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Physicochemical and antimicrobial properties of nanoencapsulated *Eucalyptus staigeriana* essential oil

Emanuele D. Herculano [b], Haroldo C.B. de Paula [a, *], Evânia A.T. de Figueiredo [b], Flayanna G.B. Dias [b], Vanessa de A. Pereira [a]

[a] Department of Analytical and Physical Chemistry, Building 940, Federal University of Ceará, Fortaleza, 60455-760, Ceará, Brazil
[b] Department of Food Science and Technology-DETAL, Building 858, Federal University of Ceará, Fortaleza, 60356-000, Ceará, Brazil

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ABSTRACT

In this work *Eucalyptus staigeriana* essential oil (ESO) was nanoencapsulated using cashew gum (CG) as wall material. The nanoparticles had their antimicrobial activity against *Listeria monocytogenes* (Gram-positive) and *Salmonella* Enteritidis (Gram-negative) evaluated by determining their Minimum Bactericidal Concentration, in addition to being characterized by infrared spectroscopy, thermal analysis, particle size distribution and zeta potential. Data from MBC showed greater activity against Gram-positive bacteria, due to a likely synergistic effect between the CG and ESO. The nanoparticles had sizes ranging from 27.70 nm to 432.67 nm and negatively charged surfaces. The ESO content varied between 4.76% and 7.12% and the encapsulation efficiencies from 24.89% to 26.80%. The aforementioned data suggest that ESO nanoparticles have potential for use as a natural food preservative.

1. Introduction

The nanoencapsulation of labile bioactive compounds represents an efficient alternative to increase their physical stabilities, protecting them from deleterious interactions with food components and the environment, besides increasing their bioactivity mainly owning to their nanometer size range (Donsì, Annunziata, Sessa, & Ferrari, 2011). Upon encapsulation, sample can now be easily handled because the active principle is protected against oxidation and other environmental harms. Moreover, its volatile ingredients are now retained in a particular matrix, exhibiting prolonged sensory perception, bioavailability and improved efficacy (Neethirajan & Jayas, 2011).

The extracts obtained from leaves of *Eucalyptus* have been approved as food additives, being also used in cosmetic formulations, although most studies have been focused on their functional properties (Gilles, Zhao, An, & Agboola, 2010).

Several properties have been assigned to the genus *Eucalyptus*, such as antimicrobial (Tyagi & Malik, 2011), antihyperglycemic (Gray & Flatt, 1998), antioxidant (Santos et al., 2012) and anthelmintic (Macedo et al. 2010). A number of researches have been done to demonstrate the antimicrobial properties of the *Eucalyptus* essential oils. However, these studies are concentrated in a few species, especially *Eucalyptus citriodora* that has a broad spectrum of antifungal activity. Only a few studies have been conducted to assess the activity against pathogenic and food spoilage bacteria, as well as yeast (Gilles et al., 2010).

Cashew gum is a heteropolysaccharide extracted from the exudate of *Anacardium occidentale*, an abundant tree in the Brazilian Northeast region, whose structure resembles gum Arabic. Cashew gum is able to interact with water and thus act as stabilizer, emulsifier and adhesive, and may be a suitable substitute for gum Arabic, which is more expensive (Mothé & Rao, 2000).

Taken into consideration the need for more research on the formulation and application of nanoparticles with natural antimicrobials in food, this work aimed at the preparation of nanoparticles of cashew gum (CG) loaded with *Eucalyptus staigeriana* essential oil (ESO), aiming to preserve the active ingredient and prolong its release to the medium. Moreover, Minimum Bactericidal Concentration of ESO, CG and ESO loaded nanoparticles on *Listeria monocytogenes* and on *Salmonella* Enteritidis bacteria were determined, in order to evaluate their antimicrobial actions.

* Corresponding author. Tel.: +55 85 3366 9961.
E-mail address: hpaula@ufc.br (H.C.B. de Paula).

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Furthermore, the nanoparticles were characterized regarding their physicochemical properties and the effect of the wall material and surfactant ratio on zeta potential, particle size, loading and encapsulation efficiency.

2. Materials and methods

2.1. Materials

E. staigeriana essential oil (FERQUIMA, Brazil), ethanol 99% and Tween 80 surfactant (VETEC, Brazil) were used as received. Cashew gum (1.1×10^5 g/mol) was extracted from native trees of Ceará, and purified as described by De Paula, Heatley, and Budd (1998).

2.2. Nanoparticle preparation

An organic phase was obtained by mixing Tween 80 (T80) and *E. staigeriana* essential oil in a vortex mixer (PHOENIX, Model AP 56, Brazil) under continuous stirring. The above mixture was dripped into an aqueous solution of cashew gum (CG) 2% (w/v) under constant stirring (18,000 rpm) in an ultrasonic homogenizer Turratec (TECNAL, model TE-101, Brazil) for 2 min. Three formulations were established: F.1, with ratios (m/m) for CG:ESO = 2:1 and ESO:T80 = 2:1; F.2, with CG:ESO = 4:1 and ESO:T80 = 2:1 and F.3, with CG:ESO = 2:1 and ESO:T80 = 1:1.

