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MICROENCAPSULATION OF CLOVE ESSENTIAL OIL WITH GELATIN AND ALGINATE

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Abstract. Clove essential oil has antioxidant and antimicrobial properties. To prevent chemical changes the oil is microencapsulated. The method used was emulsion extrusion, using a cross-linking agent, calcium chloride. The encapsulated materials can be used in leather industry. The wall material is build of alternative, inexpensive and natural polymeric, like gelatin and sodium alginate. Two microcapsules were developed: E130 and E131, with concentrations of 1% clove essential oil, 3% alginate and 0 - 1% gelatin, respectively. The microcapsules were characterized throughout morphology, encapsulation efficiency (EE), functional groups present and thermogravimetric analysis. Clove essential oil showed great antioxidant capacity of 85.37%, even with low content of total phenolic compounds (6.35 EAG mg g^{-1}). FTIR confirmed the incorporation of clove oil into the microcapsules and the chemical stability of the clove oil after encapsulation. The EE was 63% and 61% for E130 and E131, respectively. Furthermore, FTIR and thermogravimetric analysis indicated an interaction between the wall material and clove oil. Encapsulation process of clove essential oil by emulsion extrusion is an efficient technique to maintain the oil in the capsule.

1 Introduction

Essential oils have received commercial interest primarily because of their potential antimicrobial, antifungal and antioxidant properties and for being of natural origin, which generally means lower risk to the environment and human health[1]. But they are instable compounds and can suffer oxidation or volatilization or react with other formulation components[2]. Clove essential oil (CEO) not only contains many kinds of biological active substances but also has highly effective and comprehensive antibacterial functions, based on its constituents of phenolic compounds. Its essential oil contains mainly phenylpropanoids, such as eugenol, β-caryophyllene, and α-humulene organic compounds[3]. The high levels of eugenol contained in CEO are responsible for its strong biological and antimicrobial activities against a wide range of pathogenic microorganisms[4,5]. The CEO can replace some biocides, conventionally used in the leather industry, that are toxic and generate environmental risks. These biocides are used to prevent the development and growth of fungi in pickled hides, tanned leathers (from chromium or vegetable tannin) and finished products during their storage and shipping[6].

To prevent chemical changes in the essential oil, it is microencapsulated. The encapsulated materials are utilized in pharmaceutical, food, agricultural, cosmetic, textile, paper, paint, and printing industries, among others[7]. Microcapsules comprise an active agent surrounded by a natural or synthetic polymeric membrane providing isolation, entrapment, protection or controlled release. This controlled release allows to prolong its useful life, avoiding its rapid evaporation and improving its performance[8].

Emulsion extrusion is considered as the most common approach of microencapsulation and might be achieved by emulsifying or dispersing the hydrophobic components in an aqueous solution where gelation occur (ionotropic or thermal)[9]. By using emulsion extrusion for microencapsulation, abroad selection of polymer coatings (“shell”) and methods of deposition are available, which are

easily adaptable to large-scale production[10]. Polysaccharides and proteins are the most widely used wall materials for microencapsulation in industry.

Sodium alginate is a linear copolymer composed of α-L-guluronic and β-D-mannuronic acids, synthesized by brown algae found in coastal sea regions. This polymer has been used in the encapsulation of essential oils[11]. Gelatin is a biodegradable biomaterial[12] that can be extracted through the hydrolysis of hide and chromium tanned leather wastes[13]. Using this materials as wall for the microcapsule, is produced a new material from renewable sources, replacing synthetic polymers[14,15].

Microcapsules with natural antibacterial substances can be use in smart leather, which have additional functionalities. Smart scented leathers using orange and lavender oil encapsulates were developed [16] and a functional antimicrobial leather using polyurethane-microencapsulated clove oil was investigated [17], which can lead to value addition to leather.

