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Encapsulation of black pepper (*Piper nigrum L.*) essential oil with gelatin and sodium alginate by complex coacervation

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ABSTRACT

The terpenes presented in black pepper essential oil (EO) are volatile and their properties can be reduced in certain conditions. The encapsulation can protect the EO and preserve their terpenes. Complex coacervation was chosen because of several advantages. This study aimed to analyze the composition of black pepper EO, determining the most appropriate conditions for the formation of the complex between gelatin and sodium alginate. Further, we also encapsulated the black pepper EO. The main terpene identified in black pepper EO was β-caryophyllene. The ratio of 6:1 (gelatin/sodium alginate) at pH 4.0 was the ideal condition. The encapsulation efficiency varied from 49.13 to 82.36%, and the chemical composition of the encapsulated EO was identified by Gas Chromatography (GC). GC analysis indicated good core protection with the materials used. These biopolymers can serve as a potential delivery system for black pepper EO.

1. Introduction

Essential oils (EOs) are natural aromatic volatile liquids extracted from different parts of plants, such as flowers, buds, seeds, leaves, stems and bark. The composition and physicochemical properties of EOs are greatly influenced by the species, part of the plant, geographic origin, time of harvest, stage of development, age of plants and extraction method (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; Dima, Pătraşcu, Cantaragiu, Alexe, & Dima, 2016). EOs are composed of the following classes of compounds: tertiary terpene alcohols and related esters, aliphatic terpene ethers, aliphatic and aromatic terpene hydrocarbons (Bakkali et al., 2008). The identification of the compounds can be achieved by gas chromatography (GC) and nuclear magnetic resonance (NMR) (Guerrini et al., 2006). Previous studies revealed that, of these classes, a series of compounds have antioxidant and antibacterial activity. For this reason, EOs are an alternative to chemical preservatives and, therefore, are used in preparing safe foods with a positive impact on consumer's health (Dima et al., 2016).

Black pepper (Piper nigrum L.) is a plant of the Piperaceae family, largely used as a flavoring agent in foods (Chandran, Nayana, Roshini, & Nisha, 2017). The black pepper EO is sensitive to oxygen, light and high temperature. These factors contribute to the degradation of the EOs and, subsequently, to the decrease in their biological potential. Therefore, encapsulation is required to prevent this occurrence (Dima et al., 2016).

Microencapsulation is a process that retains a bioactive material (solid, liquid or gas) inside another (wall material) to protect the bioactive material against adverse environmental conditions, thereby increasing the shelf life and promoting the controlled release of the active compound in the microcapsule. As one of the microencapsulation technologies used in the food industry, the complex coacervation process has been used for encapsulating reactive, sensitive, or volatile additives or nutrients (Schmitt & Turgeon, 2011). The complex coacervation process principally consists of three basic steps: emulsification, coacervation, and shell formation and/or hardening (Zhang, Zhang, Hu, Bao, & Huang, 2012). Coacervation is the separation of a colloidal system into two liquid phases (International Union of Pure and

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Applied Chemistry-IUPAC, 1997). Complex coacervation is based on the associative interaction between oppositely charged polymers, usually a protein and a polysaccharide (Schmitt & Turgeon, 2011). Among the microencapsulation techniques, coacervation has several advantages such high encapsulation efficiency, low concentration of wall materials, the integrity of wall material and a variety of biopolymers that can be used as wall materials. Many wall materials using proteins and polysaccharides are reported in the literature: whey protein isolate/sodium alginate (Rojas-Moreno, Osorio-Revilla, Gallardo-Velázquez, Cárdenas-Bailón, & Meza-Márquez, 2018), gelatin/carboxymethylcellulose (Yuan et al., 2018), gelatin/arabic gum (Girardi, García, Passone, Nesci, & Etcheverry, 2017; Lv, Yang, Li, Zhang, & Abbas, 2014; Raksa, Sawaddee, Raksa, & Aldred, 2017), and gelatin/sodium alginate (De Matos, Scopel, & Dettmer, 2018; Wang, Yang, Cao, Zhao, & Wang, 2016). In this study, the biopolymers used as wall materials were gelatin (GE) and sodium alginate (NaAlg).

Gelatin (GE) is a natural nontoxic water-soluble protein derived from collagen. The polypeptide structure of the GE molecule facilitates its interactions with other oppositely charged ingredients, which makes it an important wall material used for capsules production by complex coacervation (Wang et al., 2016). Sodium alginate (NaAlg) is an anionic linear polysaccharide containing 1,4-linked D-mannuronic acid and L-guluronic acid residues and is obtained from brown seaweed. NaAlg is a natural, biocompatible, biodegradable, and hydrophilic polymer, which has been used in microparticulate formulations (Dima et al., 2016).

Several studies in the literature describe the potential use of the complex coacervation process in the encapsulation of EOs (De Matos et al., 2018; Girardi et al., 2017; Lv et al., 2014; Raksa et al., 2017; Rojas-Moreno et al., 2018; Wang et al., 2016; Yuan et al., 2018). However, studies using the complex coacervation for encapsulating black pepper (Piper nigrum L.) EO are scarce. Thus, the present study aimed to analyze the compositions of black pepper EO and to determine the influence of pH and biopolymer ratios on the formation of the complex coacervate obtained from GE and NaAlg, for use in the encapsulation of black pepper EO.

