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Influence of the degree of inulin polymerization on the ultrasound-assisted encapsulation of annatto seed oil

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ABSTRACT

The effect of the degree of polymerization (DP) of inulin was evaluated on its encapsulant characteristics. We assessed the influence of the average inulin DP (DP ≥10 and DP ≥23) in the ultrasound-assisted encapsulation of annatto seed oil using the freeze-drying technique for particle formation. The intensification of the homogenization process with ultrasound did not improve the characteristics of the emulsions due to the physicochemical limitations of the inulin molecular chain (molecules do not exhibit surface activity). The particle morphology, oil entrapment efficiency, encapsulation efficiency, X-ray diffraction, thermogravimetric analysis and Rancimat analyses proved the effectiveness of inulin as a wall material. The properties influenced by the DP were the surface oil, encapsulation efficiency, water activity, particle size and oxidative stability of the encapsulated oil because the highest DP promoted the formation of microparticles with lower surface oil content, greater encapsulation efficiency, low water activity, larger size and greater protection against oil oxidation.

1. Introduction

Oil extracted from annatto seeds (*Bixa orellana* L.) contains valuable bioactive compounds, such as geranylgeraniol diterpene and tocotrienols (Jondiko & Pattenden, 1989; Moraes, Zabot, & Meireles, 2015). These compounds are targeted by food and pharmaceutical product markets primarily because of their anti-cancer and antioxidant properties (Marcuzzi et al., 2012). Geranylgeraniol is the major terpenic constituent of annatto seeds and is of great importance because of its ability to promote apoptosis of various types of cancer cells (Katuru et al., 2011), its anti-inflammatory properties (Giriwono et al., 2013) and its role as an important intermediate in the synthesis of vitamins K, E and various hormones (Hyatt, Kottas, & Effler, 2002). Tocotrienols are classified as vitamin E, together with tocopherols, and both occur naturally as α-, β-, γ- and δ-tocotrienol/tocopherol isoforms. Tocotrienols are more effective antioxidant and anticancer compounds than tocopherols (Sylvester & Shah, 2005), and annatto seed oil was recently recognized as the largest natural source of δ-tocotrienol (Moraes et al., 2015).

Encapsulation of annatto seed oil is a great alternative preservation method for the availability of its bioactive compounds and can enhance its use as a nutritional supplement in foods and beverages. The use of biopolymers (starches, proteins, inulins and others) as wall materials can facilitate the application of annatto seed oil in functional foods.

Inulin is a natural fructan composed of a linear chain of fructose monomers bound together by β type (2 → 1) bonds with a terminal glucose unit bound by an α(2 → 1) bond. It has a degree of polymerization (DP) that ranges from 10 to 60, and the lengths of its molecular chains are associated with its technological properties. In humans, inulin is not digested in the upper gastrointestinal tract because there are no enzymes capable of breaking β(2 → 1) type bonds, resulting in a prebiotic effect related to fermentation under anaerobic conditions in the colon and the proliferation of bifidobacteria in the lower colon (Vervoort & Kinget, 1996). Edible plants, such as asparagus, garlic, chicory, leeks, onions and artichokes are natural sources of inulin, which are characterized as a reserve carbohydrate (Kaur & Gupta, 2002). In the manufacturing of food, depending on its DP, inulin is used in place of fat, sugar and thickeners or gelling agents (Glibowski, 2010). There are few reports in the literature in which inulin was applied as the only wall material. Most studies have focused on the use of inulin in blends with other encapsulating materials. For example, Fernandes, Borges, and Botrel (2014) assessed inulin blends (DP ≥23) with modified starch, gum Arabic and maltodextrin for encapsulation of rosemary essential oil by spray drying.

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Obtaining powdered products using the oil encapsulation process consists of two steps: emulsifying the active material and drying the continuous phase to obtain the particles. Compared to traditional mechanical agitation methods in rotor-stator type devices, the use of ultrasound in the homogenization of emulsions leads to the formation of emulsions with smaller droplet sizes and thus more stability (Jafari, Assadpoor, He, & Bhandari, 2008b). In emulsion drying, the spray drying (SD) and freeze-drying (FD) methods are widely used. FD is the best technique for obtaining high-quality powdered food products, although it is less attractive than SD because of longer processing times and power consumption (Schwegman, 2009). The FD method conditions, such as vacuum drying and low temperatures, prevent oxidation and chemical modification of the products, making it an attractive technique for bioactive compounds sensitive to heat and oxidation (Ratti, 2001).

This work aims to evaluate the properties of inulin as a wall material to clarify the effect of the DP on the encapsulant characteristics. The specific objective was to evaluate the influence of the average inulin DP (DP ≥10 and DP ≥23) on the ultrasound-assisted encapsulation of annatto seed oil using the FD technique for particle formation. The influence of the applied ultrasound power on the formation and stability of oil-in-water emulsions according to the DP was assessed. The microparticles obtained were compared in terms of their moisture content, water activity, surface oil, entrapment efficiency, encapsulation efficiency, size distribution, average diameter, morphology, X-ray diffraction patterns, thermogravimetric and oxidative stabilities.