The performance of antimicrobial agents against different fungis was evaluated[6]. For chrome leather, the antimicrobial agents 2-thiocyanomethylthio benzothiazole (TCMTB) and Aqueous dispersion of 2-n-octyl-4-isothiazolin-3-one + methyl-Nbenzimidazol- 2-ylcarbamate (OIT+BMC/water) showed antifungal capacity against different fungi tested applied in concentration of 0.2% (weight leather base). However, for vegetable tanned leather[6], the results revealed a low antifungal capacity of selected microbicides when applied at an offer of 0.2% (mass hide base) fungicides. Treatment with OIT+BMC/water (0.75%) showed satisfactory fungal protection against different fungi tested and proved to be the most suitable for the preservation of vegetable tanned leather. Thus, the development of ecological and effectively antimicrobial bactericides is essential.

The aim of this study was to develop essential oil microcapsules from sodium alginate and gelatin. The microcapsules will be characterized as morphology, encapsulation efficiency (%), functional groups present and thermogravimetric analysis.

2 Materials and Methods

2.1 Materials

Sodium alginate (Dinâmica, Brazil), Clove essential oil (Delaware, Brazil), Calcium chloride (Dinâmica, Brazil), were purchased and used as received. Gelatin type B (bloom 240), was donated from Gelita (Brazil). All other reagents were analytical grade and used without further purification.

2.2 Methods

2.2.1 Microencapsulation process

Microencapsulation of oil was performed using emulsion extrusion technique[10]. For the microcapsule with sodium alginate wall only, sodium alginate was dissolved in distilled water to produce alginate solutions with concentration of 3 w/v%. Afterwards, sodium alginate suspension and clove oil (1 w/v%) were homogenized into a 200 mL beaker with stirring at a speed of 300 rpm for 15 min by a magnetic stirrer. The oil was gradually added to the alginate suspension mixing until the desired oil loading was obtained. The alginate-oil emulsion was then dropped into a collecting water solution containing calcium chloride solution 1 w/v% using a syringe. The resulting microcapsules were allowed to harden in the $CaCl_2$ solution for 5 min. The oil-loaded alginate capsules were rinsed with distilled water and filtered. The microcapsule with sodium alginate and gelatin wall, the difference was the addition of the solution gelatin 1 w/v% in the water solution containing calcium chloride solution 1 w/v% (Table 1).

Table 1. Composition of the wall materials.

Assay	Wall material (g.100g^{-1} solution)	
	Alginate	Gelatin
E130	3	0
E131	3	1

Core material - clove essential oil: 1 g.100g^{-1} solution

2.2.2 Total phenolic content of essential oil

The total phenolic content of clove essential oil was determined by the Folin-Ciocalteau method [18]. Thus, 0.1 g of CEO was diluted in 10 mL of methanol and allowed to stand for 24 h. After that the sample was centrifuged and 500 µL of the supernatant was mixed with 4 mL of destilled water and 250 µL of Folin-Ciocalteau solution 10% (v/v) and react for 3 min. After, 500 µL of 1 M Sodium Carbonate solution was added. The reaction was stored in the dark for 1 h and was analysed at 725 nm in spectrophotometer T80+ UV/Vis (PG Instruments) using as a blank water and reagents only. Results were expressed as mg of gallic acid equivalents per gram of sample (GAE mg g^{-1}) and was determined in triplicate.

2.2.3 Antioxidant activity of essential oil

The antioxidant activity was determined using the scavenging of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical, a spectrophotometric methodology, according to the proposed method [19]. Antioxidant activity was expressed as percentage of DPPH scavenging and calculated using the following equation (1):

$$AA\ (\%) = \frac{Ac - As}{Ac} * 100 \quad (1)$$

where Ac is the absorbance of the control which contains control reaction (containing DPPH solution and methanol except the CEO), and As is the absorbance in the presence of CEO.

