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ORIGINAL ARTICLE

Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in β-cyclodextrin

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Abstract Cinnamon leaf (CLO) and garlic oils (GO) are good antimicrobials, however, their volatility complicates their application as food preservatives. Hence, microencapsulation of CLO and GO with β-cyclodextrin (β-CD) was studied at 4:96, 8:92, 12:88, and 16:84 (oil:β-CD) percent weight ratios. Microcapsule characterization included gas chromatography analysis, moisture sorption–desorption isotherms, infrared spectroscopy (IR), and antifungal activity against *Alternaria alternata*. Major oil constituents were eugenol for CLO and allyl disulfide for GO. The 16:84 ratio (CLO:β-CD) showed the highest eugenol content; the allyl disulfide content was higher, but not significantly different ($P > 0.05$) for the 12:88 and 16:84 ratios. Microcapsules showed lower moisture sorption than β-CD, although during water desorption there were no difference between them. Hydrogen bonds were detected between oil constituents and β-CD by IR spectroscopy. CLO:β-CD and GO:β-CD microcapsules displayed good antifungal activity against *Alternaria alternata*. Therefore, CLO and GO microcapsules can have important applications in the food industry as stable natural antimicrobial compound systems.

Keywords Cinnamon leaf oil · Garlic oil · β-cyclodextrin · Microcapsules · Antifungal

Abbreviation list

β-CD	β-cyclodextrin
CLO	Cinnamon leaf oil
GO	Garlic oil
GRAS	Generally recognized as safe
FDA	Food and Drug Administration
GC-MS	Gas chromatography-mass spectrometry
GC-FID	Gas chromatography-flame ionization detector
RH	Relative humidity
IR	Infrared
PDA	Potato dextrose agar
NCSS	Number Cruncher Statistical System
LSD	Least significant difference

Introduction

The β-cyclodextrin (β-CD) molecule is made up of 7 D-glucose monomers linked by α(1,4) bonds, exhibiting the shape of a truncated hollow cone. The cavities of β-CD are hydrophobic, whereas the external faces are hydrophilic [1]. These properties have made β-CD an option in the microencapsulation of several compounds. Microencapsulation in β-CD is one of the most effective methods for protecting active compounds against oxidation, heat degradation, and evaporation [2]. This protection is due to the fact that the protected molecules are tightly held within the

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β-CD cavity; therefore, microencapsulation in β-CD is considered as a molecular-complex formation. The interaction between β-CD (host) and active compounds (guests) may involve total inclusion or association with the hydrophobic or hydrophilic part of the molecule [1]. Nowadays, there are many labile compounds with important bioactive properties that can be microencapsulated and used in industrial processes.

Antimicrobial properties of herbs and spices have been recognized and used since ancient times for food preservation as well as for medicinal purposes [3]. Scientific reports on natural antimicrobial agents date back to more than a century ago, for example, Chamberlain reported in 1887 the action of essential oil vapors on anthrax spores, as cited by Maruzzella and Sicurella [4]. A renewed interest in 'natural preservation' appears to be stimulated by consumer demands of the partial or complete removal of chemically synthesized preservatives from foods; impelling the creation of 'green' image policies by food industries.

Cinnamon leaf oil (CLO) (*Cinnamomum zeylanicum*) and garlic oil (GO) (*Allium sativum*) are recognized for their flavor and aroma in addition to their antimicrobial medicinal applications [5, 6]. CLO and GO are generally recognized as safe (GRAS) natural products by the U.S. Food and Drug Administration (FDA) and it is generally accepted that their volatile compounds are the main reason for their antimicrobial properties. However, volatility can be a problem. This makes difficult the application of such oils as food preservatives. Mallavarapu et al. [7] reported that eugenol and cinnamaldehyde were the main volatiles of CLO, and mono to hexa allyl sulfides and vinyl dithiin isomers were described as the major constituents of GO [8]. Today, exploration and understanding of all of the advantages of these essential oils in nutrition and medicine are still in progress.

Considering the requirements of effectiveness and convenience of the application of natural antimicrobial products, the main goals of this study were set. The Main objectives were to microencapsulate CLO and GO within β-CD; identify and quantify the main oil constituents trapped within the β-CD. Moisture sorption isotherms and the antifungal activity of the complexes formed were also determined.

Materials and methods

Reagents

Essential oils (CLO, GO), eugenol, allyl disulfide and other GC standards were bought from Sigma-Aldrich Co. β-CD (Cavamax W7 food grade) was kindly supplied by Wacker Biochem, (Mexico). All other reagents used were analytical grade.

Microencapsulation process

Microcapsules of CLO:β-CD and GO:β-CD were prepared separately according to the precipitation method [9]. In brief: A portion of 50 g (±0.01) of β-CD was dissolved in 500 mL of an ethanol:water (1:2) mixture and maintained at 55 °C (±2 °C) on a hot stirrer plate. A predetermined quantity of each essential oil (0, 2.08, 4.35, 6.82, or 9.54 ± 0.01 g) was dissolved in ethanol (10% w/v) and then slowly added to the warm β-CD solution to obtain weight ratios of essential oils to β-CD of 0:100, 4:96, 8:92, 12:88, and 16:84, respectively. During the addition of the oil solution, the β-CD solution was continuously stirred and the temperature maintained at 55 °C (±2 °C). Afterwards, the heater was turned off and the resultant mixture was covered and stirred for 4 h. The final solution was maintained overnight at 4 °C. The precipitated oil:β-CD microcapsules were recovered by filtration and then dried in a convection oven at 50 °C for 24 h. The complexes were removed from the oven and allowed to air-dry at 25 °C in a desiccator for an additional 24 h. The final microcapsules were weighed at equilibrium. The amount of recovered microcapsules (dry basis) was calculated in percentage comparing the initial weight added of raw materials to the recovery microcapsules. Finally, the oil microcapsules were stored at 25 °C in an airtight bottle. Each starting oil ratio was prepared and analyzed in triplicate.

