Production, Characterization, and Stability of Orange or Eucalyptus Essential Oil/β-Cyclodextrin Inclusion Complex

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Abstract: The aim of this study was to produce and characterize inclusion complexes (IC) between β-cyclodextrin (β-CD) and orange essential oil (OEO) or eucalyptus essential oil (EEO), and to compare these with their pure compounds and physical mixtures. The samples were evaluated by chemical composition, morphology, thermal stability, and volatile compounds by static headspace-gas chromatography (SH-GC). Comparing the free essential oil and physical mixture with the inclusion complex, of both essential oils (OEO and EEO), it was observed differences occurred in the chemical composition, thermal stability, and morphology. These differences show that there was the formation of the inclusion complex and demonstrate the necessity of the precipitation method used to guarantee the interaction between β-CD and essential oils. The slow loss of the volatile compounds from both essential oils, when complexed with β-CD, showed a higher stability when compared with their physical mixtures and free essential oils. Therefore, the results showed that the chemical composition, molecular size, and structure of the essential oils influence the characteristics of the inclusion complexes. The application of the β-CD in the formation of inclusion complexes with essential oils can expand the potential applications in foods.

Keywords: β-cyclodextrin, essential oil, inclusion complex, volatile compounds

Introduction

Cyclodextrins (CDs) are nonreducing cyclic oligosaccharides composed of 6 (α-cyclodextrin), 7 (β-cyclodextrin), or 8 (γ-cyclodextrin) glucose units joined together by α-1,4 glycosidic linkages (Kurkov and Loftsson 2013; Yao and others 2017). The cyclodextrins are obtained from the enzymatic degradation of starch by the enzyme cyclodextrin glycosyltransferase (Abarca and others 2016), which catalyzes 4 types of reactions, the hydrolysis, transglycosylation/cyclization, coupling, and disproportionation (Dura and Rosell 2016). CDs have a peculiar structure with extremities that are essentially hydrophilic, which helps the solubilization in water and other polar substances while its interior cavity is hydrophobic (Zhu and others 2014). This characteristic is responsible for the formation of inclusion complexes with organic and inorganic substances, forming stable host-guest inclusion complexes by the force of hydrophobic and van der Waal's interaction (Aytac and others 2017; Chen and others 2018) and expanding the application of CDs in different areas including those related to food, pharmaceuticals, cosmetics, chemicals, agriculture, and biotechnology (Li and others 2014; Moriwaki and others 2014; Cheng and others 2017).

The formation and stability of the inclusion complex are dependent upon the compatibility between the cyclodextrin and the bioactive molecule. Therefore, the different types of cyclodextrins (α-CD, β-CD, and γ-CD) may have different capacities to imprison the same molecule; and thus, the kind of cyclodextrin used depends on the purpose for which it is intended and the type of molecule that it desires to host (Fathi and others 2014). Among the cyclodextrins, the β-CD is the most used, because its cavity allows it to host molecules with a molecular weight between 200 and 800 g/mol. Another factor that favors its use is the ease of production, a higher stability and lower water solubility, facilitating its separation from other cyclodextrins and reduction of its cost (Rakmai and others 2017; Shrestha and others 2017).

In the food area, cyclodextrins are mainly applied in the formation of inclusion complexes in substances with sensibility to high temperature, as essential oils that are aromatic oily plant extracts. The great interest in the food industry for these extracts is due to its antioxidant and antimicrobial activities, but its use as a natural food preservative and flavor is limited by its volatility and low water solubility (Kfoury and others 2014, 2015). The formation of the inclusion complex affects the physicochemical properties of guest molecules, as it increases its solubility in water, stability to light and temperature, provides protection against oxidation, masks or reduces undesired physiological effects, and reduces their volatility (Hill and others 2013; Kamimura and others 2014; Aytac and Uyar 2017).

The orange essential oil (*Citrus sinensis* (L.) Osbeck) consists of a mixture of terpenes, hydrocarbons, and oxygenates that, due to its unsaturated structure, consists mainly of terpenes and sesquiterpenes that are considered chemically unstable, and easily oxidized in the presence of air, light and moisture (Galvão and others 2015). The eucalyptus essential oil has a varied composition, since it has more than 900 species and subspecies, and is mainly composed of citronellal. The essential oil of *Eucalyptus citriodora* species has been used for several years by the pharmaceutical and cosmetic

industry, and its use in the food industry has increased, mainly as an antimicrobial agent, since it is classified as nontoxic and recognized for its broad biological action (Singh and others 2012). These compounds such as orange and *Eucalyptus citriodora* essential oils are very unstable due to their low molecular weight and high volatility, requiring alternatives of protection, with complexation into another chemical (Zhu and others 2014).

Although there are studies evaluating the formation of inclusion complexes with different essential oils including the orange essential oil, as Galvão and others (2015) have found who prepared inclusion complexes of *Citrus sinensis* essential oil as an alternative to control *Aedes aegypti* larvae, there are few studies comparing the ability of β-CD complexation with different oils. The same type of cyclodextrin can form inclusion complexes with different properties depending on the guest molecule characteristics, mainly due to its composition and molecular weight. Therefore, the aim of this study was to produce inclusion complexes between β-CD and essential oils from orange (β-CD-OEO) or eucalyptus (β-CD-EEO) as well as to compare the inclusion complex with the pure compounds and their physical mixtures. The samples were evaluated by chemical composition, thermal, morphological, entrapment efficiency, and the volatiles analysis of the inclusion complex by static headspace-gas chromatography (SH-GC).

Materials and Methods

Raw materials

Oranges of the Valencia variety [*Citrus sinensis* (L.) Osbeck] were collected in September 2015 located in the Southern Rio Grande do Sul, in the city of Pelotas, Brazil and eucalyptus leaves from mature trees were collected in June 2015 classified as *Eucalyptus citriodora* species, located in the southern Rio Grande do Sul, in the city of Canguçu, Brazil (latitude: 31°23'44"/longitude: 52°41'11"). The commercial β-CD (97% purity) was purchased from Sigma–Aldrich (U.S.A.).

Extraction of orange essential oil (OEO) and eucalyptus essential oil (EEO)

For the essential oil extraction process, the peels of oranges and leafs of eucalyptus were frozen and crushed, aiming to increase the contact surface, which results in a higher yield of the process. Then both essential oils were distilled by the hydrodistillation technique using a Clevenger apparatus. In a flask with a capacity of 2 L 300 g of crushed orange peel and 1 L of distilled water were added for the extraction of the orange essential oil; and 300 g of crushed eucalyptus leaf were added for the extraction of the eucalyptus essential oil. The flask was then connected to a Clevenger apparatus and heated at boiling temperature by a heating mantle during 3 h. The orange and eucalyptus essential oils were stored at −20 ± 2 °C in an amber glass bottle until analysis.