2.2.4 Encapsulation efficiency

The efficiency of encapsulation (EE) was determined by the presence of phenolic compounds in CEO, using the method proposed[19]. The amount of CEO encapsulated into the microbeads was quantified by extracting the loaded oil from 0.1 g of capsules via their dissolution with 10 mL of methanol and left for 24 h. After this period, the solution was centrifuged at 4000 rpm, for 15 min at room temperature, and 0.5 mL of the supernatant was collected for the analysis. Total phenolic content of collected fractions were evaluated as described in item 2.2.3. The EE was calculated according Equation 2, giving the percentage of phenolic compounds:

$$EE\ (\%) = \frac{Phenolic\ content\ of\ oil - Phenolic\ content\ of\ capsules}{Phenolic\ content\ of\ oil} * 100 \quad (2)$$

2.2.5 Characterization of the microcapsules

Fourier Transform Infrared spectroscopy of the microcapsules were recorded in the range 650-4000 cm^{-1} with 32 scans and 4 cm^{-1} of resolution on a Frontier ATR-FTIR spectrophotometer (Perkin Elmer, USA). Thermal analyses were carried out under N_2 atmosphere. Both the thermogravimetry (TG) and differential scanning calorimetry (DSC) (DSC 6000, Perkin Elmer) were used to investigate the thermostability of CEO and microcapsules. The temperature ranges were 40-800°C and 0-300°C, respectively, and the heating rate was 10°C min^{-1}. The morphology of microparticles was observed using stereo microscope (Model SZX16, Olympus) attached to a digital camera. Microparticles were placed onto a glass slide, observed under microscope and captured.

3 Results and Discussion

3.1 Characterization of clove essential oil

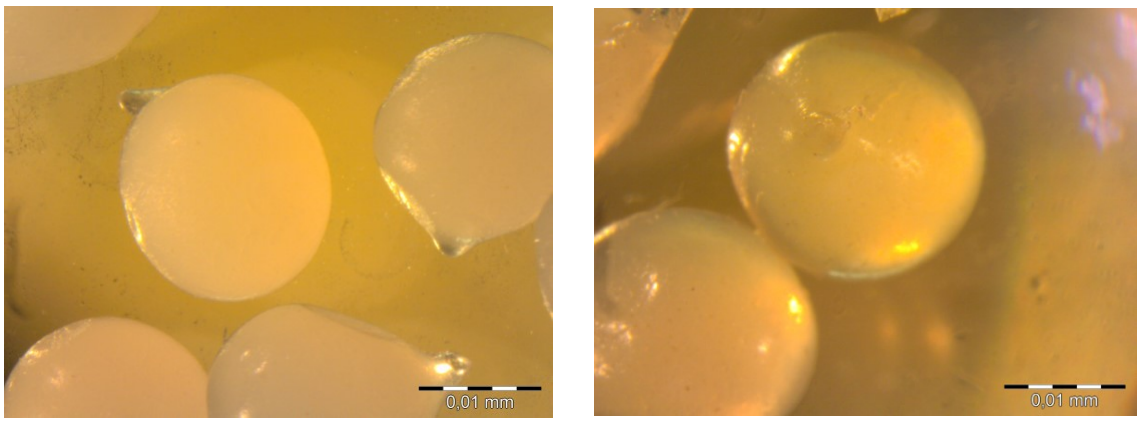
The total phenolic compounds content in the essential oil and its antioxidant capacity is presented in Table 2. Total phenolic compounds found is lower than 9.07 EAG mg g^{-1} [19] but was higher than 2.41 mg EAG g^{-1}, that showed a small concentration of phenolics[20]. These different values suggest that the concentration of phenolic compounds is dependent on the oil extraction method and characteristics of the sample. Antioxidant capacity of CEO at the concentration of 488 µg mL^{-1} was high. For instance, the literature report 45.27% of scavenging of DPPH at the concentration level of 500 µg mL^{-1} [21], while 94.86% was reported for the scavenging of DPPH at 484.7 µg mL^{-1} [19]. The high DPPH scavenging activity observed for the CEO can be explained by a synergistic effect between phenolic compounds, even at low concentrations[19].