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

Analyses of CLO and GO samples before and after microencapsulation in β-CD were performed using a Varian GC-3400 Cx, equipped with a Saturn 2100T mass-selective detector (Varian, Mexico) and a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Column temperature rose from 55 to 65 °C at a rate of 1 °C/min, and held 3 min, then the temperature rose from 65 to 290 °C at a rate of 10 °C/min rate, and was held at this final temperature for 10 min. Helium was the carrier gas, at a flow rate of 1 mL/min. For GC-MS detection an electron ionization system with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 100 and 290 °C, respectively. Identification of the compounds was based on the comparison of their mass spectra with those of the Saturn library and NIST 98 library data of the GC-MS system.

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

Quantification was determined using a Varian Star 3400-Cx chromatograph equipped with a FID detector (Varian, Mexico) and a DB-5 capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Column temperature program was the same as that of the GC-MS analysis. Nitrogen was used as the carrier gas, at a flow rate of 1 mL/min. One μL of the extract was injected manually in the splitless mode. Essential oils were diluted with dichloromethane to a concentration of 500 μg/mL. Essential oils were extracted from the microcapsules by sonication in dichloromethane. For this, 10 mg of solid complex was dissolved in 1 mL of deionized water and then 2 mL of dichloromethane was added. The mixture was sonicated for 30 min and the organic phase was recovered. This step was repeated twice and the total volume of the organic phase was used for quantification of microencapsulated compounds. The major constituent of each essential oil was quantified using standard calibration curves.

Moisture determination

The moisture contents of the pure β-CD, CLO:β-CD, and GO:β-CD microcapsules were determined by drying a sample (3–4 g) in a vacuum oven at 70 °C for 24 h and under pressure <6.7 kPa [10]. The percentage of moisture was calculated by comparing the initial and the final sample weights of the pure β-CD and the oil microcapsules. These data were used to calculate the moisture sorption–desorption isotherms as described below.

Moisture sorption–desorption isotherms

Moisture sorption–desorption isotherms for the β-CD and its microcapsules with essential oils (2 g, initial weight) were obtained using saturated salt solutions of anhydrous $Ca_3(SO_4)_2$, CsF, $MgCl_2$, NaBr, K_2SO_4, to give RH of 0, 18, 33, 66, and 96%, respectively. Initial moisture was recorded, and samples were weighted until constant weight was attained (3 weeks). After sorption, samples were saturated with 100% RH and were returned to previously established RH for 3 weeks. Data are reported as mg of sorbed or desorbed water/mg of solid.

Infrared analysis (IR)

IR spectra of free and microencapsulated oils, as well as β-CD, were recorded using an infrared spectrophotometer FTIR Nicolet, Protege 460 (Nicolet, Madison, WI). Scanning conditions were as follows: wave number range, from 4,000 to 400 cm^{-1}; resolution, 4 cm^{-1}; number of scans, 64; scan speed, 0.63; detector, DTGS. CLO and GO were recorded on KBr plates. β-CD, physical mixture of CLO, GO, and β-CD, and both microcapsules CLO:β-CD and GO:β-CD, were recorded using KBr pellets. An Aldrich Library of Infrared Spectra was used for identification [11].

Antifungal activity

Isolates of the fungus *Alternaria alternata* were obtained from naturally infected tomato fruit and identified according to Simmons [12]. CLO:β-CD or GO:β-CD ratios that showed the highest oil absorption were diluted in potato dextrose agar (PDA) at different concentrations. Then spores of *Alternaria alternata* (5×10^5/mL) were inoculated in the center of a petri dish containing the above-mentioned treatments. The colony diameter was recorded (cm) after 48 h at 25 °C, when colony diameter was asymmetrical, four diameter measures were taken and averaged. Comparisons among treatments and oil microcapsules were carried out.

Statistical analysis

This experiment was designed based on a completely randomized design with equal replication. Analysis of variance for the treatments was done using NCSS statistical software. Mean comparisons of the studied parameters among treatments were done using the least significant difference (LSD) test at the 5% level ($P < 0.05$).

Results and discussion

Microencapsulation

The recovery of the CLO and GO microcapsules at the equilibrium state is presented in Table 1. The weights of oil microcapsules that were recovered were less than the amount of β-CD and essential oil originally used. There was a significant increase ($P < 0.05$) in the recovery of the 12:88 and 16:84 ratios compared to the 4:96 and 8:92 for both oil microcapsules. Statistical comparison for CLO microcapsules indicated that there were significant differences ($P < 0.05$) among all the ratios. However, for GO microcapsules no significant differences ($P > 0.05$) between the 12:88 and 16:84 ratios were observed. These data indicate that optimum ratios of CLO and GO to β-cyclodextrin during microencapsulation existed at around 16:84 and 12:88,

Table 1 Recoveries of CLO and GO microcapsules at various ratios of essential oil versus β-CD

Ratio*	Final oil:β-CD (g, DW**)	Microcapsule yield (%)***	Relative major volatile load (%)***	Total volatile load (%)***
CLO:β-CD			Eugenol	
4:96	41.22 ± 0.09	79.16[d]	78.18[a]	6.96[d]
8:92	46.53 ± 0.04	85.61[c]	49.50[b]	7.92[c]
12:88	52.62 ± 0.13	92.62[b]	46.80[c]	11.22[b]
16:84	56.45 ± 0.18	94.82[a]	39.94[d]	12.76[a]
GO:β-CD			Allyl disulfide	
4:96	42.78 ± 0.04	82.15[c]	100[a]	11.75[c]
8:92	45.93 ± 0.05	84.51[b]	89.20[b]	14.26[b]
12:88	53.74 ± 0.04	94.58[a]	84.30[c]	20.24[a]
16:84	55.82 ± 0.11	93.76[a]	64.86[d]	20.75[a]