Essential oil composition

The characterization of the orange (OEO) and eucalyptus essential oils (EEO) was carried out by gas chromatograph equipped with a flame ionization detector (GC-FID; Varian 3400CX, Palo Alto, Calif., U.S.A.). An aliquot of 1 μL of the EO hexane solutions was injected in the GC injection port at 250 °C in split mode (1:20). The OEO compounds were separated on a nonpolar capillary column of fused silica BPX 5 (25 m × 0.22 mm × 0.25 μm) (SGE, Australia). The initial column temperature was adjusted to 40 °C and held for 0.5 min. Then, there was a temperature increase at 4 °C/min to 80 °C, then to 230 °C by at 10 °C/min,

and kept under isothermal conditions for 2 min. The EEO compounds were separated on a nonpolar capillary column of fused silica Equity TM-5 (60 m × 0.20 mm × 0.20 μm) (Supelco, Bellefonte, Pa., U.S.A.). The initial column temperature was set at 40 °C and held for 1 min, then increased at 1 °C/min to 70 °C, then to 220 °C increasing at 10 °C/min and kept isothermal for 3 min. Hydrogen was used as carrier gas at a constant pressure of 10 and 25 psi, respectively. The detector temperature was maintained at 250 °C. A homolog series of n-alkanes were analyzed under the same chromatographic conditions to calculate the linear retention index (LRI).

The qualitative analysis was performed by a Shimadzu QP2010 Plus gas chromatograph coupled to a mass spectrometer (GC/MS, Shimadzu Corporation, Kyoto, Japan). For this analysis, the same chromatographic conditions described above were used and helium was the carrier gas. The ionization source was operated in an electron impact ionization mode at 70 eV and a mass analyzer with a scan range of 35 to 350 m/z. Analytes were identified based on comparison to the experimental mass spectra with the available National Institute of Standards and Technology (NIST) Library. Additionally, the experimental Linear Retention Indices (LRI) were compared with those available in the scientific literature. The percentage of the relative amount of each identified compound was obtained from the peak area obtained from the GC-FID.

Preparation of the inclusion complex and physical mixture

The β-CD-OEO and β-CD-EEO inclusion complexes were prepared by the precipitation method, according to Bhandari and others (1998) and Petrovic and others (2010), with some modifications. The process and proportions used were the same for both oils (orange and eucalyptus). A sample of 2 g of β-CD was dissolved in 50 mL of distilled water maintained at 35 °C on a hot plate and 1.5 g of essential oil was slowly added to the hot solution. The mixture was continuously stirred and maintained at 35 °C for 3 h. When the temperature decreased spontaneously to room temperature, the solution remained during the night under refrigeration at 5 °C. The cold precipitated material was recovered by vacuum filtration. The precipitate was washed with 99% ethanol and dried in an oven with forced air circulation at 50 °C during 24 h. The inclusion complex obtained was stored and refrigerated in sealed vials.

The physical mixtures (PM) between the orange essential oil or eucalyptus essential oil and β-CD (PMOEO, PMEEO, respectively), in the ratio of 1:1 (w/w), were homogenized in a mortar and pestle. Posteriorly, the PM was stored in a tightly closed bottle and kept under refrigeration (4 °C).

Scanning electron microscopy (SEM)

The morphologies of commercial β-CD, the inclusion complex of β-CD-OEO, inclusion complex of β-CD-EEO and physical mixtures were obtained in a scanning electron microscope using a 10 kV acceleration voltage and a magnification 2000 ×. The samples were placed on copper strips and attached to a blade and then covered with a gold film.

Thermal characterization

Thermal analysis of the samples was performed using a differential scanning calorimeter (DSC model 2010, TA Instruments, New Castle, Del., U.S.A.) using approximately 3 mg of the sample in aluminum crucibles under a dynamic nitrogen atmosphere

(50 mL/min), and a heating rate of 10 °C/min, in the temperature range of 30 to 300 °C.

The thermal stability of the β-CD, PM, inclusion complexes and essential oils were evaluated using a thermogravimetric analyzer (TGA, TA-60WS, Shimadzu, Kyoto, Japan). The samples (±5 mg) were heated in the temperature range of 30 to 600 °C, using alumina crucibles at a heating rate of 10 °C/min, under a dynamic nitrogen atmosphere (50 mL/min).

Fourier transform infrared (FTIR) spectroscopy

FTIR spectra were obtained on a spectrometer (IRPrestige21, Shimadzu, Kyoto, Japan), according to the method described by Zhang and others (2015) with minor modifications, in the range of 4000 to 400 cm^{-1}, a resolution of 4 cm^{-1}, and 16 scans. The solid samples (β-CD, PM, inclusion complexes) were ground and mixed thoroughly with KBr. Then, KBr discs were produced by compressing the mixed samples in a hydraulic press. The liquid sample (OEO and EEO) was prepared using the KBr window technique.

Volatile compounds

Analysis of static headspace by gas chromatography (SH-GC). For the SH-GC analysis 110 mg of inclusion complex was used in a 20 mL glass vial, according to Kfoury and others (2015). The PM and EOs free of orange and eucalyptus were evaluated considering the inclusion complex of the content of the EOs. The vials were then sealed with a silicone/PTFE septum and subjected to a water bath at 30 ± 0.1 °C. After the 30 min of equilibrium time 1 mL of headspace was sampled using a gas-tight syringe (SGE, Australia). It was then injected directly into the GC-FID injection port under the same conditions described in Section "Essential oil composition". The peak areas were obtained from chromatograms and the identification of the compounds was performed with comparison to an experimental LRI and those obtained from the GC/MS (Section "Essential oil composition").

Stability of β-CDEOs. The SH of the samples was evaluated on the day of preparation of the inclusion complexes and 7 and 14 d after that. The percentage of retention (r) of the EOs by β-CD was determined according to Kfoury and others (2015), with some modifications, by SH-GC at 25 °C and expressed as Eq (1).

$$r \quad (\%) = \left(1 - \frac{\sum ACD}{\sum A0} \right) \times 100 \qquad (1)$$

where $\sum A0$ and $\sum ACD$ are for the sum of peak areas of each EO component in the absence and the presence of CD, respectively.

