spices. J Rapid Meth Aut Mic. 2004;12:1-55.
28. Sánchez-González L, Vargas M, González-Martínez C, Chiralt A, Cháfer M. Use of Essential Oils in Bioactive Edible Coatings: A Review. Food Eng Rev. 2011;3:1-16.
29. Weissinger WR, McWatters KH, Beuchat LR. Evaluation of Volatile Chemical Treatments for Lethality to Salmonella on Alfalfa Seeds and Sprouts. J Food Prot. 2001;64:442-450.
30. Pettersen EF, Goddard TD, Huang CC, Couch GS, GreenblattDM, Meng EC, Ferrin TE UCSF Chimera a visualization system for exploratory research and analysis. J Comput Chem. 2004;25:1605–1612.