* Initial oil:β-CD ratio weight 4:96 = 52.08 g; 8:92 = 54.35 g; 12:88 = 56.82; 16:84 = 59.54 g

** DW—Dry weight

*** Same letters indicate no significant differences among means ($P > 0.05$)

respectively. Starting ratios of GO to β-CD greater than 12:88 did not significantly affect ($P > 0.05$) the amount of GO recovered from microcapsules. This result may suggest that the maximum inclusion of β-CD with the GO had been reached. Comparing the relative absorption of the major constituent of each oil, in relation to the ratio oil: β-CD, it can be observed that increasing the oil amount added to the β-CD the absorption of major constituents decreased, being more noticeable for the CLO. This decrement could be caused by the decrement of free β-CD which has been occupied by the oil constituents and saturation occurred. This can be observed and ratified particularly in the volatile load of GO microcapsules, were in the saturation started at the ratio of 12:88. It was observed that the recovered free β-CD decreased while the amount of added oil increased, which is in agreement with the proposed saturation process (data not shown).

For the 12:88 and 16:84 ratios, in both oil microcapsules, there was a 92–94% increase in the amount of recovered product relative to the initial oil: β-CD weights. At these greater concentrations of essential oils in the starting solution, there is a major recovery of oil components to form the microcapsules. This statement was verified by gas chromatography assays detailed in the next section. Some other factors may contribute to a low recovery of essential oil microcapsules, such as essential oils remaining in the solution after forming microcapsules, and some evaporation may occur during the microencapsulation process [9]. However, it has to be remarked that a saturation of the cyclodextrin matrix is reached, and the optimization of the process can be established with respect to the major recovery, which, in this case, for CLO and GO microcapsules the optimal recovery ratios were 16:84 and 12:88, respectively.

Gas chromatography (GC-MS, GC-FID) analysis

Identification of the volatile constituents of CLO (Fig. 1) and GO (Fig. 3) before (a) and after (b) the microencapsulation process was accomplished by GC-MS analysis. Once identified the major constituents of each microencapsulated oil, chromatograms were used to compare the differences among the non encapsulated oil at the different oil ratios. Quantified major oil constituent for CLO and GO are shown in Figs. 2 and 4, respectively.

Eugenol was the major constituent detected in the CLO, as shown in Fig. 1a before and in Fig. 1b after the microencapsulation process. Eugenol accounts approximately 78% w/w of the total volatiles, as it can be appreciated in Fig. 2. In a previous study, a total of 41 volatile compounds were identified in the CLO, with eugenol as the major constituent with about 70% w/w of total volatiles [13]. Other minor CLO constituents detected were cinnamaldehyde, copaene, and β-caryophyllene. Figure 2 shows the eugenol concentration for the 4:96, 8:92, 12:88, and 16:84 treatments (CLO:β-CD) expressed

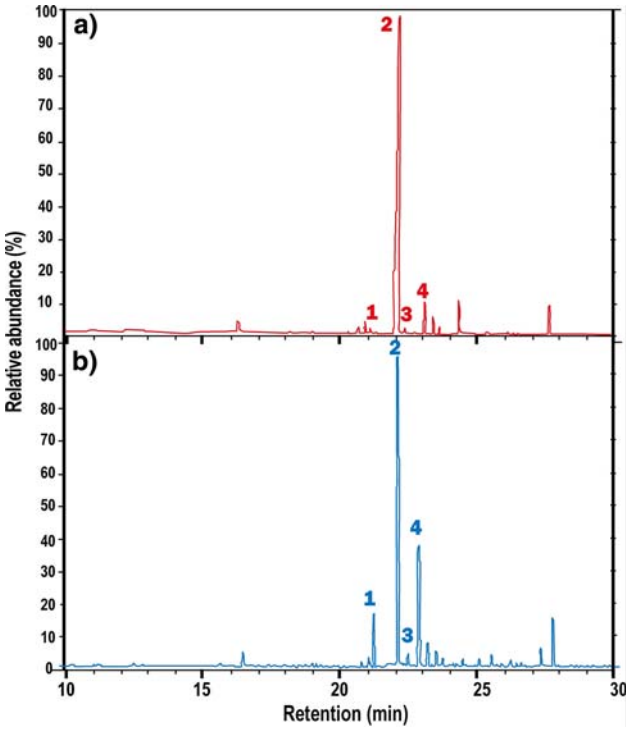

Fig. 1 GC-MS volatile profile of CLO before (**a**) and after (**b**) microencapsulation in β-CD. 1: Cinnamaldehyde, 2: Eugenol, 3: Copaene, 4: β-caryophyllene. The 16:84 (CLO:β-CD) weight ratio was selected for this assay

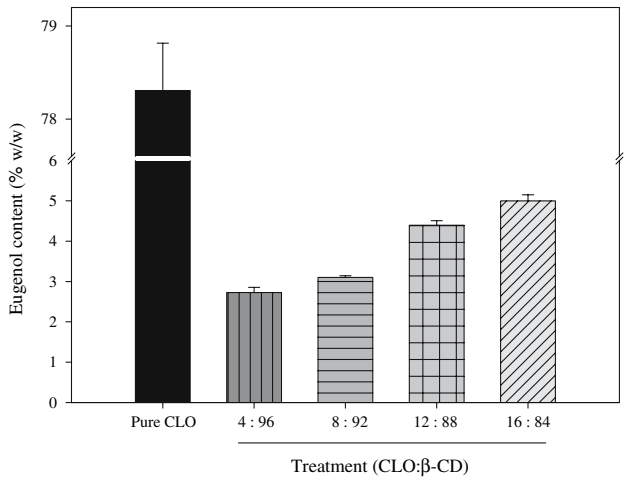

Fig. 2 Eugenol content in pure CLO and the different oil weight ratios microencapsulated in β-CD

as % of eugenol content in the analyzed microcapsules. Eugenol content increased from 2.7 to 5% w/w, being significantly different ($P < 0.05$) among them.