Results and Discussion

Gas chromatography of the OEO and EEO

Analysis of the orange essential oil allowed the identification of 7 compounds; the limonene (95.95%) was the major component, followed by β-myrcene (1,76%), β-linalol (0.98%), α-pinene (0.52%), cis-cimene (0.44%), 4-thujene (0.30%), and finally, α-terpineol (0.05%). These results were similar to those found by Galvão and others (2015), who characterized the orange essential oil and also identified 7 compounds, where the limonene (96.30%) was the main component, followed by myrcene (2.11%), α-pyrene (0.66%), and including some other components at lower concentrations. According to Hashtjin and Abbasi (2015) the limonene

and myrcene compounds are the main constituents of the orange essential oil, representing 95% and 2%, respectively.

Ten compounds were identified for eucalyptus essential oil. The higher component was 1,8-cineole (65.59%), followed by p-cymene (8.37%), γ-terpinene (7.73%), α-pinene (6.99%) β-myrcene (4.06%), limonene (3.92%), β-pinene (1.46%), α-phellandrene (1.06%), where the remainder of the compounds were below 1.0% each. Elaissi and others (2011) analyzed the chemical composition of 20 different species of eucalyptus, including *E. citriodora*, and found 1,8-cineole (54.0%) and α-pinene (23.0%) as the major constituents. The chemical composition of essential oils can vary between the same species due to the weather, location, type of soil, and the old leaves station; this is the method used for drying the leaves and essential oil extraction (Batish and others 2008).

Scanning electron microscopy (SEM) of inclusion complex and physical mixtures

The surface morphologies of the β-CD, as well as the inclusion complex (β-CD-OEO and β-CD-EEO) and physical mixtures (PMOEO and PMEEO), are shown in Figure 1A, B, C, D and E, respectively. The hydrophobic cavity of the β-CD has the ability to accommodate various kinds of guest molecules to form inclusion complexes with different morphology (Zhang and others 2015). The pure β-CD has a particle size and irregular shape (Figure 1A). On the other hand, the inclusion complex (β-CD-OEO and β-CD-EEO) showed changes in particle size and shape (Figure 1B and C, respectively), suggesting a change of the structure, which may indicate the formation of a complex, also differing from the structure observed in the physical mixtures (Figures 1D and E, respectively). A similar result was found by Galvão and others (2015), who also evaluated the morphology of an inclusion complex between β-CD and orange essential oil; they observed changes in the form and particle size when compared to the pure β-CD.

Wen and others (2016) evaluated the morphology of the inclusion complexes of β-CD and cinnamon essential oil and reported that the β-CD appeared with particles of different sizes in rectangular blocks, whereas the sample relating to the inclusion complex was presented in the form of a multilayered crystal. Thus, the different morphology of the inclusion complex and the β-CD indicates the formation of an inclusion complex.

Thermal characterization of the inclusion complex and physical mixtures

Differential scanning calorimetry (DSC). DSC thermal analysis is widely used for the evaluation of inclusion complexes. The DSC curves of all samples are shown in Figure 2A. The pure β-CD sample presented a single endothermic peak at 144.65 °C, which according to Wang and others (2011), is related to water loss inside the hydrophobic cavity of β-CD. The β-CD-OEO inclusion complex presented an endothermic peak at 159 °C less pronounced than pure β-CD. Zhang and others (2015) found a peak of reduced intensity in the inclusion complex of trans-anethole and β-cyclodextrin. They also suggested that part of the water that was initially present in the β-CD had been displaced by the oil, due to its greater affinity for the hydrophobic cavity of β-CD, confirming the complex formation.

The endothermic peak at 234 °C observed in the thermogram of orange essential oil was not detected in the thermograms of the β-CD-OEO inclusion complex; this indicates a greater stability of the OEO complex when compared to the free essential oil.
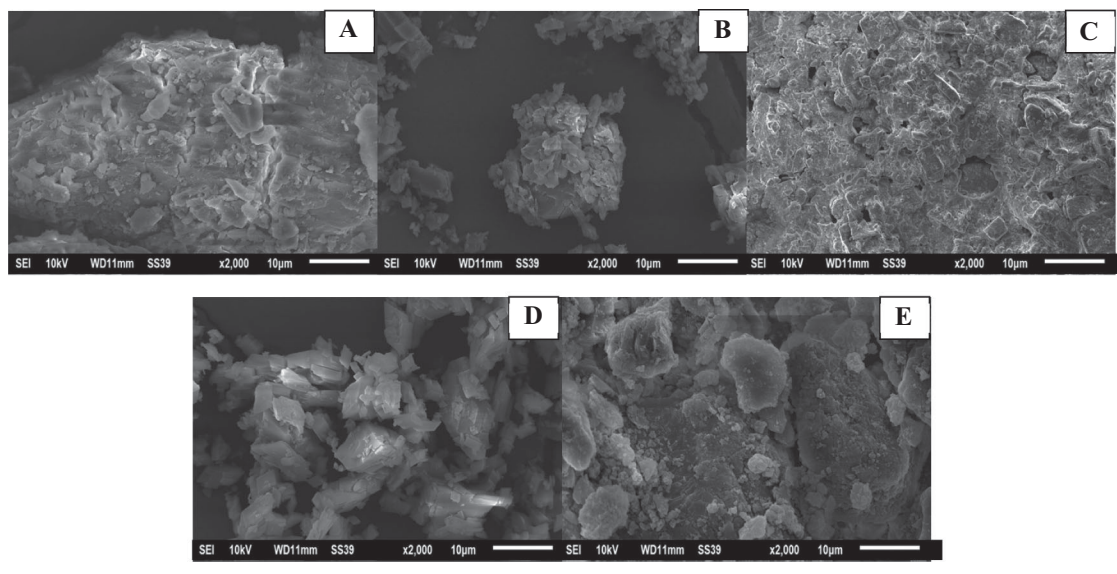
Figure 1–SEM of the β-CD (A) inclusion complex β-CD-OEO (B), inclusion complex β-CD-EEO (C) physical mixture PMOEO (D), and physical mixture PMEEO (E).

Tao and others (2014) also found an endothermic peak of 268 °C only in thyme oil. This peak was not observed in the thermogram of the inclusion complex, indicating that thyme oil had been protected in the hydrophobic cavity of β-CD, confirming the formation of the inclusion complex.