2. Materials and methods

2.1. Annatto seed oil extraction

The annatto seed oil used as the active material was obtained from whole annatto seeds through extraction with supercritical carbon dioxide, as described by Silva, Gomes, Hubinger, Cunha, and Meireles (2015).

2.2. Inulins used as wall material

The inulins used as wall materials in the encapsulation of the annatto seed oil were chicory inulin Orafti®GR (DP ≥10) and Orafti®HP (DP ≥23) (BENEO-Orafti, São Paulo, Brazil). The materials were characterized chemically following the AACC (2000) methodology. GR inulin: moisture content 4.7 ± 0.3 g/100 g, wet basis; lipids 0.11 ± 0.06 g/100 g; protein 0 g/100 g; ash 0.02 ± 0.00 g/100 g. HP inulin:moisture content 4.9 ± 0.0 g/100 g, wet basis; lipids 0.02 ± 0.01 g/100 g; protein 0 g/100 g; ash 1.07 ± 0.04 g/100 g.

2.3. Preparation of annatto seed oil emulsions

The emulsions were prepared with a volumetric fraction (ϕ) of 0.04. The annatto seed oil to encapsulant material ratio was maintained at 1:4 (w/w) (Jafari, Assadpoor, He, & Bhandari, 2008a). Each emulsion was prepared by dissolving 16% (w/w) HP or GR in deionized water (Millipore®). The inulin suspensions were prepared by dissolving the materials in deionized water at 80 °C. After dissolution of the wall material, the suspensions were cooled to 40 °C. The annatto seed oil was gradually added to the cooled suspension with stirring in a rotor–stator type homogenizer (Fisatom, model 713D, São Paulo, Brazil) at 1000 rpm. After complete incorporation of the active material, the system was homogenized at 2000 rpm to form the pre-emulsion.

Aliquots of 25 cm^3 of the pre-emulsions were then subjected to ultrasonication to completely emulsify the annatto seed oil. An ultrasonic probe with 13 mm diameter and 19 kHz (Unique, Desruptor 800 W Indaiatuba, Brazil) was used for 3 min. The height of the ultrasonic probe in contact with the emulsions was standardized at 40 mm.

The effects of inulin DP and ultrasonication energy on the stability of the emulsions, average droplet diameter and size distribution were evaluated at two (DP ≥10 and DP ≥23) and five (0 W, 160 W, 320 W, 480 W and 640 W) levels, respectively, using a full factorial experimental design. The experiments were performed in duplicate, and a total of 20 emulsions were evaluated.

2.4. Characterization of the emulsions

2.4.1. Emulsion droplet size

The size distribution and average droplet diameter of the emulsions were determined by light scattering using laser diffraction (Mastersizer 2000 Malvern Instruments Ltd., Malvern, UK). The average diameter was calculated based on the average diameter of an area of a similar sphere, the surface mean diameter (D_{32}) represented by Eq. (1) and the polydispersity index (PDI) determined according to Eq. (2). The samples were analyzed in triplicate for each repetition of the evaluated emulsion by the wet method with dispersion in water and a refractive index of 1.52. The measurements were performed at 25 °C.

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

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

where d_i is the average droplet diameter; n_i is the number of drops; and d_{10}, d_{50} and d_{90} are the diameters at 10%, 50% and 90% cumulative volume, respectively.

2.4.2. Emulsion stability

Immediately after the emulsification process, 10 cm^3 aliquots of each emulsion were transferred to 25 cm^3 graduated cylinders (internal diameter, 1.8 cm, and height, 16.5 cm), sealed and stored at 25 °C. The volume of the oil phase was measured 24 h after preparation of the emulsions. The analyses were performed in duplicate for each replication of the experiment. The stability was expressed in terms of the separation index:

$$\mathrm{Separation}(\%) = \left(\frac{H_S}{H_T}\right) \times 100 \quad (3)$$

where H_T represents the total height of the emulsion and H_S is the height of the upper phase.

2.5. Freeze-drying

The homogenized emulsions were immediately frozen on aluminum plates at −40 °C for 3 h and then freeze-dried in a Liobrás, model L 101 freeze-dryer (São Carlos, Brazil). The dried emulsion was macerated into a fine powder.

2.6. Particle characterization

2.6.1. Moisture and water activity

The moisture contents of the annatto seed oil microparticles were gravimetrically determined in a forced circulation oven to constant weight at 105 °C (AOAC, 1997). The water activity (a_w) of the particles at 25 °C was measured by an AquaLab Water Activity Meter device (Series 3TE, Decagon, Pullman, USA).

2.6.2. Entrapment efficiency (oil retention)

The total content of the encapsulated annatto seed oil was determined by distillation of the microparticles in a Clevenger-type