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Antimicrobial activity of microencapsulated lemongrass essential oil and the effect of experimental parameters on microcapsules size and morphology

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ABSTRACT

Lemongrass (*Cymbopogom citratus*) essential oil, known due to its broad-spectrum antimicrobial activity, was microencapsulated by simple coacervation. Poly(vinyl alcohol) (PVA, 78,000 Da and 88 mol% degree of hydrolysis) crosslinked with glutaraldehyde was used as wall-forming polymer. The influence of stirring rate and oil volume fraction on the microcapsule size distribution were evaluated. Sodium dodecil sulphate (SDS) and Poly(vinyl pirrolidone) were tested in order to avoid microcapsules agglomeration during the process. Depending on the experimental conditions, microcapsules in the range of 10 μm to 250 μm were obtained. Microcapsules presenting no agglomeration were obtained when SDS at 0.03 wt.% was used. The composition and the antimicrobial properties of the encapsulated oil were determined, demonstrating that the process of microencapsulation did not deteriorate the encapsulated essential oil.

1. Introduction

Many active agents present in cosmetics and food products (essential oils and flavors) are instable compounds. They can suffer oxidation or volatilization or react with other formulation components causing skin irritation. Microencapsulation is a feasible alternative to increase the stability of these compounds. There are several works available in the literature that discuss essential oil microencapsulation using methods as spray-drying [1–3] complex coacervation [4,5], simple coacervation [6–8] and extrusion [9]. The mayor advantage of simple coacervation over the other methods is that it readily allows the production of microcapsules containing hydrophobic substances, such as essential oils. In this method, the wall-forming polymer plays an important role because it is responsible for the protection of the encapsulated essential oil. Poly(vinyl alcohol) (PVA) is a hydrophilic polymer that can be used as wall-forming material in microcapsules. In presence of sulphuric acid, acetic acid and methanol, PVA can be crosslinked with glutaraldehyde, forming a hydrogel. Many drug delivery systems are based on hydrogels since they do not dissolve in water and maintain their three-dimensional networks [10]. PVA is also interesting because of its relatively simple chemical structure, ease of processing, and potential use in pharmaceutical and biomedical fields [11].

Studies on lemongrass (*Cymbopogom citratus*) essential oil have been reported in the last years due to its applicability in food and pharmaceutical industry. It can be use as a sedative [12] or as an antimicrobial agent [12–14]. The antimicrobial activity of the lemongrass essential oil was investigated by Onawunmi et al. [13], demonstrating that α-citral (geranial) and β-citral (neral) components individually exhibited antibacterial action on gram-negative and gram-positive organisms. Sacchetti et al. [15] evaluated eleven essential oils for the use as a food functional ingredient concluding that lemongrass essential oil presented the most broad-spectrum activity and showed satisfactory effectiveness. Its minimum inhibitory concentration (MIC) was comparable to that provided by the reference oil (*Thymus vulgaris*). The authors pointed out that geraniol and citral isomers should probably account for such efficacy.

The critical aspect in the microencapsulation of a given essential oil is to prevent the deterioration of the oil during the encapsulation step. Baranauskien et al. [1] observed changes in the composition of oregano, citronella and marjoram flavors after encapsulation into milk protein-based matrices by spray-drying. Those changes could be explained by loss of flavor during encapsulation and a high amount of non-entrapped flavors. Ramos [2] studied the therapeutic efficiency of copaiba oil encapsulated with gum arabic, showing that the efficiency of the essential oil was not affected by the encapsulation process.

There is only a few works in literature [16–18] on the encapsulation of lemongrass essential oil. Some aspects of the microencapsulation process still need to be better understood as well as the influence of the process on the oil characteristics. If the encapsulation is carried out by coacervation, it is of key importance to evaluate the antimicrobial activity of the oil after the process because potentially aggressive reagents are used, such as methanol, sulfuric acid and

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acetic acid. It is also important to know the influence of some operational parameters on the microcapsule size distribution because it directly influences end-use properties [6]. In addition, the mechanisms responsible for microcapsules agglomeration during coacervation must be better investigated because it may reduce encapsulation efficiency and influence release characteristics.

In the present work, the microencapsulation of lemongrass essential oil by the simple coacervation method was investigated, using crosslinked PVA as the wall-forming material. The influence of stirring rate and oil volume fraction on the microcapsule size distribution and on the release characteristics was evaluated. The agglomeration of the microcapsules was investigated using an ionic surfactant and a steric stabilizer. Observations using an optical microscope were carried out during the process to determine the moment in which the microcapsules begun to agglomerate. The microencapsulated essential oil was extracted by hydrodistillation and its composition and minimum inhibitory concentration were compared to those of the pure oil.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA, Polysciences Inc., Mw = 78,000 Da, degree of hydrolysis of 88 mol%) was used as the wall-forming polymer. Sodium sulphate (Nuclear, reagent grade) was employed as phase separation inducer. Lemongrass essential oil (EO) was purchased from Ferquima. A glutaraldehyde solution (Nuclear, 25 vol.%) was used as crosslinking agent under acidic conditions (sulphuric acid from Ecibra, anhydrous methanol and glacial acetic acid from Nuclear, all of analytical grade). Poly(vinyl pyrrolidone) (PVP, Sigma-Aldrich, Mw = 40,000 Da) was used as stabilizing agent. Sodium dodecil sulphate (SDS, Vetec, 99% purity) was used as surfactant. Ethanol (Nuclear, reagent grade) was used to wash the microcapsules. Sodium hydroxide (Vetec, reagent grade) was used to adjust the pH. All the reagents were used as received.

