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Microencapsulation of lipophilic bioactive compounds using prebiotic carbohydrates: Effect of the degree of inulin polymerization

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ABSTRACT

This paper presents novel outcomes about the effect of degree of inulin polymerization (DP) on the technological properties of annatto seed oil powder obtained by freeze-drying. Inulins with two DP's were evaluated: GR-inulin (DP ≥ 10) and HP-inulin (DP ≥ 23). Micrographs obtained by confocal microscopy were analyzed to confirm the encapsulation of bioactive compounds using both inulins, especially the encapsulation of the natural fluorescent substance δ-tocotrienol. Microparticles formed with both inulins presented the same capacity for geranylgeraniol retention (77%). Glass transitions of microparticles formed with GR-inulin and HP-inulin succeeded at 144 °C and 169 °C, respectively. Regarding water adsorption isotherms, microparticles formed with HP-inulin and GR-inulin presented behaviors of Types II (sigmoidal) and III (non-sigmoidal), respectively. Reduction of water adsorption capacity in the matrix at high relative moistures (> 70%) was presented when HP-inulin was used. At low relative moistures (< 30%), the opposite behavior was observed.

1. Introduction

The crescent interest of developing prebiotic substances is aimed at non-digestible oligosaccharides. One of the prebiotics is inulin, a fructooligosaccharide generally extracted from chicory (Pandey et al., 1999), but also from other sources as artichoke (Ruiz-Aceituno, García-Sarrió, Alonso-Rodriguez, Ramos, & Sanz, 2016), and used for stabilizing proteins (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015) or converted into other functional ingredients by inulinases (Mazutti et al., 2010). Longer chain lengths make inulin as dietary fiber useful for food and pharmaceutical applications. Examples of applications include as low caloric sweetener, as substance that provides solid dispersion for increasing dissolution rate, as an agent to form gels and to increase the viscosity of solutions, and as a non-digestible fiber (Mensink et al., 2015). Inulin also presents the ability to change the gut flora composition after a short feeding period based on results from in vitro studies and human subjects (Kolida & Gibson, 2007).

The use of inulin depends on its degree of polymerization (DP). The molecular chain length can range some extent, commonly between 4 and 80 (Mensink et al., 2015). The degree of inulin polymerization depends on the harvest time, storage time/temperature and growing conditions (Saengthongpinit & Sajjaanantakul, 2005). Consequently, the degree of inulin polymerization determines the physicochemical characteristics to a substantial extent, as morphology (i. e., crystal morphology, crystal structure and structure in solution), solubility, rheology (i. e., viscosity, hydrodynamic shape and gelling), thermal characteristics and physical stability (i. e., glass transition temperature, vapor sorption and melting temperature) and chemical stability (Mensink et al., 2015).

Following the scenario of using inulin as prebiotic substance (Zabot, Silva, Azevedo, & Meireles, 2016), bioactive compounds extracted from vegetal sources are also of recent interest because of their functional properties. Annatto seed oil is one of the sources of value-added bioactive compounds, as δ-tocotrienol (Albuquerque & Meireles, 2012; Moraes, Zabot, & Meireles, 2015) and geranylgeraniol (Silva, Zabot, & Meireles, 2015). Tocotrienol-rich fractions obtained from annatto seeds act as natural antioxidants by inhibiting lipid oxidation of fish oil and lipid-based formula emulsions (Zou & Akoh, 2015). In addition, geranylgeraniol modulates the apoptosis of carcinogen cells (Marcuzzi et al., 2012).

In this context, the objective of this study was to evaluate the influence of the degree of inulin polymerization (DP ≥ 10 and DP ≥ 23) on the physical properties of annatto seed oil microparticles obtained by freeze-drying. Inulin was used as encapsulating matrix and the effect of its degree of polymerization on the recon-

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stitution properties of the emulsions was assessed. Furthermore, the morphology and δ-tocotrienol distribution in the particles microstructures, geranylgeraniol retention by the encapsulating matrices, glass transition of microparticles and physical stability through water adsorption isotherms were also the responses taken into account when evaluating the effect of the degree of inulin polymerization.

2. Material and methods

2.1. Annatto seed oil extraction

Annatto seed oil used as active material was extracted with supercritical CO_2 using a pilot scale equipment (Thar Technologies, Pittsburgh, USA) containing an extraction vessel of 5 L with internal diameter of 10.2 cm. Solvent flow rate was equal to 200 g CO_2/min and the bed was maintained at 40 °C and 20 MPa, as described in detail by a previous study (Silva, Gomes, Hubinger, Cunha, & Meireles, 2015).

2.2. Inulins and materials for chromatographic analyses

Inulins used as active materials for encapsulating annatto seed oil were both from chicory: Orafti®GR (GR-inulin, DP ≥ 10) and Orafti®HP (HP-inulin, DP ≥ 23) (BENEO-Orafti, São Paulo, Brazil). Materials for chromatographic analyses were: methanol, ethanol and hexane (Chemco, Hortolândia, Brazil); geranylgeraniol (purity > 85%, Sigma–Aldrich, Steinhein, Germany) and ammonium acetate (P.A., Dinâmica, Campinas, Brazil).