The β-CD-EEO inclusion complex presented a peak of high intensity at 135.07 °C, which might be attributed to the EEO (107.78 °C), and the peak shift evaporation, showing a higher thermal stability of this compound, compared to the essential oil free; there was also another endothermic peak at 145.14 °C, attributed to loss of water molecules of β-CD (Wen and others 2016). According to Mura (2015), modifications in the shape and temperature peak of the CD dehydration band, coinciding with the disappearance of the guest molecule melting peak, is related to the inclusion complex formation. Moreover, in the thermogram of the PMOEO the appearance of 2 endothermic peaks was observed, suggesting that there was not a good interaction between the β-CD and OEO. This demonstrates the precipitation method is necessary for the formation of the inclusion complex.

For the physical mixture (PMEEO), there were 2 endothermic peaks, one at 107.17 °C and another at 143.19 °C, which refer to the evaporation of the essential oil and release of the β-CD water molecules, respectively (Galvão and others 2015). According to Mura (2015), the presence of 2 peaks in the DSC curve of the physical mixture refers to the peak melting of the EEO molecules and β-CD dehydration.

In the thermogram of OEO, a peak at 234 °C was presented, probably related to the limonene, the major OEO component, confirmed by the analysis of GC-MS. Lower intensity peaks at 82 and 100 °C were also found relating to the volatilization of the remaining oil constituents. The EEO showed an intense endothermic peak at 107.78 °C corresponding to the evaporation process of essential oil compounds. However, it is noteworthy that endotherms start from 66.69 °C, coinciding with the high volatility of the compound (Abarca and others 2016).
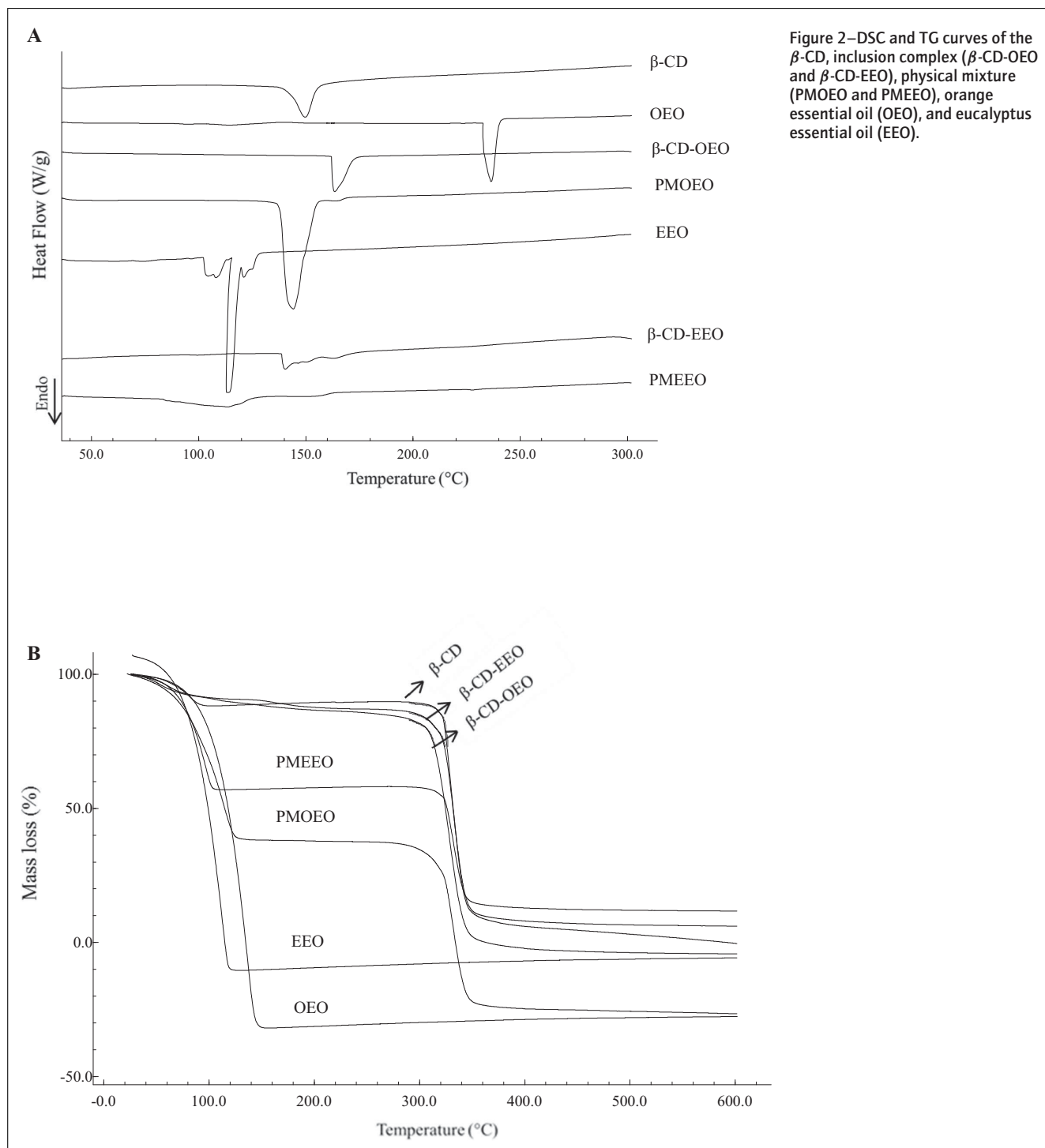
Thermogravimetric analysis (TG). The TG profiles of the β-CD, the inclusion complexes (β-CD-OEO and β-CD–EEO), the physical mixture (PMOEO and PMEEO) and the essential oils

(OEO and EEO) are shown in Figure 2B. The thermal decomposition of β-CD exits 2 weight loss steps at 60 to 90 °C and 324 to 341 °C. The first stage with a 9.86% mass loss can be attributed to the evaporation of the water binding to the β-CD and the second one is associated with the decomposition of the β-CD. At this point, 73.6% of the mass is reduced. According to Mura (2015) and Trotta and others (2000), the TG curves of β-CD are characterized by 2 separate weight losses. The first stag occurs at temperatures around 100 °C, and is due to the CD dehydration, while the second stage occurs at temperatures higher than 300 °C, due to the decomposition of the macrocycles.

The TG curves of the inclusion complexes (β-CD-OEO and β-CD-EEO) showed the same behavior, but the β-CD-EEO inclusion complex showed a higher thermal stability, with 75.1% of the mass loss and the temperature of decomposition around 323 °C; whereas the β-CD-OEO presented 82.3% of the mass loss with the temperature of decomposition in 315 °C.