The emulsion obtained was fed into a Mini Spray Dryer B-290 (BÜCHI, Switzerland), at an inlet temperature of 160 ± 10 °C and an outlet temperature of 70 ± 10 °C, pump feed flow of 5 mL/min, air volume flow of 35 m^3/h, and aspirator flow 84 L/h (Paula, Sombra, Abreu, & De Paula, 2010). The nanoencapsulated sample was stored in amber glass at room temperature (25 °C).

2.3. Minimum Bactericidal Concentration

The Minimum Bactericidal Concentration (MBC) of the nanoparticles was determined in order to evaluate its antimicrobial action. The nanoparticle concentrations tested ranged from 0.5 g/L to 7 g/L of the active ingredient (D-limonene). It was also determined the MBC of bare essential oil, and of cashew gum solutions. As controls were employed culture media and culture media + bacterial inoculum. The experiments were performed in duplicate on three different days ($n = 6$) according to Sahm, & Washington (1991). Briefly, selected strains of Gram-positive pathogenic bacteria, *L. monocytogenes* (ATCC 19115- Microbiologics) and Gram-negative, *Salmonella* Enteritidis (IAL 1132- Adolfo Lutz Institute, Brazil) were used. The lyophilized strains were activated as recommended by the manufacturer.

In test tubes containing 3.95 mL of Tryptone Soy Broth (TSB/Difco) sterile for *L. monocytogenes* and Infusion Brain Heart Broth (BHI/Difco) for *S.* Enteritidis was added 50 μL of bacterial suspension in concentration of 10^5 UFC/ml. For each culture, were added separately the *E. staigeriana* essential oil, the nanoparticle solution and cashew gum solution. The tubes were incubated at 35 °C for 24 h under stirring at 200 rpm.

After incubation, 100 μL of culture were inoculated on plates containing the Listeria Oxford Medium (OXA/Oxoid) for *L. monocytogenes* and Hecktoen Enteric Agar (HE/Difco) for *S.* Enteritidis, and spread with handle Dringalsky. The plates were incubated at 35 °C for 24 h and observed for the presence of bacteria growth. The lowest concentration at which no growth occurred was taken as the MBC. Determination of Minimum Inhibitory Concentration was not possible due to the occurrence of turbidity of the medium when the samples were added.

2.4. Nanoparticles characterization

2.4.1. GC–MS analysis

The composition of ESO was determined by gas chromatography-mass spectrometry (GC–MS) using an SHIMADZU equipment model QP2010 SE, (Japan), equipped with a capillary column Rtx-5MS, Restek, Germany (30 m, ID 0.25 mm, film thickness 0.25 μm) and auto injector AOC-20i. Helium was used as carrier gas (1 mL/min). The initial column temperature was kept at 60 °C for 3 min and then was increased to 280 °C at a rate of 3 °C/min. The mass selective detector (SHIMADZU) was used in the electron ionization mode (70 eV), the mass range between 35 and 500. Ion source (260 °C) and transfer line (300 °C) temperatures were employed throughout analyses.

2.4.2. Essential oil loading and encapsulation efficiency

The encapsulated oil content was determined by absorption spectroscopy in the UV–Vis region (Micronal, model B582, Brazil), at a wavelength of 210 nm, according to Paula et al. (2010). Oil concentration was determined by crushing a 10 mg sample in ethanol and calculating its concentration using a calibration curve (Eq. (1)). All analysis were performed in triplicate and conventional statistical methods (ANOVA, followed by comparison with the Tukey test) were used to calculate average and standard deviations. Differences between means were considered to be significant when $p < 0.05$.

$$abs = 0.02602conc + 0.0807 \quad R^2 = 0.991 \tag{1}$$

Where *abs* signifies absorbance and *conc* represents the oil concentration in ppm. Oil loading is given by the ratio of corrected ESO mass obtained from the calibration curve of Eq. (1) and the sample mass.

The encapsulation efficiency (E.E) was calculated according to Eq. (2):

$$E.E(\%) = (M/M_0) \times 100 \tag{2}$$

Where M is the amount of ESO in the loaded sample (mg), as determined from Eq. (1) and M_0 is the initial added ESO amount (mg).

2.4.3. FT-IR spectroscopy

The particles were characterized by Fourier transform infrared spectroscopy (FT-IR) using KBr pellets on a Perkin Elmer device, using wavenumbers in the range 400–4000 cm^{-1}, with a resolution of 4 cm^{-1}.

2.4.4. Thermal analysis

The thermal stabilities of the nanoparticles was determined by thermogravimetric analysis (TGA) in a Shimadzu-TGA-50 (Japan) under a nitrogen atmosphere, applying a heating rate of 10 °C min^{-1}, from 25 to 900 °C, and by differential scanning calorimetry (DSC) in a Shimadzu-DSC-50 (Japan), with a heating rate of 10 °C min^{-1}, from 25 to 400 °C according to Paula et al. (2010).

2.4.5. Particle size distribution and zeta potential

Particle size distribution and zeta potential of the sample solution were determined by a Zetasizer Nano (Malvern, model 3600) using a red light beam with a wavelength of 633 nm and measuring an angle of 175°. Data were expressed as the averages of 3 readings (Paula, Sombra, Cavalcante, Abreu, & De Paula, 2011).