When the profile of volatiles of the CLO:β-CD microcapsules (Fig. 1b) was analyzed, several interesting aspects could be commented. (i) It is possible to observe that eugenol is still the major CLO constituent; however, its percentage in the CLO:β-CD microcapsules decreases with respect to the non encapsulated CLO (Fig. 2). (ii) The relative abundance of cinnamaldehyde, copaene, and β-caryophyllene was higher in microcapsules than in free CLO (Fig. 1a); this effect was more evident for cinnamaldehyde and β-caryophyllene. This increment on both compound concentrations in the CLO:β-CD microcapsules can be explained considering that cinnamaldehyde and β-caryophyllene can stoichiometrically form 1:1 stable complexes with β-CD and, consequently, a competitive equilibrium between β-CD and all volatiles (eugenol, cinnamaldehyde, copaene, and β-caryophyllene) is established [14]. These results may be supported by the fact that some of these aromatic compounds had already been separated by enantioselective gas chromatography using β-CD derivatives as the stationary phase [15].

Garlic essential oils are reported to consist primarily of allyl, dimethyl, and allyl methyl mono-, di-, trisulfides, and a few minor components. Allyl disulfide accounts for 30–50% of the total mixture [16]. The main compounds detected by GC-MS in GO (Fig. 3) were allyl disulfide, allyl trisulfide and allyl tetrasulfide. Organosulfur compounds from garlic such as diallyl mono-, di-, and trisulfide have been reported to show antimicrobial activity [17].

Figure 3 shows that the presence of the GO compounds was not affected by the microencapsulation process, however, the proportion of these compounds was. Allyl disulfide was the major constituent of GO with 21% w/w.

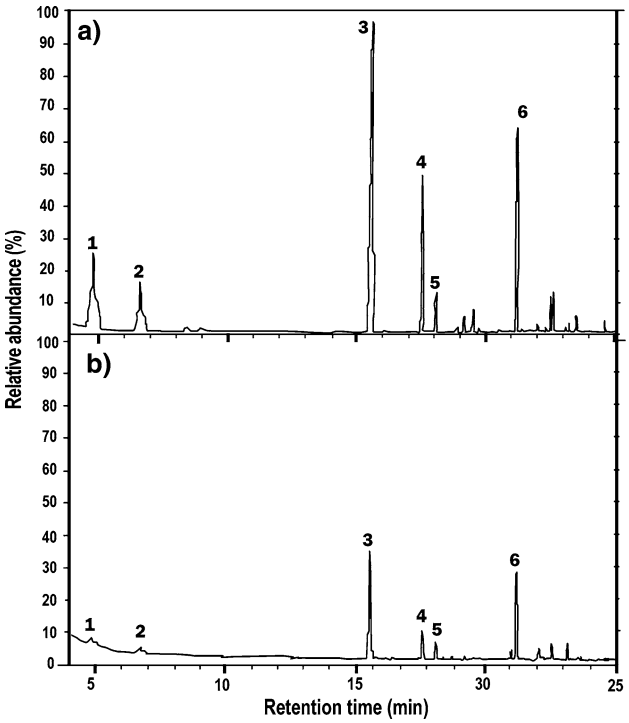

Fig. 3 GC-MS volatile profile of GO before (**a**) and after (**b**) microencapsulation in β-CD. 1: Methyl disulfide, 2: Allyl sulfide, 3: Allyl disulfide, 4: Allyl trisulfide, 5: Trimethylene trisulfide, 6: Allyl tetrasulfide. The 12:88 (GO:β-CD) weight ratio was selected for this assay

From an examination of Fig. 3a and b, it is possible to see that the concentration of all GO compounds decreased comparing to the non encapsulated with the encapsulated oil. This effect was more evident for methyl disulfide and allyl sulfide.

The effect of the GO:β-CD weight ratio on the allyl disulfide content is presented in Fig. 4. At high GO:β-CD ratios (12:88, 16:84) no significant differences ($P > 0.05$) were observed (2.13 and 2.19% w/w, respectively); however, at lower concentrations a concentration-dependent pattern was observed, decreasing the allyl disulfide percentage as the ratio decreases. When the percentage of retained disulfide was analyzed (allyl disulfide represents 21% in the original GO), it was possible to observe that as the oil ratio increased, the retained allyl disulfide also increased. As in the case of CLO, it seems that multiple competitive complexes or that stoichiometries different to 1:1, such as 2:1: (guest:β-CD) are formed [14]. The similarity in composition for the main volatiles between the non encapsulated and the encapsulated oils is likely to be due to the small size and polarity characteristics of all the volatile molecules studied, which can be easily included in the β-CD.

