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# Obtaining annatto seed oil miniemulsions by ultrasonication using aqueous extract from Brazilian ginseng roots as a biosurfactant

M. Thereza M.G. Rosa [a,*], Eric Keven Silva [a], Diego T. Santos [a], Ademir J. Petenate [b], M. Angela A. Meireles [a]

[a] LASEFI/DEA/FEA (School of Food Engineering)/UNICAMP (University of Campinas), R. Monteiro Lobato, 80, 13083-862 Campinas, SP, Brazil
[b] DE, IMEC-UNICAMP, Cx. Postal 6065, CEP: 13083-970 Campinas, SP, Brazil

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ABSTRACT

The purpose of this study was to produce an oil-in-water (O/W) emulsion of δ-tocotrienol-rich oil obtained from annatto seeds by a supercritical fluid extraction process. The effects of emulsification by ultrasound (US) were evaluated and compared to emulsification by dispax reactor (DR) at similar energy densities. Saponin-rich extract from Brazilian ginseng roots (BGR) was obtained from BGR by pressurized liquid extraction and used as a biosurfactant. A model O/W emulsion system was prepared with soybean oil and commercial saponin. The influence of the emulsification process, energy density, oil type, biosurfactant type and biosurfactant concentration on the size and stability of the resulting droplets was examined through the experimental design and proper statistical analysis. The results showed that US produced more stable emulsion with smaller droplet sizes in comparison with the DR device at the same energy density. In general, increasing the energy density helped to reduce the emulsion droplet size. The minimum average droplet size observed in the mini-emulsions was 0.35 μm. The data show that both biosurfactants were capable of forming emulsions containing relatively small droplets (<0.83 μm) and were rather stable (96–99%), with some creaming. The emulsion droplets also showed a surface potential of approximately −49 mV because of the adsorbed biosurfactants, which minimized the flocculation of the oil droplets. These results indicate that BGR-extracted saponin might be an attractive biosurfactant choice for emulsion formulations for use in food and beverage products.



## 1. Introduction

Food industries have focused on nutritive and healthy food products that meet consumer demand for a healthy lifestyle, which are intended to prevent nutrition-related diseases in consumers (Menrad, 2003). Annatto (*Bixaorellana* L.) seeds contain carotenoid pigments, which are the most commonly used colorants in food processing, for example in coloring butter, cheese, ice cream, bakery products and edible oils (Smith, 2006). Moreover, the lipid fraction of these seeds is rich in tocotrienol, which health benefits are very interesting for the food industry. Tocotrienols, which are related to the tocopherol family, have received a large amount of attention recently for their important biological activities, especially for inhibiting tumor development and reducing the risk of cardiovascular disease (Sylvester and Theriault, 2003). Aggarwal et al. (2010) published an excellent review paper about tocotrienol effects on cancer, bone resorption, diabetes, and cardiovascular and neurological diseases at both preclinical and clinical levels.

Tocotrienols consist of a chromanol ring linked to a 15-carbon tail, with three trans double bonds. These compounds are made of a group of four amphipathic molecules (α-, β-, γ- and δ-) that differ in the number and position of methyl groups on the chromanol rings (Aggarwal et al., 2010; Schauss et al., 2013). Tocotrienols are present in only a very small number of plants and are especially abundant in rice bran, palm oil and annatto seeds, for which the ratio of tocopherol-to-tocotrienol in each is 50:50, 25:75 and 0.1:99.9, respectively. Tocotrienol-containing products can be a considerable source of vitamin E when consumed. Annatto seeds are described by Tan (2011) as the richest source of the most potent tocotrienol, that is, δ-tocotrienol. Tocopherol interferes with tocotrienol benefits. Annatto stands out as a superior tocotrienol source because of its unique composition of 90% δ-tocotrienol and 10% γ-tocotrienol, without any tocopherols (Schauss et al., 2013).

By focusing on an increase in the high added value of δ-tocotrienol-rich extracts, which were obtained by using the supercritical fluid extraction process, a nonpurified aqueous extract from Brazilian ginseng roots (BGR) (*Pfaffia glomerata*) was used as a biosurfactant in the emulsification process. This extract

* Corresponding author.
*E-mail address:* mt_msgomes@yahoo.com.br (M.T.M.G. Rosa).

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**Table 1**
The experimental conditions for the emulsification experiments.