2.3. Microencapsulation of annatto seed oil

Annatto seed oil was encapsulated with GR- and HP-inulins through emulsification assisted by ultrasound with nominal power of 160 W during 3 min using a 13 mm diameter, 19 kHz ultrasonic probe (Unique, Disruptor, 800 W, Indaiatuba, Brazil) for each 30 mL of emulsion. Total concentration of solids in the emulsion (emulsifying + oil) was equal to 20 g/100 g of emulsion. Annatto seed oil was added to the suspensions containing GR- and HP-inulins and the concentration of oil was maintained at 20% relative to the amount of solids, that is, 4 g of oil per 100 g of emulsion. Immediately after homogenization, the emulsions were frozen in aluminum plates at −40 °C for 3 h and then subjected to freeze-drying (FD) process. Drying was performed in a freeze-dryer system (Liobras, L 101, Sao Carlos, Brazil). The dried emulsions were converted into fine powders through maceration. Detailed description of obtaining annatto seed oil microparticles is reported by a previous study (Silva & Meireles, 2015).

2.4. Reconstitution properties of emulsions

The wettability of the powders was determined as the method reported by Fuchs et al. (2006), with a few modifications. The samples of powders (0.1 g) were dispersed over water surface of a Becker containing 100 mL of ultrapure water under stirring at 25 °C. The time spent for immersing or wetting the last particle of powder was used as wettability response.

The annatto seed oil microparticles obtained by freeze-drying were reconstituted in ultrapure water. An amount of 2 g of powder was mixed with 8 g of ultrapure water and the solution was stirred during 30 s in a vortex type homogenizer (PHOENIX, AP-56 model, Araraquara, São Paulo, Brazil) at 25 °C.

Droplet size distribution and mean diameter of the emulsion droplets, after reconstitution and before drying, were determined by light scattering technique using laser diffraction (Mastersizer 2000 Malvern Instruments Ltd, Malvern, UK). The measurements were performed at 25 °C. The mean diameter was calculated based on the mean diameter of a sphere of similar area, superficial mean diameter (D_{32}), as Eq. (1). Polydispersity index (PDI) was calculated as Eq. (2). All samples were analyzed through the wet method, with dispersion in water and refractive index of 1.52.

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (1)$$

$$PDI = \frac{(d_{90} - d_{10})}{d_{50}} \quad (2)$$

Where: d_i is the mean diameter of the droplets; n_i is the number of droplets; and d_{10}, d_{50} and d_{90} are the diameters at 10%, 50% and 90% of cumulative volume, respectively.

2.5. Scanning electron microscopy (SEM)

Micrographs were taken in a scanning electron microscope with Energy Dispersive X-ray Detector (SEM) (Leo 440i, EDS 6070, SEM/EDS: LEO Electron Microscopy/Oxford, Cambridge, England). Analyses were performed with 5 kV accelerating voltage and 50 pA beam current for obtaining the micrographs.

2.6. Confocal scanning laser microscopy (CSLM)

CSLM analysis was performed using a Zeiss LSM 780-NLO confocal on an Axio Observer Z.1 microscope (Carl Zeiss AG, Germany) with a 40 × objective. Images were taken by exciting δ-tocotrienol molecules with lasers at 488 nm wavelength, without any previous preparation of the samples as a consequence of the fluorescent properties of δ-tocotrienol, similarly to the procedure described by a previous study (Silva, Zabot, Cazarin, Maróstica Jr., & Meireles, 2016).

2.7. Geranylgeraniol retention

Geranylgeraniol content in annatto seed oil (before and after encapsulation) was determined by high-performance liquid chromatography (HPLC). Chromatographic analyses were accomplished using an HPLC-PDA (Waters, Alliance E2695, Milford, USA) system, consisting of a separation module with an integrated column heater, an autosampler and a photodiode array (PDA) detector. Separation of geranylgeraniol was fulfilled using a fused-core type column (Kinetex, C_{18}, 100 mm × 4.6 mm × 2.6 μm; Phenomenex, Torrance, USA). An aliquot of 10 μL of each sample diluted to 500 ppm (w/w) in hexane (Chemco, Hortolandia, Brazil) and filtered using nylon membrane (0.45 μm) was injected. A solution of methanol:ammonium acetate 50 mM (90:10, v/v) was the mobile phase. The column was maintained at 40 °C. Mobile phase flow rate was 1 mL/min and the analytical run time was 7 min. Detector wavelength range was 200–400 nm. Geranylgeraniol was detected at 210 nm and at 2.4 min, and its quantification was performed using external standard calibration curve.

Intending to quantify geranylgeraniol entrapped in the microparticles after using inulins with two degrees of polymerization, four procedures were carried out to break the structure for releasing such compound:

I ***Centrifugation***: Approximately 0.1 g of particles from each treatment was mixed with 4 mL of ultra-pure water. The samples were maintained static during 24 h; thereafter, they were manually agitated for reconstituting the emulsions. Aliquots of 0.5 mL were transferred to an Eppendorf tube of 2 mL containing 1.3 mL of hexane. The mixtures were then centrifuged at 5000 rpm for 20 min and at 10,000 rpm for 5 min. This procedure was performed to break the emulsion and to capture the