Eugenol and Allyl disulfide content of CLO and GO were used as a pattern to evaluate the efficiency of the

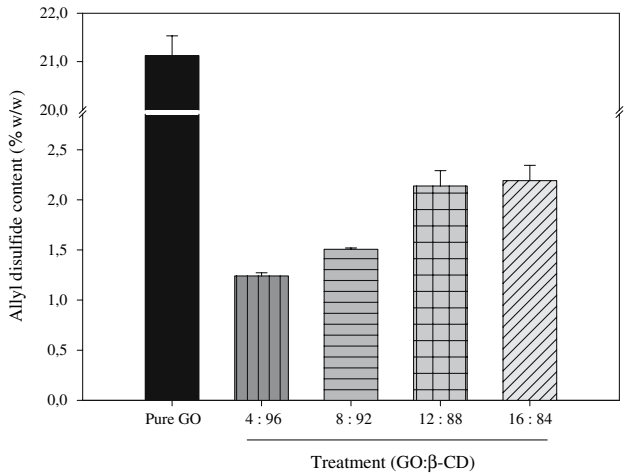

Fig. 4 Allyl disulfide content in pure GO and the different oil weight ratios microencapsulated in β-CD

tested ratios in the microencapsulation process, respectively. In summary, for the microencapsulated CLO and GO, the more efficient ratios were 16:84, and 12:88 with 5, and 2.19% of eugenol and allyl disulfide, respectively. These ratios were used to perform the IR and antifungal capacity assays.

Moisture sorption isotherms

The moisture sorption (a) and desorption (b) isotherms of β-CD and different ratios of CLO:β-CD and GO:β-CD microcapsules at 20 °C are shown in Figs. 5 and 6. The pure β-CD showed the highest moisture uptake at 100% RH (about 0.15 mg water absorbed per mg β-CD). A constant uptake was observed up to 40% RH, showing a plateau; thereafter, this moisture content value was consistent with the 14–15% water content reported for β-CD [18]. It has been reported that a cluster of 7 molecules of water occupies the cavity of the macrocyclic of a fully hydrated β-CD molecule, and 5.4 water molecules occupy interstitial spaces [18]. It has to be mentioned that no significant effect of the moisture determination assay on the microencapsulated volatile content was found, which guaranteed that only water loss was measured.

When the sorption isotherms of both CLO:β-CD and GO:β-CD microcapsules were analyzed (Figs. 5a and 6a), it was observed that both essential oils presented type II isotherms, which are characteristic of hydrophilic polymers having both surface adsorption and absorption in the solid phase [19]. At high RH values, the moisture uptake of the microcapsules approximates to those reported for β-CD. These results are in agreement with those reported by Suihko et al. [20] for the sorption isotherm of the tolbutamide-hydrxypropyl-β-cyclodextrin complex.

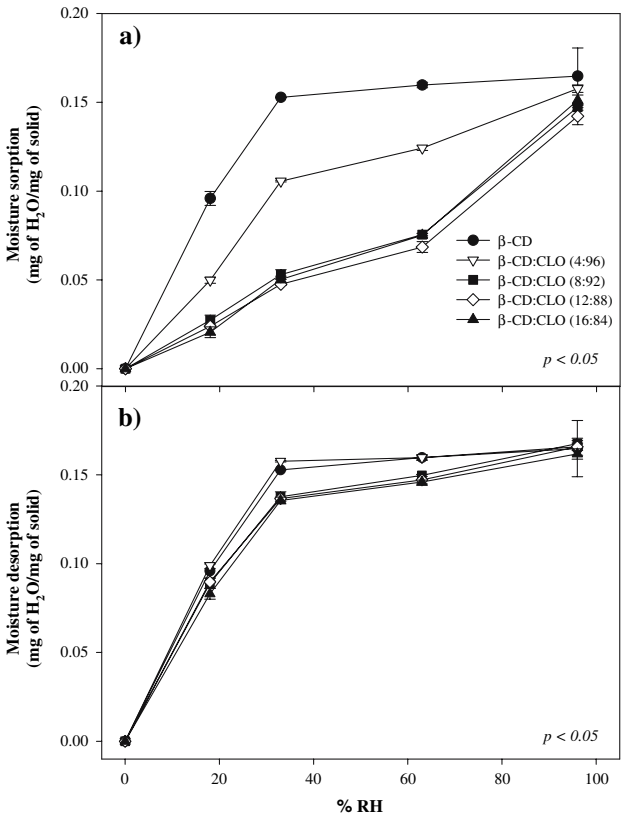

Fig. 5 Moisture sorption (**a**) desorption (**b**) isotherms for CLO microencapsulated in β-CD at different ratios

Figure 5a shows that the sorbed moisture level decreased for those microcapsules with the highest CLO oil ratios, at RH values below 60%. The isotherm of the 4:96 weight ratio microcapsules was statistically different from that of β-CD and the rest of the ratios. No differences in the isotherms of the 8:92, 12:88, and 16:84 weight ratios were observed. These results may be explained considering that during the microencapsulation of CLO by β-CD, the oil constituents are positioned in the hydrophilic sites of the β-CD molecule and, consequently, the capacity of microcapsules to adsorb water is reduced [21]. These results could sustain the theory that CLO constituents are interacting with the β-CD molecule through hydrogen bonds.

GO microcapsules also showed that an increment in the oil weight ratio decreased the sorbed moisture (Fig. 6a). However, at all weight ratios studied, this effect was less evident than that for CLO. These results could be explained in terms of complexation stability. If we consider that the main component of CLO is eugenol, which is an *o*-diphenol derivative and can be easily complexed by the β-CD [15], whilst in the case of GO the main constituents are smaller aliphatic sulfur compounds. It is expected that the complexation process in the case of CLO will take place in a higher degree than in the case of GO, because of the molecular size of the constituents and, consequently,