2. Materials and methods

2.1. Materials

Gelatin (code 48723), sodium alginate (code 180947), calcium chloride (code C1016) and Tween 20 (code P8341) were obtained from Sigma-Aldrich (St. Louis, USA). Black pepper EO was obtained from Ferquima (São Paulo, Brazil) with the following characteristics: transparent/straw yellow, density (20 °C) of 0.870–0.900 g cm^{-3}, and refraction index (20 °C) of 1.480–1.500. Ultrapure water with a conductivity of 0.05 μS/cm was used (Master System P&D, Gehaka, Brazil).

2.2. Methods

2.2.1. Chemical composition of black pepper (Piper nigrum L.) EO

2.2.1.1. GC analysis. Black pepper (Piper nigrum L.) *EO was solubilized in dichloromethane in a proportion of* 50 mg/mL. Analysis of volatile oils were carried out on a gas chromatography coupled with a mass spectrometer (QP2010, Plus-Shimadzu, Japan) with a Factor Four-VF-5ms capillary column (Varian) (30 m × 0.25 mm, 0.25 ìm film thickness). The injection volume of the sample was 1 μL (split 1:30) and the injector, gas chromatography coupled with a mass spectrometer (GC-MS) interface and ion source temperatures were maintained at 220, 310, and 250 °C, respectively, with an ionization energy of 70 eV. The oven temperature was programmed as follows: 60 °C at 2 min, 60–260 °C at 3 °C min^{-1}, 260–290 °C at 10 °C min^{-1}, and 290 °C at 5 °C min^{-1}. The EO constituents were identified by visual comparison of their spectra in the literature (Adams, 2007) and by the spectra provided by the device database (NIST21 and NIST107). A standard solution of n-alkanes (C_8–C_{20}) was injected under the same chromatographic conditions as the sample to obtain the retention indices (IK value).

A gas chromatograph (5890-series II, Hewlett Packard, USA) coupled with a flame ionization detector (CG-FID) and equipped with a Factor Four-VF-5ms capillary column (Varian) (30 m × 0.25 mm, 0.25 μm film thickness) was used for the quantification of EO. The injector and detector temperatures were maintained at 220 and 250 °C, respectively. The amount of the injection of the samples was 1 μL in split mode (1:30). The carrier gas was helium at a flow rate of 1 mL min^{-1} (12 psi). The oven ramp temperature was programmed as follows: 60 °C for 2 min, increased to 260 °C at 3 °C min^{-1}, and 260–290 °C at 10 °C min^{-1}. The relative percentage of the oil constituents was expressed as a percentage by area normalization.

2.2.1.2. NMR analysis. 10 mg of the black pepper (*Piper nigrum L.*) EO was dissolved in 500 μL of the deuterated chloroform ($CDCl_3$) with tetramethylsilane as the reference. The NMR spectra were recorded on a spectrometer (Advance III, Bruker, USA) operating at 500.13 MHz, the sample was measured at 298.15 K. The acquisition parameters of 1D NMR experiments employed were: TD (time domain data) = 65536, AQ (acquisition time) = 124 3.17 s, NS (number of scans) = 16, SW (spectral width) = 7500 Hz, D1 (relaxation delay between successive/transients) = 1.0s, LB (exponential line broadening prior to Fourier transformation) = 0.3 Hz, as reported by Vicente, de Carvalho, and Garcia-Rojas (2015). The spectra were processed using the MestreNova® Software 9.0 (Mestrelab research).

2.2.2. Determination of the molecular weights of the biopolymers

The viscosimetric method was used to determine the molecular weights for the biopolymers. The molecular weight of the NaAlg was determined in a previous study (Bastos, De Carvalho, & Garcia-Rojas, 2018). The molecular weight of the GE was determined according to the methodology adapted from Masueli (2014).

The dynamic viscosity was calculated from the data obtained using Cannon-Fenske viscosimetric capillaries, (Schott-Gerate, Germany). The capillaries were immersed in a thermostatic bath (CT-52, Schott-Gerate, Germany) to control the temperature, which was maintained at approximately 25 °C± 0.05. The GE solutions were prepared ranging from 0.1 to 1.0% with a variation of 0.1% (Masueli, 2014). The solutions and solvent densities were measured in a densimeter (DMA 4500M, Anton Paar, Austria) with an autoinjector (Xsample 452, Anton Paar, Austria). The intrinsic viscosity ([η]) was estimated by extrapolation of Martin curves to concentration "zero" (Guo et al., 2017), using the following equations:

$$\ln\left(\eta_{sp}/c\right) = ln[\eta] + K[\eta]c \quad (1)$$

$$\eta_{sp} = \frac{\eta - \eta_0}{\eta} \quad (2)$$

where $[\eta]$ is the intrinsic viscosity (cm^3/g), η_{sp} is the specific viscosity, c is the concentration of the biopolymers (g/mL), η is the viscosity of the solution of the biopolymers (g/cm.s), η_0 is the viscosity of the solvent (g/cm.s), and K is Martin's constant.

The molecular weight was determined by the following viscosimetric equation (Houwink, 1940; Mark, 1938):

$$[\eta] = K \cdot M^a \quad (3)$$

where $[\eta]$ is the intrinsic viscosity (cm^3/g), M is the molecular weight, and K and a are constants that depend on the polymer, the solvent, and the temperature. The GE constants $a = 0.62$ and $K = 2.9 \times 10^{-3}$ cm^3/g were used as reported by Masueli (2014).