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ORANGE ESSENTIAL OIL INCLUSION COMPLEX WITH β-CYCLODEXTRIN

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ABSTRACT – Cyclodextrins due to its structure composed of internal cavities hydrophobic and essentially hydrophilic extremities has the ability to form inclusion complexes with various substances. Thus, the objective of this study was to produce an inclusion complex of β-CD with *Valência* orange essential oil (OEO), using the precipitation method and characterizing it through the scanning electron microscopy analysis (SEM) and differential scanning calorimetry (DSC), and to characterize the orange essential oil by gas chromatography-mass spectrometry (CG-EM). The *d*-limonene (95.56%) was the major component, followed by β- Myrcene (2.35%), octanal (0.55%), α-pinene (0.54%), β-linalol (0.45%), cyclohexene (0.29%) and finally, decanal (0.26%) of OEO. The results show that was possible to produce an inclusion complex between orange essential oil and β-CD. The inclusion complex showed greater interaction with the orange essential oil when compared to the physical mixture.

KEYWORDS: cyclodextrins, inclusion complex, orange essential oil.

1. INTRODUCTION

Cyclodextrins are cyclic oligosaccharides non reducing composed of six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) glucose units joined together by α-1,4 glycosidic linkages, obtained from the enzymatic degradation of starch by the enzyme cyclodextrin glycosyltransferase (Abarca et al., 2016). Cyclodextrins have a peculiar structure with its internal hydrophobic cavities and extremities essentially hydrophilic. This characteristic is responsible for the formation of inclusion complexes with organic and inorganic substances, altering its physical and chemical characteristics (Shao et al., 2014). The formation and stability of the inclusion complex is dependent upon the compatibility between the cyclodextrin and the bioactive molecule. Therefore, different types of cyclodextrins (α-CD, β-CD e γ-CD) may have different capacities to imprison the same molecule, and thus, the kind of cyclodextrin produced depends on the purpose for which it is intended and the type of molecule that is desired to host (Fathi et al., 2014). Among the cyclodextrins, the β-CD is the most used, because its cavity allows to host molecules in the molecular weight range of most molecules of interest, between 200 and 800 $g.mol^{-1}$, and still, another factor that favoring its use, is the reasonable price of this cyclodextrin compared to others (Tao et al., 2014).

In the food area, cyclodextrins are mainly applied in the formation of inclusion complexes in substances with sensibility to temperature, as antioxidants in essential oils (García-Segovia et al. 2011). The formation of the inclusion complex of such compounds increases its solubility in water, its

stability to light and temperature, provides protection against oxidation, masks or reduces undesired physiological effects and reduce their volatility (Aguiar et al., 2014).

Essential oils are aromatic oily plant extracts of great interest in the food industry due to its antioxidant and antimicrobial activities (Kfoury et al., 2015). The orange essential oil (*Citrus sinensis* (L.) Osbeck) consists of a mixing of terpenes, hydrocarbons and oxygenates, that due to its unsaturated structure, consisting mainly of terpenes and sesquiterpenes are considered chemically unstable, easily oxidized in the presence of air, light and moisture (Galvão et al., 2015). This fact justifies the increasing research looking for alternatives for the protection of the orange essential oil. The objective of this work was produce and characterize a β-CD inclusion complex with orange essential oil.

2. MATERIAL AND METHODS

2.1 Material

Oranges of Valencia variety [*Citrus sinensis (L.)* Osbeck] were collected in September 2015 in the city of Pelotas, Brazil. The commercial β-CD (97% purity) was purchased from Sigma- Aldrich (USA).

2.2 Extraction of orange essential oil (OEO)

For the essential oil extraction process the peels of oranges were frozen and crushed, aiming to increase the contact surface, which results in a higher yield of the process. The method used was to hydrodistillation using Clevenger extractor. In a flask with a capacity of 2 L was added 300 g of crushed orange peel and 1 L of distilled water, The flask was then connected to Clevenger equipment and heated until boiling temperature (100 °C) by a heating mantle during 3 h. The orange essential oil was dehydrated by filtration funnel with sodium sulfate (Na_2SO_4), and stored at -20 °C ± 2 in amber glass bottle sealed with parafilm until the time of analysis.

2.3 Gas chromatography of the orange essential oil

Analysis of orange essential oil was carried out by gas chromatography coupled to Mass Spectrometry (GC-MS), using capillary column nonpolar (30 m x 0.25 mm, film of 0.25 μm). The carrier gas used was helium, under flow 1.2 $mL.min^{-1}$. The temperature was maintained at 60 °C for 2 min and then gradually increased to 4 $°C.min^{-1}$ until reaching 220 °C, the total running time was 41 min. Mass spectra were recorded in the range of 30 to 450 m/z and the injected volume was 0.1 μL. The individual components were identified by matching their mass spectra, obtained by comparison with the internal library. The percentage composition has been obtained from the measurement of integration of the areas under the peaks.