Table 2. Total phenolic compounds and antioxidant capacity (%) of CEO.

Assay	Total phenolic (EAG mg g^{-1})	Antioxidant capacity (%)
CEO	6.35±0.5	85.37

3.2 Characterization of microcapsules

The microcapsules E130 and E131 (Fig. 1a and 1b) were regular and spherical and measured 2 mm.



(a) (b)

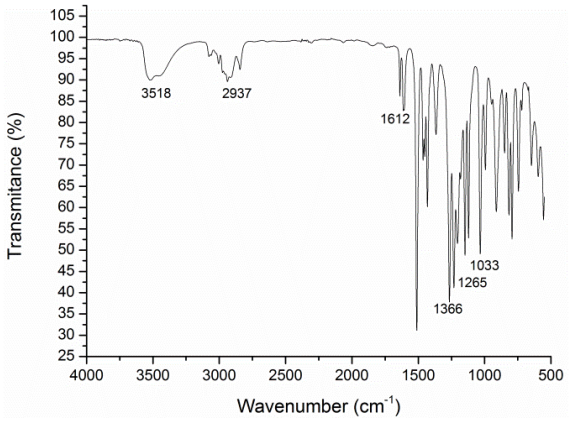
Fig. 1. Microscopy images (x40 magnification) of microcapsules: (a) E130; (b) E131.

The encapsulation efficiency (EE) of microcapsules is presented in Table 3. Microcapsule without gelatin shows better EE. EE results reported in the literature are dependent of the wall material, emulsifier and encapsulated oil. Clove essential oil was encapsulated with alginate (2%) and obtained EE of 90.02%[19], but when encapsulated with soybean phospholipids EE was 57.9% to 84.6%[5].

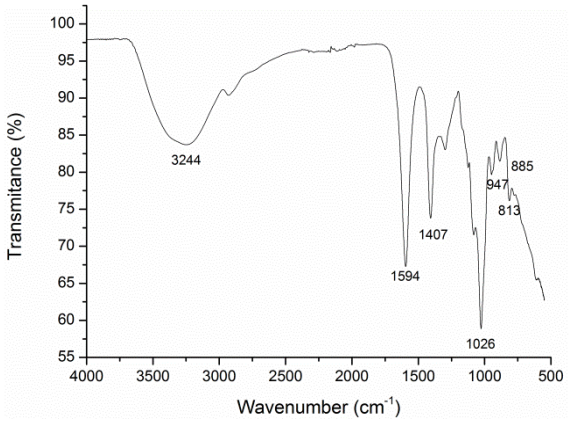
Table 3 – Encapsulation efficiency of microcapsules