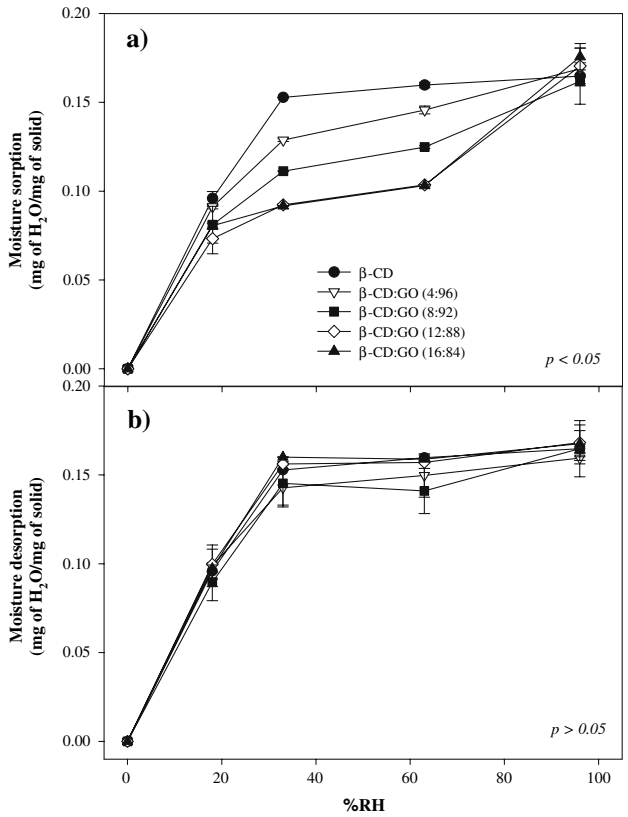

Fig. 6 Moisture sorption (**a**) desorption (**b**) isotherms for GO microencapsulated in β-CD at different ratios

the amount of water displacement in the latter complex will be lower.

The desorption isotherms of both CLO:β-CD and GO:β-CD microcapsules were carried out after equilibration of the complexes at 100% RH (3 weeks at 20 °C), and are presented in Figs. 5b and 6b. From an inspection of these figures it can be observed that β-CD sorption–desorption isotherms were exactly the same. Though, for both essential oils a concentration-dependent hysteresis loop was observed. As in the case of the sorption isotherm, the hysteresis loop was more evident for the CLO than for the GO, indicating once again a better complexation process. These results showed that water molecules are replacing oil constituents when humidity increases.

Oil-cyclodextrin interactions are mainly hydrophobic–hydrophilic. In this context, a release of the hydrophobic volatiles constituents of the microcapsule could be intimately related to hydration. When hydrophobic interactions play a major role in a binding process, the hydration process of the microcapsule must be accompanied by a substantial release of the hydrophobic volatile constituents from the cyclodextrin matrix. On the other hand, when water availability increases around the microcapsules, water starts to interact with the polar groups of the β-CD unbalancing the equilibrium and oil constituents are displaced [22]. As water penetrates the encapsulating matrix; the system turns energetically unfavorable for the hydrophobic antimicrobial guest that could migrate to a lower water content medium. Therefore, in this study oil constituents could be interacting with β-CD polar groups via hydrogen bonding. This hypothesis is supported by the IR assay shown in the next section. And the probably release of the hydrophobic volatile constituents by hydration is recommended as further research.

IR analysis

The IR spectra of β-CD (a), CLO (b), their physical mixture (c), and CLO microcapsules (d) are presented in Fig. 7. The 16:84 weight ratio was selected for this assay because of its high eugenol content. High similarity of the CLO spectra can be found compared to the free eugenol spectra reported in the website of Sigma-Aldrich. Free CLO showed the major IR absorptions at 3,521 cm^{-1} (OH stretch), 2,842–3,076 cm^{-1} (C–H stretch), 1,600–1,680 cm^{-1} (alkene C=C), and 1,400–1,600 cm^{-1} (aromatic C=C), similar to those of eugenol, its major constituent. IR spectral analysis shows differences in some band positions of the microcapsule with respect to CLO and β-CD, and their physical mixture. These differences in the IR spectra are typical of β-CD solid state complexes, due to the loss of vibrating and bending of the guest molecule during complex formation [23]. Patterns of physical mixtures show approximate superimposition of the individual patterns of both β-CD and CLO. In the IR spectra of the CLO microcapsules, CLO bands are almost completely obscured by very intense and broad β-CD bands, which are hardly influenced by molecular complex formation. However, absorption bands for OH groups at 3,490 and 3,244 cm^{-1} experienced a dramatic broadening of the spectra of the prepared CLO microcapsules, and the peaks were shifted toward these lower frequencies from 3,521 to 3,375 cm^{-1} found in the free CLO and β-CD, respectively. This change may be related to the formation of intramolecular hydrogen bonds between the guest and host molecules [23, 24].

The IR spectra of the β-CD (a), GO (b), their physical mixture (c) and GO microcapsules (d) are compared in Fig. 8. The 12:88 weight ratio was selected for this assay because of its high allyl disulfide content. Typically GO presents 4 major peaks in the region between 3,100 and 2,900 cm^{-1}. The first at 3,081 cm^{-1} corresponds to asymmetric stretch vibration of $=CH_2$, the second at 3,011–3,007 cm^{-1} to C–H stretching, the third at 2,980–2,978 cm^{-1} to symmetric stretch vibration of $=CH_2$, and the fourth at 2,914–2,912 cm^{-1} to $-CH_2-$ stretching. The second region from 1,700 to 1,000 presents 5 peaks in GO. In this area, the very intense peak at 1,633 cm^{-1} is