2.4 Preparation of the inclusion complex and physical mixture

The inclusion complex between orange essential oil (OEO) and β-ciclodextrina (β-CD) was prepared by the precipitation method, according to the method described by Bhandari et al. (1998) and Petrovic et al. (2010), with some modifications. 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 decreases 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 ethanol P.A. and dried in an oven with forced air circulation 50 °C during 24 h. The obtained inclusion complex was stored refrigerated in sealed vials.

The physical mixture (PM) between the orange essential oil and β -CD, in the ratio of 1:1 (w/w), was homogenized in a mortar and pistil. Posteriorly, the PM was stored in tightly closed bottle and kept under refrigeration.

2.5 Characterization of inclusion complex and physical mixture

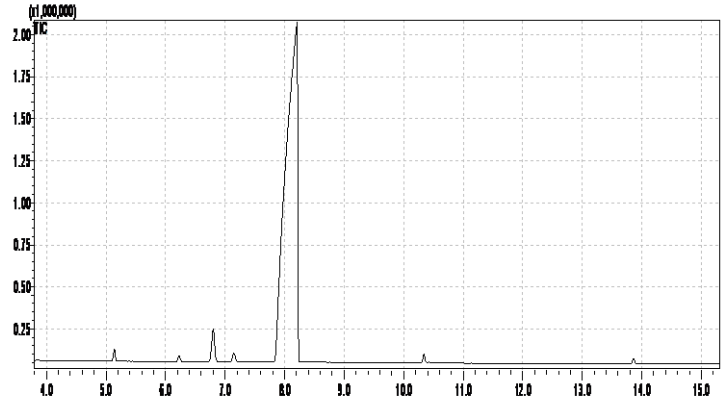
The morphologies of commercial β-CD, the inclusion complex of β-CD-OEO and physical mixture were obtained in a scanning electron microscope using a 10 kV acceleration voltage and a magnification 2000x. Thermal analysis of samples was performed using differential scanning calorimeter (DSC, 2920 TA Instruments) under an atmosphere of nitrogen with flow of 50 $mL.min^{-1}$. A sample of approximately 5.5 mg was weighed into a pan and subjected to heating 30 to 300 °C, under a heating rate of 10 $°C.min^{-1}$.

3. RESULTS AND DISCUSSION

3.1 Gas chromatography of the orange essential oil

Analysis of orange essential oil by GC-MS allowed the identification of 100% of the integrated components as shown in Figure 1. It was possible to identify seven constituents, the *d*-limonene (95.56%) was the major component, followed by β-myrcene (2.35%), octanal (0.55%), α-pinene (0.54%), β-linalol (0.45%), cyclohexene (0.29%) and finally, decanal (0.26%), very close result to the findings of Galvão et al. (2015). These authors characterized the orange essential oil and also identified seven compounds, being the limonene (96.30%) the main component, followed by myrcene (2.11%), α- pyrene (0.66%), including some other components at lower concentrations.

Figure 1. Chromatographic profile of orange essential oil.



According to Maróstica Júnior and Pastore (2007), the *d*-limonene is the main component of orange essential oil, and it can reach concentrations which may vary from 90 to 96%. Similar results were found by O´Bryan et al. (2008) who also reported that the *d*-limonene and *β*-mircene compounds are the main constituents of the orange essential oil, representing 93.96% and 2.12%, respectively.

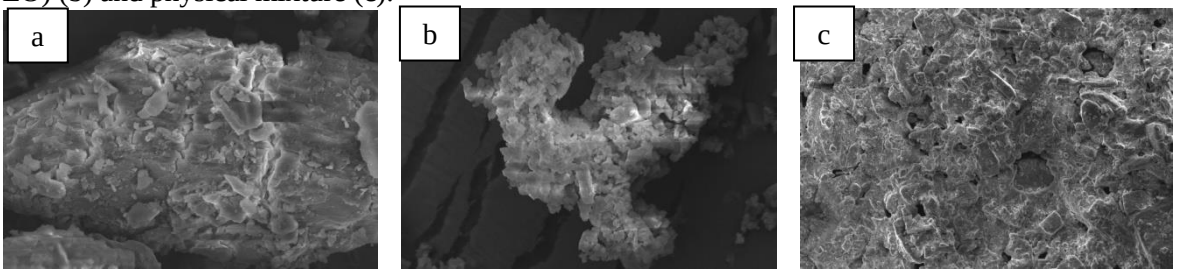
According to Gonçalves et al. (2015), citrus essential oils are characterized as mixtures of many components, including terpenes, sesquiterpenes, aldehydes, alcohols and esters, and can be

described as a mixture of terpene hydrocarbons, oxygenates compounds and non-volatile residues. However, factors such as the harvest season, the form of cultivation, weather conditions, genetics, extraction method, water stress, and the period and storage conditions can influence the chemical composition of essential oils (Dima & Dima, 2015). According to Calo et al. (2015), approximately 400 compounds have been identified in citrus essential oils, and the amount of these compounds depends mainly on the particular cultivar and citrus extraction and separation methods.