Assay	EE (%)
E130	63.09
E131	61.92

The Fourier transform-infrared (FTIR) was used to confirm the presence of CEO, sodium alginate and gelatin in the microcapsules. FTIR spectra of CEO show (Fig. 1a) peaks at 3518 cm^{-1} of stretching vibrations O-H, at 2937, 1612, 1366 cm-1 attributed to C-H, C=C, O-H, respectively and some special peaks at 1265 cm^{-1} (C-OH axial) [22] and at 1033 cm^{-1} (C-O-C axial symmetric) [17]. The FTIR spectrum of gelatin is shown in Fig. 1b. The band appearing at 3279 cm^{-1} represented the N–H stretching vibrations of amide groups designated as amide A. The carbonyl C=O stretching vibrations with contributions from in-phase bending of the N–H bond and stretching of the C–N bond occurred at 1629 cm^{-1} and are referred as the amide I band. The amide II band appeared at 1526 and 1446 cm^{-1}. The small peak representing the amide III band was also found at 1236 cm^{-1} [23]. The FTIR spectrum of sodium alginate (Fig. 1c) showed characteristic absorption bands at 3244, 1594, 1407 and 1026 cm^{-1} that corresponded to stretching vibration of O−H, COO− (asymmetric), COO− (symmetric) and C−O−C. These similar findings were reported in the literature [24]. Besides, peaks at 947, 885 and 813 cm^{-1} are attributed to the C-H vibration of the pyranose (ring of the alginate) group[25]. Microcapsule E130 (Fig. 1d) presented peak at 3252 cm^{-1} (O-H vibration) due to the presence of the great amount of water. The peak at 1415 cm^{-1} is attributed to the asymmetrical stretching of COO-. The bands at 1081 and 1029 cm^{-1} correspond to guluronic units of C-H stretching present in sodium alginate. The peak at 947 cm^{-1} corresponds to the C-H group of the ring pyranose. The CEO is present at 1265 cm^{-1} (C-OH axial). In case of microcapsule E131, the spectra (Fig. 1e) showed peaks 1266 and 1033 cm^{-1}, the same peaks present in CEO, showing that the encapsulation did not alter the structure of the oil's main assets. In addition, the peak at 1637 cm^{-1} is characteristic of the $CONH_2$ group and indicate the interaction between alginate and gelatin. The results indicate the successful incorporation of clove oil into the microcapsules and the chemical stability of the clove oil after encapsulation.



(a)



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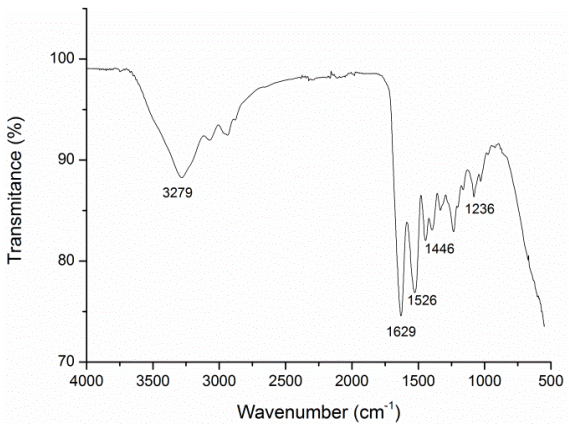

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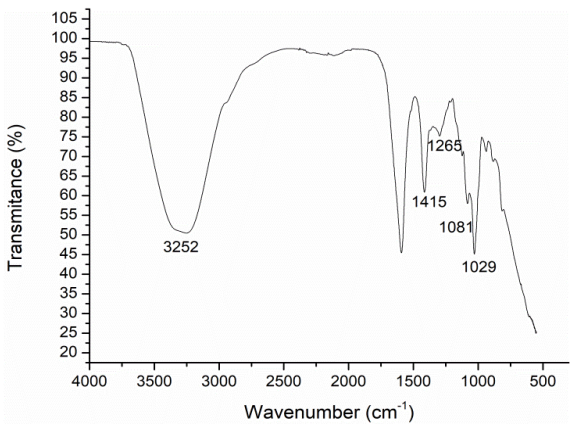
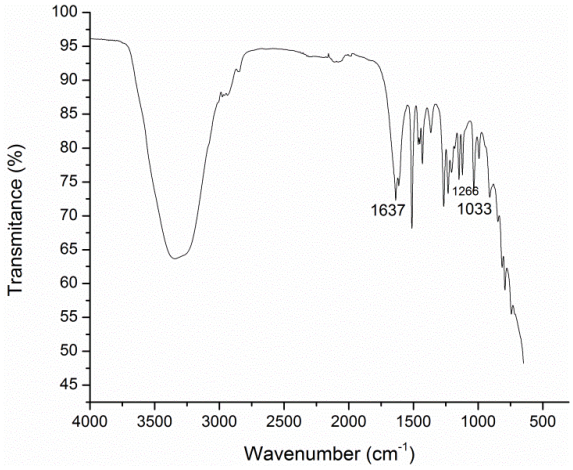