The thermograms of the physical mixtures are very different from those of the inclusion complexes. The curve from both inclusion complexes show that the shift and decrease of the original small peak attributed to evaporation of water binding to the β-CD; this can be observed due to the redistribution and reduction of the type of water molecules and the water concentration, respectively. According to Zhang and others (2015), the substitution of the water molecules binding to the β-CD by an appropriate hydrophobic ligand is the principal "driving force" for complex formation. Furthermore, the decomposition peak of the inclusion complex in a higher temperature than that of physical mixture and pure β-CD indicates that the inclusion complex may be more thermally stable than the physical mixture and β-CD (Menezes and others 2012).

The TG curves of the physical mixtures show 2 weight loss steps at 30 to 150 °C for the both physical mixtures, related to the oil degradation and the second step in the 325 to 343 °C range for the PMEEO and in the 324 to 342 °C range for the PMOEO, similar to the stage of degradation of the β-CD. Due to the high volatility of the free essential oils (OEO and EEO), this showed 100% mass loss at 30 to 150 °C, attributed to the evaporation the essential

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A

B

Figure 2–DSC and TG curves of the *β*-CD, inclusion complex (*β*-CD-OEO and *β*-CD-EEO), physical mixture (PMOEO and PMEEO), orange essential oil (OEO), and eucalyptus essential oil (EEO).

oils. However, there is no evidence of the presence of the essential oils in the respective thermograms of inclusion complexes. This finding suggests the formation of inclusion complexes increasing the thermal stability of the essential oils when they are complexed with the *β*-CD (Mura 2015; Abarca and others 2016).

FTIR spectroscopy of inclusion complex and physical mixtures

The FTIR spectra of the inclusion complexes (*β*-CD-OEO and *β*-CD-EEO), physical mixtures (PMOEO and PMEEO), es-

sential oils (OEO and EEO) and the pure *β*-CD are presented in Figure 3. The spectrum related to the inclusion complex shows that there was a marked decrease of the band in 3408 cm^{-1} observed in the spectrum of the pure *β*-CD. Furthermore, the bands in 1447 and 1448 cm^{-1}, characteristics of OEO and EEO, respectively. These may be associated with C=C elongation vibrations of the olefinic groups of essential oils, where they were not observed in the spectrum of their inclusion complexes, indicating that these bonds could be involved in the formation of these complexes. Changes in the characteristic bands of the guest molecule,
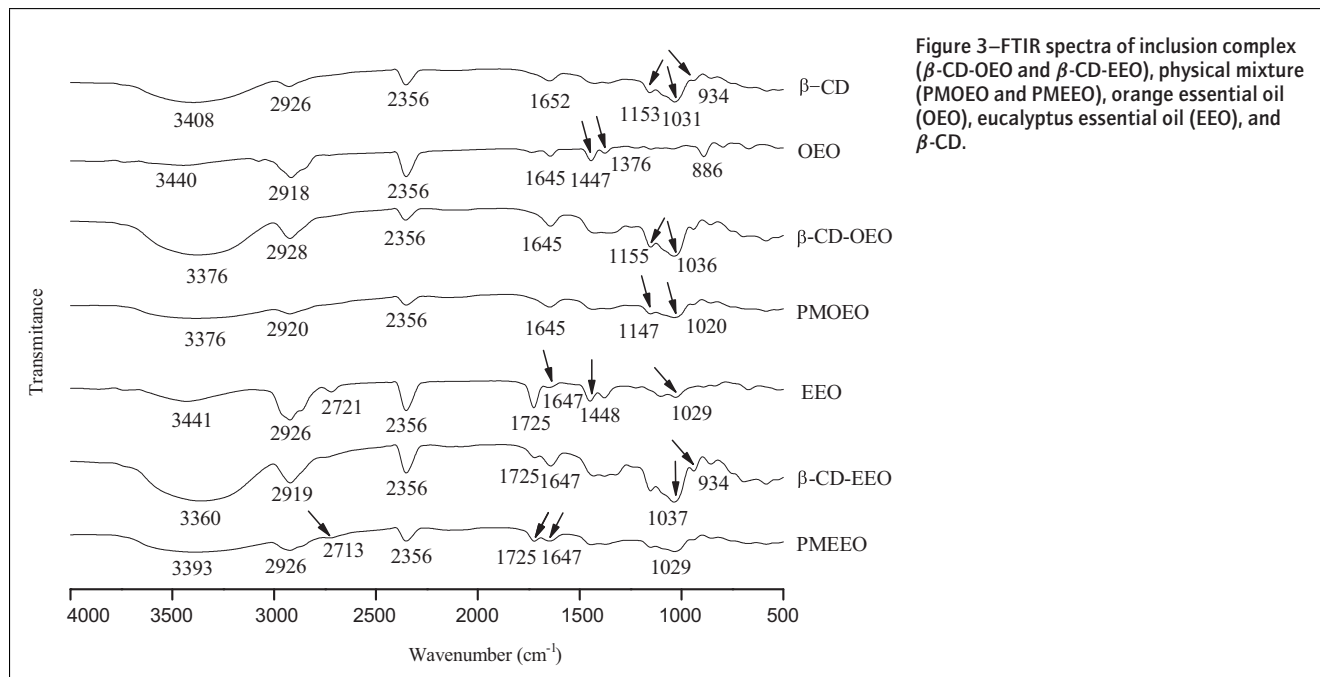
Figure 3–FTIR spectra of inclusion complex (β-CD-OEO and β-CD-EEO), physical mixture (PMOEO and PMEEO), orange essential oil (OEO), eucalyptus essential oil (EEO), and β-CD.

such as disappearance, broadening, and variations in peak intensity, are results of restriction of the stretching vibrations of the guest molecule caused by inclusion into the CD cavity (Mura 2015). According to Oliveira and others (2011), the insertion of the guest molecule into the β-CD cavity can cause a conformational constraint, which consequently reduces the movement of the encapsulated molecules, reducing the signal strength. A similar result was found by Galvão and others (2015), who evaluated the inclusion complex of the *Citrus sinensis* (L.) Osbeck essential oil and β-CD. These authors observed a reduction of the intensity of some bands characteristic of essential oil and disappearance of others, indicating the interaction of essential oil inside the cavity of the β-CD.

