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Ultrasound-assisted encapsulation of annatto seed oil: Retention and release of a bioactive compound with functional activities

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ABSTRACT

This paper brings forward the encapsulation of annatto seed oil (rich in geranylgeraniol) assisted by high intensity ultrasound using gum Arabic (GA) as stabilizing agent. We studied the effects of time (min) and ultrasonication power (W) over the emulsion characteristics. After forming microparticles from the best emulsion using freeze-drying (FD) and spray-drying (SD) techniques, we evaluated particle size distribution, moisture, water activity, surface oil, entrapment efficiency, encapsulation efficiency, geranylgeraniol retention, oxidative stability and kinetic release of geranylgeraniol, a biocompound with functional activities. The combined intensification of time and ultrasonication power reduced the superficial mean diameter (D_{32}) and polydispersity (PDI) of emulsions. Drying the continuous phase of the optimized emulsion (smallest $D_{32} = 0.69 \pm 0.03$ μm) using FD and SD formed microparticles with different morphological characteristics, Brouckere diameter (D_{43}), particle size distribution, moisture and water activity. SD process led to microparticles with the highest oil encapsulation efficiency (85.1 ± 0.1 wt.%) as a consequence of their lowest surface oil (SO). However, GA-FD microparticles presented the highest oil entrapment efficiency (97 ± 1 wt.%). Geranylgeraniol retention (80–86 wt.%) was similar for both drying techniques. GA-FD microparticles were more stable against oxidation through accelerated test Rancimat, even though presenting higher SO. This behavior is associated with the likely phase transition on the GA-SD matrix. The difference on the kinetic release of geranylgeraniol is linked to the difference on the particles morphology and particle size distribution.

1. Introduction

Annatto (*Bixa orellana* L.) is industrially desirable in the food, textile, cosmetic and pharmaceutical fields because the annatto seed is pigmented and a rich source of natural red and orange coloring substances. Several carotenoids were identified from annatto seeds, as bixin, the major colorant comprising 80–90% of total carotenoids (Balaswamy, Prabhakara Rao, Satyanarayana, & Rao, 2006; Prabhakara Rao, Jyothirmayi, Balaswamy, Satyanarayana, & Rao, 2005).

Recently, Albuquerque and Meireles (2012) showed the oil obtained by supercritical fluid extraction (SFE) as the richest natural source of δ-tocotrienol, containing approximately 14.6 ± 0.4 g/100 g of oil. A great content of tocotrienols from annatto seeds was also extracted by Moraes, Zabot, and Meireles (2015). Tocotrienols and tocopherols are antioxidant compounds commonly known as vitamin E. They are found in eight isoforms of α-, β-, γ- and δ-tocopherols and tocotrienols (Pierpaoli et al., 2013). However, tocotrienols are classified as the more effective anticancer and antioxidant compounds when compared with tocopherols (Sylvester & Shah, 2005).

Annatto seeds are also rich in geranylgeraniol, the major terpenic constituent (Jondiko & Pattenden, 1989). Geranylgeraniol presents several biological activities, as therapeutic action against Chagas disease (Menna-Barreto et al., 2008), inhibition of the microorganism that causes tuberculosis (Vik, James, & Gundersen, 2007) and apoptosis of carcinogen cells (Katuru et al., 2011; Marcuzzi et al., 2012).

Stabilizing the bioactive compounds contained in the annatto seed oil by encapsulation is a promising alternative for adding value to the processing chain of annatto. Encapsulation expands the application of functional products in the global market. Until the date, the application of ultrasound technology for food extraction and extraction of bioactive compounds has been widely studied (Rombaut, Tixier, Bily, & Chemat, 2014; Roselló-Soto et al., 2015; Santos, Aguiar, Barbero, Rezende, & Martínez, 2015). However, the information regarding the use of ultrasounds as a potential tool to encapsulate bioactive compounds is more limited (Mura et al., 2015; Rosa, Silva, Santos, Petenate, & Meireles, 2016; Silva & Meireles, 2015). The feasibility of using ultrasounds to encapsulate bioactive compounds is based on the ability of this technology to reduce the droplet size of the emulsion to a range smaller than 1 μm. Obtaining particles from fine emulsions results in increased oil encapsulation efficiency (Hernandez Sanchez, Cuvelier, & Turchiuli, 2016).

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In this context, the objective of this study was to evaluate the retention and release of geranylgeraniol in different particulate systems obtained from a fine and kinetically stable emulsion optimized by high intensity ultrasound using gum Arabic (GA) as stabilizing agent.

2. Material and methods

2.1. Extraction of annatto seed oil

We extracted annatto seed oil by SFE using CO_2. The procedures and details of the extraction process are described by Silva, Gomes, Hubinger, Cunha, and Meireles (2015).