3.1 Morphology

The surface morphologies of the commercial β-CD, inclusion complex (β-CD-OEO) and physical mixture can be seen in Figure 2a, 2b and 2c, respectively.

Figura 2. Scanning electron microscopy (SEM) of commercial β-CD (a), inclusion complex (β-CD-OEO) (b) and physical mixture (c).



The pure β-CD has particle size and irregular shape (Figure 2a). On the other hand, the inclusion complex (β-CD-OEO) showed changes in particle shape (Figure 2b), suggesting a change of the structure, which may indicate the formation of a complex, also differing from the structure observed in the physical mixture (Figure 2c). A similar result was found by Galvão et al. (2015) who also evaluate the morphology of an inclusion complex between β-CD and orange essential oil and observed changes in the form and particle size when compared to the pure β-CD.

Wen et al. (2016) who evaluated the morpholgy (SEM) of inclusion complex of β-CD and cinnamon essential oil, reported that the β-CD appeared with particles of different sizes in rectangular blocks, whereas the sample relating to the inclusion complex presented in the form of a multi-layered crystal. Thus, the results showed that the morphology of the inclusion complex was relatively different from β-CD, demonstrating the formation of an inclusion complex.

3.2 Differential scanning calorimetry (DSC)

The thermal behavior of all materials examined is shown in Figure 2. The β-CD thermogram shows a sharp endothermic peak at 144 °C, which according to Wang et al. (2011) is related to water loss inside the hydrophobic cavity of β-CD. For β-CD/OEO an endothermic peak was observed at 159 °C less pronounced than pure β-CD. Similar results were found by Aguiar et al. (2014), that found a peak of reduced intensity in the inclusion complex of β-CD and cinnamon essential oil, suggesting that part of the water that was initially present in the β-CD have been displaced by the oil, due to its greater affinity for hydrophobic cavity of β-CD, confirming the complex formation. The endothermic peak at 234 °C observed in the thermogram of orange essential oil does not appear in β-CD/OEO, which indicates a greater stability of the OEO complexed when compared to the free essential oil. Tao et al. (2014) also found an endothermic peak of 268 °C only in thyme oil, this peak not being observed in the thermogram of inclusion complex, indicating that thyme oil has been protected in the hydrophobic cavity of β-CD, confirming the formation of the inclusion complex. Moreover, in thermogram of the PM was observed the appearance of two endothermic peaks, suggesting that there was not a good

interaction between β-CD and OEO. This demonstrates that is necessary the precipitation method for the formation of the inclusion complex.

In the thermogram of OEO (Figure 2), a peak at 234 °C was presented, probably related to the d-limonene, major component OEO, confirmed by the analysis of GC-MS (Figura 1), were also found lower intensity peaks at 82 °C and 100 °C relating to the volatilization of the remaining oil constituents.

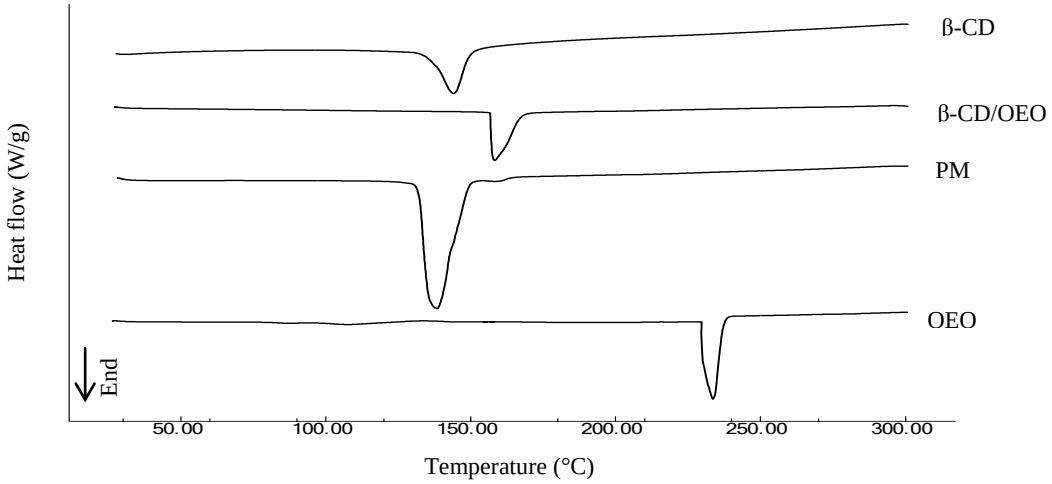


Figure 2. DSC thermogram of the β-CD samples, inclusion complex (β-CD/OEO) physical mixture (PM) and orange essential oil (OEO).

4. CONCLUSION

The main constituents of the OEO are *d*-limonene β-myrcene, octanal, α-pinene, β-linalol, cyclohexene and decanal. This work shows the formation of the inclusion complex between orange essential oil and β-CD, by precipitation method. The inclusion complex shows greater interaction with the orange essential oil when compared to the physical mixture.

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