In the spectrum of the physical mixtures characteristic bands of both β-CD and essential oils (OEO and EEO) were observed, indicating almost no interaction between the molecules

(Figure 4). The FTIR spectrum of pure EEO showed characteristic bands at 3441 cm^{-1} corresponding to the stretching of the O–H alcohol components of the essential oil. The bands at 2721 and 1725 cm^{-1} correspond to the stretching modes of C–H and C=O, respectively, of citronellal. The absorption bands at 1647 and 1448 cm^{-1} may be associated with vibration of C=C stretching of olefinic groups of the essential oil and a double CH$_3$ and CH$_2$ antisymmetric curvature scissors. Various absorption bands common to all essential oil components were found, such as double bonds of methyl and methylene groups (Montenegro and others 2013).

The FTIR spectrum of pure β-CD showed characteristic bands at 3408 and 2926 cm^{-1} corresponding to stretching the vibration groups of O–H and C–H, respectively. Absorption bands at 1652, 1153, 1031, and 934 cm^{-1} correspond to H–O–H bending, stretching C–O groups, and the symmetrical C–O–C stretching
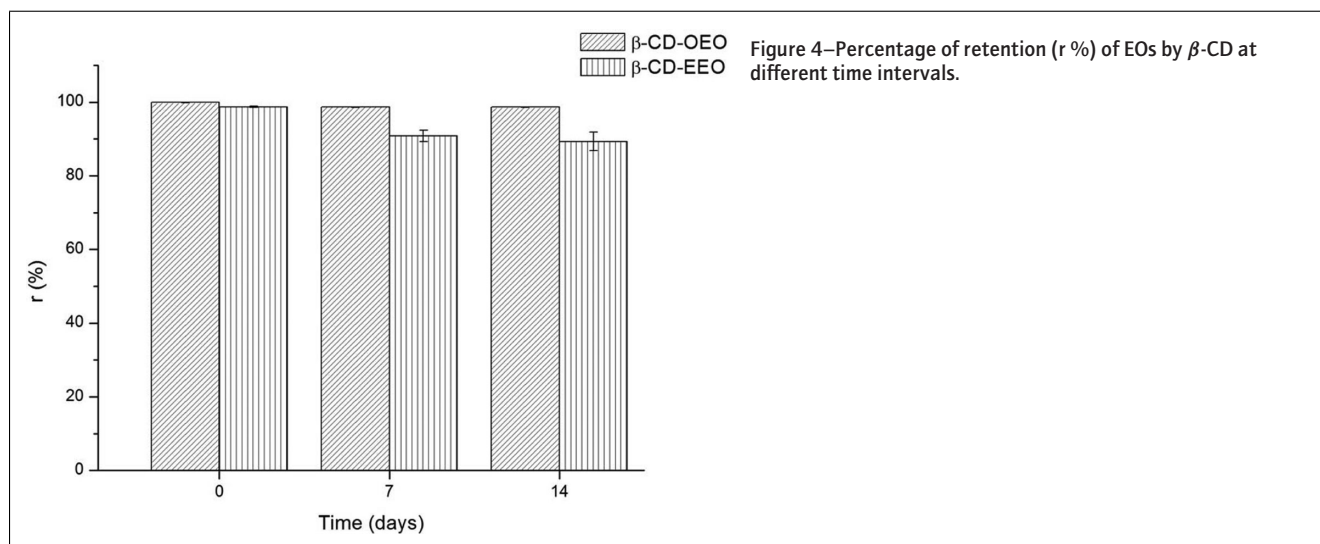

Figure 4–Percentage of retention (r %) of EOs by β-CD at different time intervals.

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vibration of α-1,4 linkages, respectively (Zhang and others 2015; Abarca and others 2016; Wen and others 2016).

Identification of the volatile composition of EOs

The area and the concentration of the volatile compounds, in relation to the total area of the peaks, of free essential oils, physical mixtures and the inclusion complexes, are shown in Table 1. For both essential oils (OEO and EEO) the chromatographic profile of the free essential oil, its physical mixture and inclusion complex, showed qualitative and quantitative differences; this is because each oil constituent can form an inclusion complex with greater or lesser affinity for the β-CD.

Moreover, the relatively low abundance of the compounds of the inclusion complex of both essential oils showed the high stability of the inclusion complex, with the loss of volatilization of only 0.02% for β-CD-OEO, 22.70% for PMOEO, 0.93% for β-CD-EEO, and 73.47% of PMEEO (Table 1). The difference in volatile components between the constituents of the essential oils can be justified by molecular size. When the guests have a similar molecular dimension, the more hydrophobic molecule or residue has the higher affinity for the CD cavity (Marques, 2010). Moreover, the binding between CDs and the aroma compounds depends on both hydrophobicity of the guest molecule and their geometric accommodation into the CD cavity. (Ciobanu and others 2013). Entrapment of the molecule inside the cavity is through chemical forces, such as van der Waals forces, dipole–dipole interactions, and hydrogen bonding (Gupta and others 2016).

Retention of EOs by β-CD

The retention of the EOs by the β-CD was quantified by comparing the sum of the chromatographic peak areas of the EO components in the presence of the β-CD to that of the blank experiment. Retention values are shown in Figure 4. The retention values showed that the EOs (OEO and EEO) were efficiently retained by the β-CD, due to the guests' retention after inclusion of the complex formation. This reflected the reduction of the vapor pressure and volatility of essential oils due to its inclusion within the cavity (Kfoury and others 2015).

According to Ho and others (2011) the release of guests from the solid inclusion complexes may be influenced by factors such as temperature and relative humidity. Kfoury and others (2016) showed in their study that an increase in temperature in a humid atmosphere accelerated the release of guests.

The results of this study also suggest that inclusion complexes with cyclodextrins would be an efficient and stable encapsulation even at high temperatures, due to the ability of the β-CD in the efficient retention of the essential oils that consequently protect them from evaporation, even 14 d after their elaboration. The β-CD-OEO showed 1.29% volatiles loss while the β-CD-EEO lost 9.45% of the volatiles after 14 d since they were produced. Therefore the β-CD-OEO was more stable than the β-CD-EEO (Figure 4). This difference is probably related to the higher affinity of the orange essential oil with the β-CD cavity, due to the limonene, a major component that is more apolar than the 1,8-cineole of the eucalyptus essential oil (Marques 2010). Moreover, the performance of the CDs depends on geometric complementarity (size and shape) between their cavity and the guest (Kfoury and others 2015).