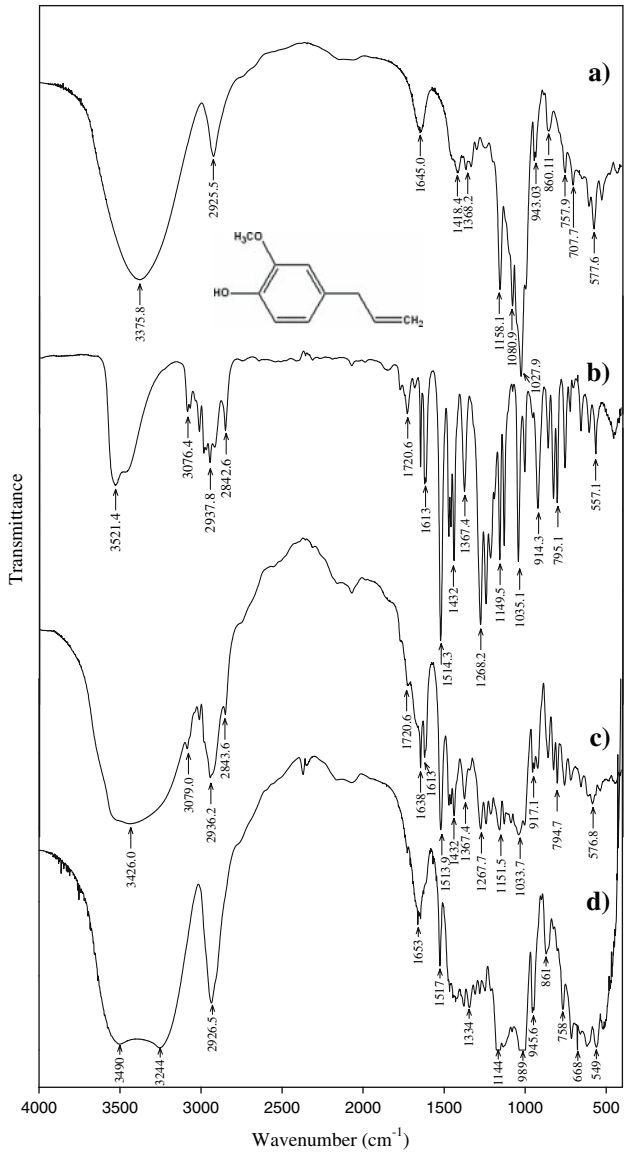

Fig. 7 IR spectra of β-CD (**a**), CLO (**b**), CLO and β-CD physical mixture (**c**), CLO:β-CD (16:84% w/w) microcapsules (**d**). Inset in Fig. 7b shows the structure of eugenol

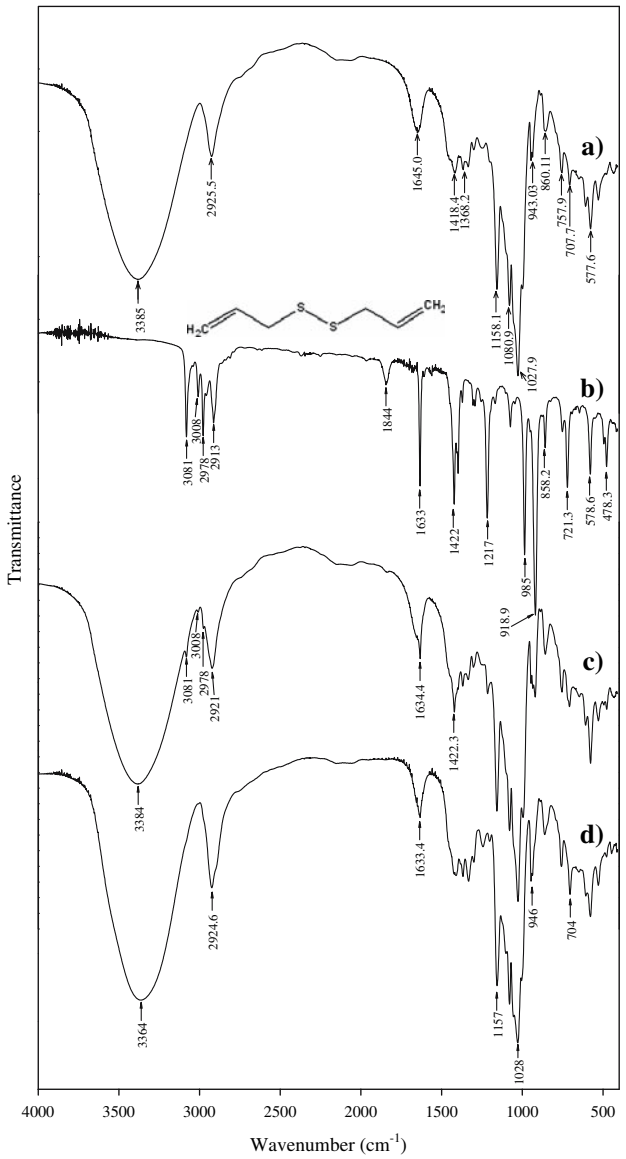

Fig. 8 IR spectra of β-CD (**a**), GO (**b**), GO and β-CD physical mixture (**c**), GO:β-CD (12:88% w/w) microcapsules (**d**). Inset in Fig. 8b shows the structure of allyl disulfide

assigned to C=C stretching vibration of the allyl group. The double peak at 1,428–1,401 cm^{-1} may be assigned to the stretching of $–CH_2–$ group while CH_2=CH– stretching is shifted to 1,217 cm^{-1}. The above peaks were assigned according to Baranska et al., and Dean [25, 26].

IR spectral analysis shows differences in some band positions of the microcapsules with respect to the free compounds and their physical mixture. First, the microcapsule's band centroid in the spectrum is shifted in the range of valence OH vibrations towards lower frequencies compared to the position of the similar band in the spectrum of β-CD. This indicates that an interaction has occurred between the oils, separately and β-CD, via hydrogen bonding and that the complexes are not just physical mixtures.