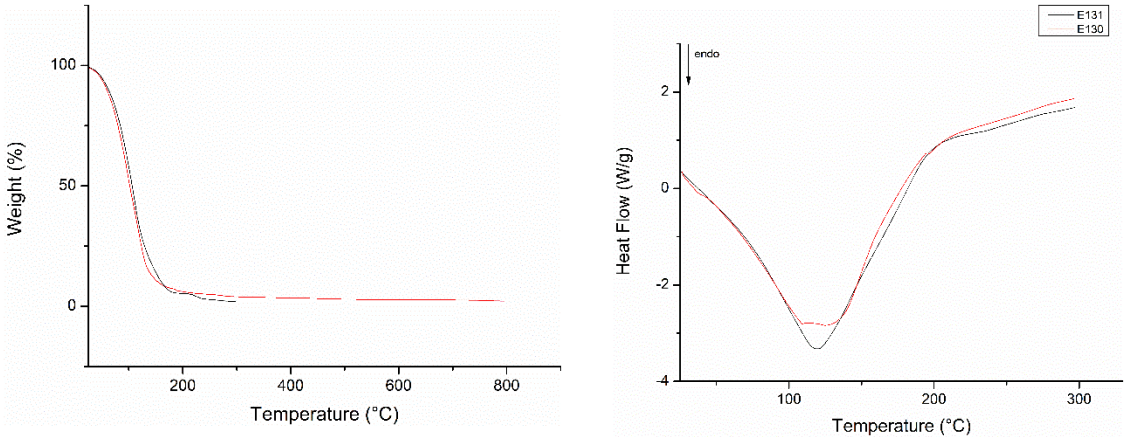
(d)



(e)

Fig. 1. FTIR spectra of (a) CEO, (b) sodium alginate, (c) gelatin, (d) E130 and (e) E131.

Thermal analysis (TGA and DSC curves) of E130 and E030 are given in Figure 3. TGA curve (Fig. 3a) showed the weight loss. This is related to moisture, corresponding to water hydrogen bounded to the saccharide structure of polymeric system[26]. The end of the thermal event occurs at a temperature above 100°C and this event may be associated with a bound water molecule. According to Lopes *et al.*[25], sodium alginate has two types of water in its structure, a portion of unbound water related to moisture and another portion of water bound to the polymer. The curve indicates that up to 100°C the samples contained a low relative tenor of organic matter, and starting from this temperature the samples degraded quickly during the heating up to 800°C. The presence of gelatin in the composition of microcapsules didn't improve the chemical stability of the microcapsules. DSC curve (Fig. 3b) indicates a thermal event between 30 and 100°C, characteristically endothermic. Thermal data of capsules analyzed by TGA was consistent with their DSC behavior. Initially, by increasing temperature, the samples lost weight until about 160°C. Accordingly, DSC measurements show the first endothermic peak below 150˚C (Fig. 3a). Endothermic peak of sodium alginate is presumably due to the cleavage of the carboxylate-calcium bond, formed in the reaction between sodium alginate and calcium chloride during encapsulation process[19]. Thus, an appropriate microencapsulation assures that the CEO remain viable throughout storage.



(a) (b)

Fig. 2. TGA (a) and DSC (b) curves of E130 and E131.

4 Conclusion

Clove essential oil has a great antioxidant capacity of 85.37%, even with low content of total phenolic compounds (6.35 EAG mg g^{-1}). Sodium alginate and gelatin showed great efficiency of clove essential oil encapsulation, 63.09% for E130 and 61.92% for E131. FTIR confirmed the incorporation of clove oil into the microcapsules and the chemical stability of the clove oil after encapsulation. Furthermore, FTIR and thermogravimetric analysis indicated an interaction between the wall material and clove oil. Encapsulation process of clove essential oil can be considered as inexpensive and efficient technique to maintain the oil in the capsule.

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