2.2. Emulsifying/wall material and materials for chromatographic analyses

Emulsions of annatto seed oil were obtained using GA instant gum BB (Nexira Comercial Ltda., Sao Paulo, SP, Brazil) as encapsulating agent, which the physic-chemical characteristics are described by Silva et al. (2015). The material for chromatographic analyses comprised of methanol (Chemco, Hortolandia, Brazil), hexane (Chemco, Hortolandia, Brazil), geranylgeraniol standard (purity >85%, Sigma-Aldrich, Steinhein, Germany) and ammonium acetate P.A. (Dinâmica, Campinas, Brazil).

2.3. Preparation of annatto seed oil emulsions

We added GA to the ultra-pure water supplied by a Milli-Q Advantage water purifier system (Millipore, Bedford, USA). We prepared the biopolymer suspension 24 h before the emulsification process. The suspension was maintained static at approximately 25 °C during 24 h to ensure the complete hydration of GA. After hydration, we added 20% of annatto seed oil (relative to the mass of total solids) to the GA suspension. Then, the emulsion was formed by 4 wt.% of oil and 16 wt.% of GA, accounting 20 wt.% of total solids (Jafari, Assadpoor, He, & Bhandari, 2008a).

The dispersed phase was slowly incorporated to the GA suspension by mechanical stirring at 2000 rpm (rotations per minute) for 5 min using a homogenizer rotor-stator type (Fisatom, model 713D, Sao Paulo, Brazil). A pre-emulsion was obtained and sequentially submitted to the ultrasonication process applying different conditions to reach a complete emulsification.

Aliquots of 25 mL of the pre-emulsion were sonicated using a 13 mm diameter, 19 kHz ultrasonic probe (Unique, Disruptor, 800 W, Indaiatuba, Brazil) for obtaining the emulsions. The probe height contacting the emulsions was standardized to 40 mm. We used an ice bath for preventing overheating the emulsions. We recorded 44.5 °C as the final temperature after ultrasonication at the more intense process condition. Fig. 1 shows the scheme used for obtaining annatto seed oil emulsions by ultrasonication.

We evaluated the effects of nominal power (W) and process time (min) on the mean diameter of the oil droplets and creaming stability using a full factorial experimental design (4^2) with nominal powers of 160, 320, 480 and 640 W, and processing times of 0.5, 1.0, 3.0 and 5.0 min. Experimental runs were carried out in duplicate, accounting 32 runs.

2.4. Emulsion characterization

2.4.1. Droplet distribution size

The droplet distribution size and mean diameter of the emulsion droplets were determined by the light scattering technique using laser diffraction (Mastersizer 2000 Malvern Instruments Ltd., Malvern, UK). 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 in triplicate, using the wet method, with dispersion in water and refractive index of 1.52:

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

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

where d_i is the 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.

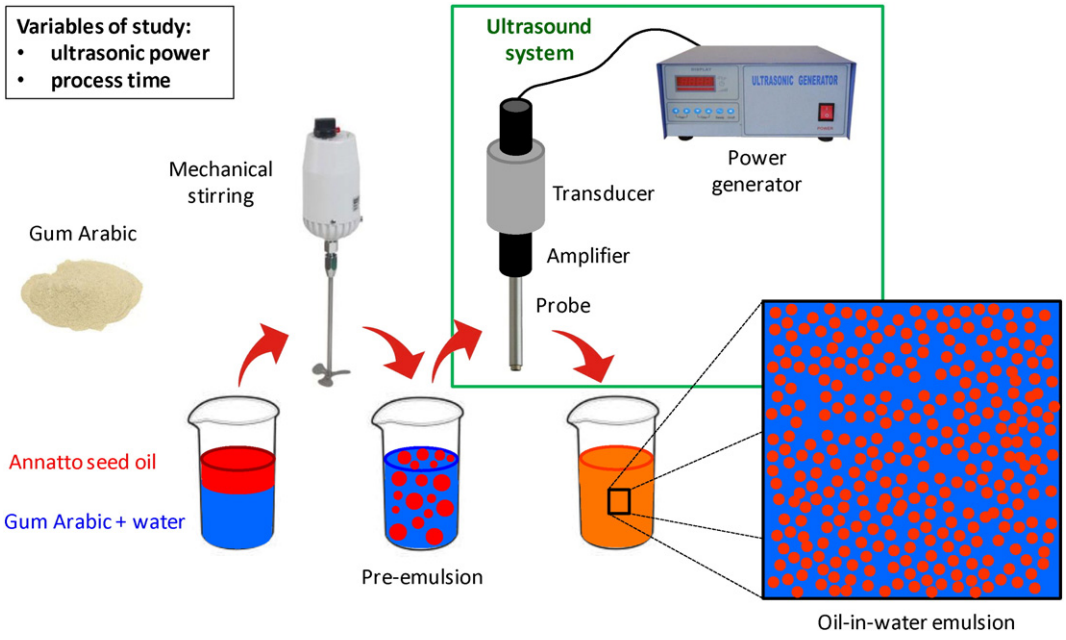

Fig. 1. Experimental procedure for ultrasound-assisted encapsulation of annatto seed oil.