According to Marques (2010), in general, the smaller the guest molecule the easier the complexation as long as interactions occur between the guest molecule and the host cavity. On the other hand, large molecules are dependent on the presence of a suitable group or ring capable of introduction into the cavity (1 or 2 benzene rings, or even larger ones, carrying an aromatic moiety, a side chain or other groups of comparable size); only some lipophilic part of the molecule is able to fit into the lipophilic CD cavity and not the entire molecule.

Decock and others (2008) studied the molecular modeling for 2 compounds with a similar hydrophobicity but from different chemical families, limonene and methyl heptine carbonate. They observed the weak empty space between the β-CD cavity; and limonene suggests that this CD is more suitable to accommodate such a substrate than α-CD and γ-CD. Regarding the sizes of cavities, for methyl heptine carbonate, a linear molecule, the empty space is larger than in the case of limonene. In this case, the α-CD is more fitting because of its smaller cavity size.

Ciobanu and others (2013) studied the controlled release of aromatic compounds from *Mentha piperita* essential oil by cyclodextrins (CD) and CD polymers in both the gaseous and phases. The β-CD presented a high retention for all studied aroma compounds in the aqueous phase. These results suggest that inclusion within the β-CD cavities gives protection against evaporation for aroma compounds.

Table 1–Area of the peaks and concentration (%) of the volatile components of essential oil, physical mixture (PM-OEO or PM-EEO), and inclusion complex (β-CD-OEO or β-CD-EEO) for orange essential oil (OEO) and eucalyptus essential oil (EEO).

Compound	OEO Area[a] (SD)	PM-OEO Area (SD)	β-CD-OEO Area (SD)
4-Thujene	1.99 (0.02)	0.73 (0.01)	
β-Myrcene	14.45 (0.39)	3.73 (0.05)	
cis-Ocimene	3.40 (0.07)	0.92 (0.01)	
Limonene	680.67 (16.29)	150.72 (2.30)	0.17 (0.003)
β-Linalol	2.81 (0.15)	2.39 (0.03)	
Total	708.40 (16.97)	160.75 (2.40)	0.17 (0.003)
Concentration (%)[b]	100	22.70	0.02

Compound	EEO Area[a] (SD)	PM-EEO Area (SD)	β-CD-EEO Area (SD)
p-Xylene	53.18 (3.51)	35.71 (8.07)	
α-Pinene	169.32 (20.21)	126.55 (18.88)	
β-Pinene	20.10 (7.72)	3.00 (0.79)	
β-Myrcene	27.93 (2.36)	14.36 (1.64)	
α-Phellandrene	17.30 (2.21)	8.48 (2.07)	
p-Cymene	49.67 (4.32)	36.82 (1.83)	1.01 (0.13)
NI	2.10 (0.21)	2.84 (0.53)	0.15 (0.01)
Limonene	29.36 (2.80)	28.66 (7.62)	0.82 (0.11)
1,8-Cineole	417.76 (28.75)	315.36 (68.94)	4.62 (0.71)
γ-Terpinene	3.77 (0.26)	10.23 (5.04)	0.88 (0.02)
Total	788.38 (58.24)	579.16 (58.70)	7.32 (0.95)
Concentration (%)[b]	100	73.47	0.93

[a]Mean of the absolute areas of 3 replicates multiplied by 10^4. SD: standard deviation.
[b]Concentration of the volatile components (%) in relation to the total area of the peaks.

Conclusions

The complexation of the β-CD with orange essential oil or eucalyptus essential oil was confirmed by the SEM, DSC, TG, and FTIR analyses. Both essential oils showed a higher stability when complexed with the β-CD rather than the free essential oils. The chemical composition, molecular size and structure the essential oils influenced directly the morphological and thermic characteristics of the inclusion complexes and their physical mixtures. The β-CD-OEO was responsible for higher volatile retention even after 14 d of storage.

In the inclusion complexes only 0.02% and 0.93% of the volatile compounds were identified for the β-CD-OEO and β-CD-EEO, respectively, when compared to 22.70% and 73.47% of their respective physical mixtures (PM-OEO and PM-EEO). Thus, the application of β-CD in the formation of inclusion complexes showed a high potential for the applications of essential oils as highly volatile compounds in foods.

Acknowledgments

References

Abarca RL, Rodríguez FJ, Guarda A, Galotto MJ, Bruna JE. 2016. Characterization of beta-cyclodextrin inclusion complexes containing an essential oil component. Food Chem 196:968–75.

Aytac Z, Uyar T. 2017. Core-shell nanofibers of curcumin/cyclodextrin inclusion complex and polylactic acid: enhanced water solubility and slow release of curcumin. Intl J Pharm 518:177–84.

Aytac Z, Ipek S, Durgun E, Tekinay T, Uyar T. 2017. Antibacterial electrospun zein nanofibrous web encapsulating thymol/cyclodextrin-inclusion complex for food packaging. Food Chem 223:117–24.

Batish DR, Singh HP, Kohli RK, Kaur, S. 2008. Eucalyptus essential oil as a natural pesticide. For Ecol Manage 256:2166–74.

Bhandari BR, D'Arcy BR, Bich LLT. 1998. 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 46:1494–9.

Chen J, Cao S, Xi C, Chen Y, Li X, Zhang L, Wang G, Chen Y, Chen Z. 2018. A novel magnetic β-cyclodextrin modified graphene oxide adsorbent with high recognition capability for 5 plant growth regulators. Food Chem 239:911–9.

Cheng J, Hu Y, Luo Z, Chen W, Chen H, Peng X. 2017. Preparation and properties of octenyl succinate β-cyclodextrin and its application as an emulsion stabilizer. Food Chem 218:118–21.

Ciobanu A, Mallard I, Landy D, Brabie G, Nistor D, Fourmentin S. 2013. Retention of aroma compounds from *Mentha piperita* essential oil by cyclodextrins and crosslinked cyclodextrin polymers. Food Chem 138:291–7.

Decock G, Landy D, Surpateanu G, Fourmentin S. 2008. Study of the retention of aroma components by cyclodextrins by static headspace gas chromatography. J Incl Phenom Macrocycl Chem 62:297–302.

Dura, A., Rosell, C. M. (2016). Physico-chemical properties of corn starch modified with cyclodextrin glycosyltransferase. Intl J Biol Macromol 87:466–72.

Elaissi A, Salah KH, Mabrouk S, Larbi KM, Chemli R, Harzallah-Skhiri F. 2011. Antibacterial activity and chemical composition of 20 Eucalyptus species' essential oils. Food Sci 129:1427–34.

Fathi M, Martin A, Mcclements DJ. 2014. Nanoencapsulation of food ingredients using carbohydrate based delivery systems. Trends Food Sci Technol 39:18–39.