Antifungal activity

To study its antimicrobial effectiveness, selected concentrations of the CLO and GO microcapsules (16:84 and 12:88 weight ratio, respectively) were added to PDA agar which was then inoculated with *Alternaria alternata* and compared to a control and β-CD (Fig. 9). In this figure, it is possible to observe that β-CD itself increased the fungal growth of *Alternaria alternata*. The reason for this

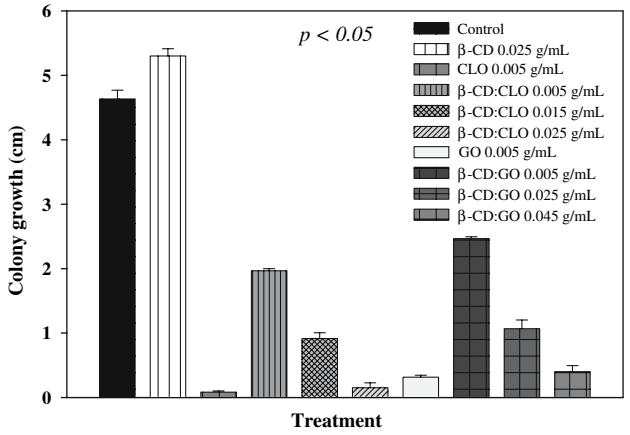

Fig. 9 Antifungal activity of CLO:β-CD (16:84% w/w) and GO:β-CD (12:88% w/w) microcapsules against *Alternaria alternata*. Each value is the mean of three replications ± standard error

increment was probably because β-CD acts as a carbohydrate source for the fungus. Both free oils inhibited the growth of *Alternaria alternata*; however, CLO was more effective than GO. CLO showed to be about 4 times more effective than GO. Microcapsules of CLO and GO maintained antifungal activity in a concentration-dependent pattern.

For both microencapsulated oils, the inhibition increased as the microcapsule concentration in the agar medium increased. For CLO, microcapsules concentrations ranging from 0.005 to 0.025 g complex/mL of agar were compared with a free oil concentration of 0.005 g/mL, GO was tested at a free oil concentration of 0.005 g/mL, and the tested microcapsules concentrations were 0.005, 0.025, and 0.045 g/mL of agar. Both free and complexed CLO inhibited the growth of *Alternaria alternata* more effectively than free and complexed GO, at all the concentrations studied. When considering the total oil concentration present in the microcapsules, similar amounts of the major constituents in the free and encapsulated oils were needed to get similar antifungal inhibition values (i.e. at the highest CLO:CD microcapsule concentration, the total amount of CLO oil present is 0.004 mg/mL, similar to that of the free oil). However, other non major constituents can be involved in the showed antifungal capacity of the microcapsules. Therefore, the essential oil complexes in β-CD showed good antifungal activity at low concentrations by delaying *A. alternata* growth.

Singh et al. [27] demonstrated that *Cinnamomum zeylanicum* bark oil had fungitoxic properties against fungi involved in respiratory tract mycoses such as *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Aspergillus flavus*. Huang and Ho [28] reported that a methylene chloride extract of cinnamon, (*Cinnamomum aromaticum* Nees), showed insecticidal activity toward *Tribolium castaneum* (Herbst) and *Sitophilus zeamais* Motsch. Valverde et al. [29] reported a reduction on total mesophilic aerobic, mold and yeast counts after 35 days in table grapes treated with eugenol, thymol, or menthol, with a more effective reduction of yeast and mold counts than the mesophilic aerobes.

Park and Shin [30] reported that garlic and clove bud oil had antitermitic activities. By GC-MS analysis, allyl disulfide, allyl trisulfide and eugenol were reported as the major constituents for garlic and for clove bud oil, respectively. The most toxic compound to the Japanese termite was allyl trisulfide, followed by allyl disulfide and eugenol, whereas allyl sulfide and β-caryophyllene showed weak antitermitic activity. The lipophilic character of the compounds used in this study suggests interactions with microorganism membranes; in fact, the hydrophobicity of these molecules enables them to partition the lipids of the bacterial cell membrane, thus disturbing its structure and rendering it more permeable [3].

In general, 0.025, and 0.045 mg/mL for CLO and GO microcapsules were the concentration that showed the lowest fungal growth for both microencapsulated oils, respectively. This proved that the antimicrobial property of the essential oils is preserved after the microencapsulation process and that the microencapsulated oil constituents were enough to preserve the antifungal activity against *A. alternata.* In this context, to avoid using synthetic preservatives in foods, the microencapsulated CLO and GO might be an adequate alternative to synthetic chemical additives [31].

The use of non synthetic chemicals to enhance the safety of many foods is of great interest to the food industry. Chemical preservatives vary in their ability to kill or retard growth of microorganisms. Effectiveness depends on the types of microorganisms and the physical and chemical characteristics of foods. However, the presence of chemical residues in foods and the labeling of preservatives on food packages are major concerns to consumers these days. Therefore, the need for natural products and naturally derived compounds with antimicrobial properties has to be explored [3].

Conclusion

CLO and GO microcapsules can be successfully produced using β-CD. Infrared and moisture sorption isotherms on the solid complex demonstrated that microcapsules are molecular inclusion complexes mainly formed by hydrogen bonding between oil and β-CD. Microcapsules of CLO showed a higher antifungal activity than GO. Isotherm data showed that stability of essential oils could be affected by water uptake in the β-CD surface. This complex with antimicrobial active substances can have important applications in the food industries, because of an improvement of stability, solubility, and bioavailability of the guest

molecules in the inclusion complex avoiding growth of deleterious microorganisms.

Future research has to be done on the application of these capsules. Controlled released of the microencapsulated compounds through water interaction may be hypothesized. If controlled release of the antimicrobial is possible, this system could be used to generate active packaging materials. Another important aspect that needs to be studied is the combination of flavors between the microcapsules and the treated food, therefore sensorial assays will need to be done.

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