| Experiment | Process | Oil | Biosurfactant | Biosurfactant concentration (%) | Energy density (J $mL^{-1}$) |
|---|---|---|---|---|---|
| 1 | DR[a] | Soybean | Commercial[c] | 1.5 | 1200 |
| 2 | US[b] | Soybean | BGR[d] | 1.5 | 600 |
| 3 | DR | Annatto | Commercial | 1.5 | 1200 |
| 4 | DR | Annatto | BGR | 3.0 | 600 |
| 5 | US | Soybean | Commercial | 1.5 | 600 |
| 6 | US | Soybean | Commercial | 3.0 | 1200 |
| 7 | DR | Soybean | BGR | 3.0 | 1200 |
| 8 | US | Annatto | Commercial | 3.0 | 600 |
| 9 | DR | Soybean | BGR | 1.5 | 1200 |
| 10 | US | Soybean | Commercial | 1.5 | 1200 |
| 11 | US | Soybean | BGR | 3.0 | 1200 |
| 12 | US | Annatto | Commercial | 1.5 | 1200 |
| 13 | DR | Soybean | BGR | 3.0 | 600 |
| 14 | DR | Soybean | Commercial | 1.5 | 600 |
| 15 | US | Annatto | BGR | 1.5 | 1200 |
| 16 | DR | Annatto | Commercial | 1.5 | 600 |
| 17 | US | Annatto | BGR | 3.0 | 1200 |
| 18 | DR | Annatto | BGR | 1.5 | 1200 |
| 19 | US | Annatto | Commercial | 1.5 | 600 |
| 20 | US | Soybean | BGR | 1.5 | 1200 |
| 21 | US | Annatto | Commercial | 3.0 | 1200 |
| 22 | DR | Soybean | Commercial | 3.0 | 1200 |
| 23 | DR | Soybean | Commercial | 3.0 | 600 |
| 24 | DR | Annatto | Commercial | 3.0 | 600 |
| 25 | DR | Soybean | BGR | 1.5 | 600 |
| 26 | US | Soybean | BGR | 3.0 | 600 |
| 27 | US | Annatto | BGR | 1.5 | 600 |
| 28 | DR | Annatto | BGR | 3.0 | 1200 |
| 29 | DR | Annatto | Commercial | 3.0 | 1200 |
| 30 | US | Annatto | BGR | 3.0 | 600 |
| 31 | DR | Annatto | BGR | 1.5 | 600 |
| 32 | US | Soybean | Commercial | 3.0 | 600 |

[a] Dispax reactor.
[b] Ultrasound.
[c] Commercial saponin.
[d] Saponin-rich extract from Brazilian ginseng roots.

is rich in saponins, a natural class of compounds used as a food additive for its amphiphilic properties (Mitra and Dungan, 2000).

Saponins are predominantly made of glycosides that possess one, two or three sugar chains attached to the aglycones, also called sapogenins, which are the nonpolar parts of the molecule (Oleszek and Hamed, 2010). The presence of hydrophobic and hydrophilic areas (the aglycone and sugar resides) in the saponin molecules account for the ability of this compound to reduce surface tension at phase boundaries (Mironenko et al., 2010). These compounds have been used in foods as natural surfactants; they serve as preservatives to control the microbial spoilage of food. Because of consumer preferences for natural substance, saponins have more recently been used as a natural small molecule surfactant in beverage emulsions to replace synthetic surfactants such as polysorbates (Cheok et al., 2014).

There are a number of mechanisms available to produce emulsions. According to the literature, ultrafine emulsions can be prepared by high- and low-energy emulsification methods (Silva et al., 2015; Yang et al., 2012). Emulsification is a process in which a system is made from two immiscible liquids (usually oil and water), one of which is dispersed as small droplets within the other (Chandrapala et al., 2012). The high-energy methods employ mechanical or ultrasound devices that generate shearing (rotor–stator) or pressure differences (a high-pressure homogenizer or power ultrasound) to decompose the emulsion structures (Spinelli et al., 2010). Anyway, the reduction of the droplet size makes emulsions more stable.

The objective of the present work was to generate food grade oil-in-water (O/W) emulsions through mechanical stirring by the dispax reactor (DR) or power ultrasound (US), with particular emphasis on identifying equipment-related constraints. A model O/W emulsion system was prepared with soybean oil and commercial saponin as biosurfactant. The best conditions for fabricating an O/W mini-emulsion of δ-tocotrienol-rich oil with a saponin-rich extract from BGR as a biosurfactant were identified. This system may be suitable for applications in food and beverage formulations.

## 2. Materials and methods

### 2.1. Material

Annatto seeds (*Bixa orellana* L.) of the *Piave* variety were obtained from the Agronomic Institute of Campinas, Department of Agriculture and Supply of the State of São Paulo, Brasil. The centesimal composition of this annatto was determined by using the official methods published by the AOAC (1997) for measuring the moisture, protein, ash and total lipid contents. The carbohydrate content was determined by difference. The γ-, δ-, α- and β-tocotrienol contents were determined according to AOCS (2004) method Ce 8–89.

The tocotrienol-rich oil used in this study was obtained from annatto seeds by using supercritical carbon dioxide (99% $CO_2$, Air Liquide, Campinas, SP, Brazil) as an extracting solvent with pilot scale equipment (Thar Technologies, model SFE-2 × 5LF-2-FMC, Pittsburgh, PA, USA) that was equipped with two 5.15 L extraction vessels and three 1 L separators displayed in a series. The process conditions were selected from the ones that were optimized by Albuquerque and Meireles (2012). The results showed that the extract with the most concentrated δ-tocotrienol and the lowest bixin content was obtained at 313 K and 20 MPa. The static extraction time was 20 min. The extraction was performed until the