Galvão JG, Silva VF, Ferreira SG, França FRM, Santos DA, Freitas LS, Alves PB, Araújo AAS, Cavalcanti SCH, Nunes, RS. 2015. β-cyclodextrin inclusion complexes containing *Citrus sinensis* (L.) Osbeck essential oil: an alternative to control *Aedes aegypti* larvae. Thermochim Acta 608:14–9.

Gupta S, Khan S, Muzafar M, Kushwaha M, Yadav AK, Gupta AP. 2016. Encapsulation: entrapping essential oil/flavors/aromas in food. In: Grumezescu AM, editor. San Diego, USA: Encapsulations Academic Press. p 229–68.

Hashtjin AM, Abbas S. 2015. Nano-emulsification of orange peel essential oil using sonication and native gums. Food Hydrocoll 44:40–48.

Hill LE, Gomes C, Taylor TM. 2013. 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 51:86–93.

Ho BT, Joyce DC, Bhandari BR. 2011. Release kinetics of ethylene gas from ethylene-α-cyclodextrin inclusion complexes. Food Chem 129:259–66.

Kamimura JA, Santos EH, Hill LE, Gomes CL. 2014. Antimicrobial and antioxidant activities of carvacrol microencapsulated in hydroxypropyl-beta-cyclodextrin. LWT – Food Sci Technol 57:701–9.

Kfoury M, Auezova L, Greige-Gerges H, Fourmentin S. 2014. Complexation of estragole as pure compound and as main component of basil and tarragon essential oils with cyclodextrins. Carbohyd Polym 118:156–64.

Kfoury M, Auezova L, Greige-Gerges H, Fourmentin S. 2015. Promising applications of cyclodextrins in food: improvement of essential oils retention, controlled release and antiradical activity. Carbohyd Polym 131:264–72.

Kfoury M, Auezova L, Greige-Gerges H, Larsen KL, Fourmentin S. 2016. Release studies of trans-anethole from β-cyclodextrin solid inclusion complexes by multiple headspace extraction. Carbohyd Polym 151:1245–50.

Kurkov SV, Loftsson T. 2013. Cyclodextrins, review. Intl J Pharm 453:167–80.

Li Z, Chen S, Gu Z, Chen J, Wu J. 2014. Alpha-cyclodextrin: enzymatic production and food applications. Trends Food Sci Technol 35:151–60.

Marques HMC. 2010. A review on cyclodextrin encapsulation of essential oils and volatiles. Flavour Frag J 25:313–23.

Menezes PP, Serafini MR, Santana BV, Nunes RS, Quintans LK, Silva GF, Medeiros IA, Marchioro M, Fraga BP, Santos MRV, Araújo AAS. 2012. Solid-state β-cyclodextrin complexes containing geraniol. Thermochim Acta 548:45–50.

Montenegro LMP, Griep JB, Tavares FC, Oliveira DH, Bianchini D, Jacob RG. 2013. Synthesis and characterization of imine-modified silicas obtained by the reaction of essential oil of *Eucalyptus citriodora*, 3-aminopropyltriethoxysilane and tetraethylorthosilicate. Vib Spectrosc 68:272–8.

Moriwaki C, Mangolim CS, Ruiz GB, Morais GR, Baesso ML, Matioli G. 2014. Biosynthesis of CGTase by immobilized alkalophilic bacilli and crystallization of beta-cyclodextrin: effective techniques to investigate cell immobilization and the production of cyclodextrins. Biochem Eng J 83:22–32.

Mura P. 2015. Analytical techniques for characterization of cyclodextrin complexes in the solid state: a review. J Pharm Biomed Anal 113:226–38.

Oliveira VE, Almeida EWC, Castro HV, Edwards HGM, Santos HF, Oliveira LFC. 2011. Carotenoids and β-cyclodextrin inclusion complexes: Raman spectroscopy and theoretical investigation. J Phys Chem 115:8511–9.

Petrovic GM, Stojanovi´c GS, Radulovic NS. 2010. Encapsulation of cinnamon oil in β-cyclodextrin. J Med Plants Res 4:1382–90.

Rakmai J, Cheirsilp B, Mejuto JC, Torrado-Agrasar A, Simal-Gándara J. 2017. Physico-chemical characterization and evaluation of bio-efficacies of black pepper essential oil encapsulated in hydroxypropyl-beta-cyclodextrin. Food Hydrocoll 65:157–64.

Shrestha M, Ho TM, Bhandari BR. 2017. Encapsulation of tea tree oil by amorphous beta-cyclodextrin powder. Food Chem 221:1474–83.

Singh HP, Kaur S, Negi K, Kumari S, Saini V, Batish DR, Kohli RK. 2012. Assessment of in vitro antioxidant activity of essential oil of *Eucalyptus citriodora* (lemon-scented Eucalypt; Myrtaceae) and its major constituents. LWT – Food Sci Technol 48:237–41.

Tao F, Hill LE, Peng Y, Gomes CL. 2014. Synthesis and characterization of β-cyclodextrin inclusion complexes of thymol and thyme oil for antimicrobial delivery applications. LWT – Food Sci Technol 59:247–55.

Trotta F, Zanetti M, Camino G. 2000. Thermal degradation of cyclodextrins. Polym Degrad Stab 69:373–9.

Wang J, Cao Y, Sun B, Wang C. 2011. Physicochemical and release characterisation of garlic oil-β-cyclodextrin inclusion complexes. Food Chem 127:1680–5.

Wen P, Zhu D, Feng K, Liu F, Lou W, Li M, Zong M, Wu, H. 2016. Fabrication of electrospun polylactic acid nanofilm incorporating cinnamon essential oil/β-cyclodextrin inclusion complex for antimicrobial packaging. Food Chem 196:996–1004.

Yao D, Zhu Z, Cai H, Chen X, Sun W, Barba FJ, Li F, Shen W, Ding W. 2017. Inhibition of cyclodextrins on α-galactosidase. Food Chem 217:59–64.

Zhang W, Li X, Yu T, Yuan L, Rao G, Li D, Mu C. 2015. Preparation, physicochemical characterization and release behavior of the inclusion complex of trans-anethole and β-cyclodextrin. Food Res Intl 74:55–62.

Zhu G, Xiao Z, Zhou R, Zhu Y. 2014. Study of production and pyrolysis characteristics of a sweet orange flavor-β-cyclodextrin inclusion complex. Carbohyd Polym 105:75–80.

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