2.2. Microencapsulation procedure

All experiments were carried out in a 1000 ml borosilicate jacketed reactor (FGG Instruments Inc) using a propeller impeller. Reaction temperature was controlled by a thermostatic water bath using a j-thermocouple to measure the temperature inside the reaction vessel. The reagents used are presented in Table 1.

Initially, 600 ml of a 2 wt.% PVA aqueous solution were added to the reactor and temperature was set to 10 °C. Stirring rate was set according to the experiment (500, 700 or 900 rpm). Then, the essential oil (EO) was slowly added to the reactor. Nitrogen was used to remove atmospheric oxygen from the reaction medium. Subsequently, sodium sulphate (20 wt.% aqueous solution) was added. Temperature was increased at l °C/min to 50 °C (the phase separation temperature of the PVA in the solution is in the range of 35 °C–40 °C). When the reactor reached 50 °C, the crosslinking solution (glutaraldehyde, methanol, glacial acetic acid and sulphuric acid) was added. The system was allowed to react for 3 h.

Table 1
Reagents used in the lemongrass EO microencapsulation

Reagent	Mass (g)
Water	735.90
Lemongrass EO	23.90/54.00
PVA	12.00
Sodium sulphate	12.00
Glutaraldehyde	2.40
Sulphuric acid	0.30
Methanol	13.20
Acetic acid	5.25

After this, pH was adjusted to 7.0 using NaOH (0.5 N aqueous solution) and stored under refrigeration.

2.3. Microcapsules characterization

Optical observations of the microcapsules were carried out with the aid of an optical microscope (Bioval L-2000A) attached to a digital camera. The microcapsule size distribution was determined measuring the microcapsules diameter using an image analysis software. About 300 microcapsules were measured for each experiment.

2.4. Equilibrium degree of swelling (EDS)

Swelling behavior of the crosslinked microcapsules was determined by first removing the essential oil from the microcapsules with ethanol for 24 h. After this, the microcapsules were dried and put in 50 ml of distilled water for 24 h. The swollen samples were removed from water and their mass (Ws) was determined after removal of the excess of water with a filter paper. The sample was then dried in an oven until no mass variation could be detected. This mass was defined as dry polymer mass (Wd). The equilibrium degree of swelling (EDS) is expressed as the mass of water in the hydrogel per mass of the dry polymer.

$$\mathrm{EDS}\left(g_{H_2O}/g_{\mathrm{polymer}}\right) = (\mathrm{Ws}-Wd)/\mathrm{Wd} \quad (1)$$

2.5. EO release

A hydrodistillation apparatus (Clevenger) was used to evaluate the essential oil release from the microcapsules. The sample was filtered and washed three times with distilled water and one time with ethanol to remove any not encapsulated EO. The EO was collected from the apparatus at intervals of time. The released percentage was determined using Eq. (2), were m_∞ is the EO mass accumulated at the end of extraction and m_t is the mass accumulated in the respective interval of time.

$$\text{released oil } (\%) = (m_t \cdot 100)/m_\infty \quad (2)$$

2.6. Gas chromatography/mass spectrometry analysis

Essential oil composition was determined using a Varian CP-3800 gas-chromatograph equipped with a CP-Sil 8 CB Low Bleed/MS (30 m×0.25 mm) column. Equipment conditions were set as follows: injector temperature at 250 °C; Helium as carrier gas (flow rate of 1 ml/min); oven temperature initially at 50 °C and then raised to 240 °C at 3 °C/min. Quantification was computed as the percentage contribution of each compound to the total amount present. EO constituents were then analyzed by Mass Spectrometry — MS (ion trap temperature at 220 °C; manifold temperature at 80 °C, transfer line temperature at 240 °C). The MS fragmentation pattern was checked by matching the MS fragmentation patterns with NIST mass spectra libraries.

2.7. Antimicrobial assay

The antibacterial activity of the lemongrass EO was investigated by employing a microdilution method [19]. The assay was carried out with two bacterial species, including the Gram-negative bacteria *Escherichia coli* ATCC 25922 (American Type Culture Collection) and the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923. Both the pure EO and the microencapsulated EO (extracted from the microcapsules by hydrodistillation) were evaluated. 200 μl of dimethyl sulfoxide (DMSO) were added to 200 μl of sample previously sterilizated in an autoclave. Subsequently, 600 μl of Mueller Hinton broth were added. Serial dilutions were prepared in the concentration range from 0.349 mg/ml to 89.300 mg/ml. 100 μl of each dilution were distributed in 96-well plates, as well as the sterility control (growth control contained Mueller–Hinton broth and DMSO, without antimicrobial substance). Each test