Food Research International 99 (2017) 393–402

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Obtaining bixin from semi-defatted annatto seeds by a mechanical method and solvent extraction: Process integration and economic evaluation

Sylvia C. Alcázar-Alay, J. Felipe Osorio-Tobón, Tânia Forster-Carneiro, M. Angela A. Meireles*

LASEFI/DEA/FEA (School of Food Engineering), UNICAMP (University of Campinas), Rua Monteiro Lobato, 80, Campinas, SP CEP: 13083-862, Brazil

A R T I C L E I N F O

Keywords:
Bixa orellana L.
Bixin
Biocompound recovery
Mechanical separation
Low-pressure solvent extraction
Cost of manufacture

A B S T R A C T

This work involves the application of physical separation methods to concentrate the pigment of semi-defatted annatto seeds, a noble vegetal biomass rich in bixin pigments. Semi-defatted annatto seeds are the residue produced after the extraction of the lipid fraction from annatto seeds using supercritical fluid extraction (SFE). Semi-defatted annatto seeds are use in this work due to three important reasons: i) previous lipid extraction is necessary to recovery the tocotrienol-rich oil present in the annatto seeds, ii) an initial removal of the oil via SFE process favors bixin separation and iii) the cost of raw material is null. Physical methods including i) the mechanical fractionation method and ii) an integrated process of mechanical fractionation method and low-pressure solvent extraction (LPSE) were studied. The integrated process was proposed for processing two different semi-defatted annatto materials denoted Batches 1 and 2. The cost of manufacture (COM) was calculated for two different production scales (5 and 50 L) considering the integrated process vs. only the mechanical fractionation method. The integrated process showed a significantly higher COM than mechanical fractionation method. This work suggests that mechanical fractionation method is an adequate and low-cost process to obtain a rich-pigment product from semi-defatted annatto seeds.

1. Introduction

Annatto (*Bixa orellana* L.) is a native shrub from South America that has been used as a cosmetic and an ingredient in traditional food since pre-Columbian times. Annatto is also cultivated in Central America, Africa and South Asia (Smith & Wallin, 2006). Annatto pigments are widely used in the food, chemical, cosmetic and pharmaceutical industries (Chisté, Yamashita, Gozzo, & Mercadante, 2011). Currently, in addition to their use as coloring agents, annatto pigments are being investigated because of their biological activity and possible health benefits (Giorgi, De Marinis, Granelli, Chiesa, & Panseri, 2013).

Annatto seeds are typically composed of 2.0 to 4.8% lipids, 1.0 to 6.3% pigments, 9.6 to 13.3% moisture, 12.1 to 17% proteins, 5.4 to 6.9% ash and approximately 50% total carbohydrates (starch and lignocellulosic material) (Albuquerque & Meireles, 2012; Silva, Gamarra, Oliveira, & Cabral, 2008). Annatto seeds contain carotenoids and phenolic compounds (Cardarelli, Benassi, & Mercadante, 2008) as well as saponins and tannins (Vilar et al., 2014).

The major coloring matter in annatto seeds is the apocarotenoid bixin, a dicarboxylic monomethyl ester (9′-cis-6,6′-diapocarotenoid-6,6′-dioato methyl hydrogen) derived from 9′-cis-norbixina dicarboxylic acid (Scotter, Wilson, Appleton, & Castle, 1998). Bixin represents > 80% of the colorant substance content in annatto seeds. Bixin is normally present in its cis form but is present in minor proportion in trans-bixin, cis-norbixin and trans-norbixin forms (Preston & Rickard, 1980). Depending of its pigment concentration, the color of annatto pigments ranges between yellow and red (Castro, Mariutti, & Bragagnolo, 2011). The total content of pigment present in seeds varies according to the variety, culture, and pre- or post-harvest technique used (Smith & Wallin, 2006). Annatto pigments can be separated from seeds by various techniques, including immersion in hot vegetal oil, dilute alkaline solutions and other organic solvents (Scotter et al., 1998; Smith & Wallin, 2006; Vilar et al., 2014).

Industrial processes of annatto pigment extraction commonly use alkaline solutions of sodium hydroxide and potassium hydroxide. In the industrial process, bixin pigment reacts with the basic solution, modifying its structure to norbixin. Norbixin and bixin are chemically similar, although norbixin is yellowish; they have different levels of solubility, stereochemistry and stability (Scotter et al., 1998). Generally, bixin extraction techniques produce a crude concentrate with a low quantity of bixin. Key factors for the bixin extraction process are to obtain an increase in the bixin yield and minimize contamination by sub-products that affect the extract composition, which harms the stability and power of coloring (Scotter, 2009). The extraction involving

* Corresponding author.
E-mail address: maam1953@unicamp.br (M.A.A. Meireles).

http://dx.doi.org/10.1016/j.foodres.2017.05.032
Received 22 September 2016; Received in revised form 27 May 2017; Accepted 29 May 2017
Available online 29 May 2017

the use of solvents is considered to be an indirect extraction method. The microstructure of the vegetal matrix is complex. It is formed by cells, intracellular and capillary spaces and pores, so the extraction is influenced by the molecular structure, molecular size, and location of the solute and its ligation with other components (Veggi, Cavalcanti, & Meireles, 2011). The low-pressure solvent extraction (LPSE) technique is used in the chemical industry and is also called leaching, decoction or elution (Wang & Weller, 2006). The solid–liquid extraction process is conducted at atmospheric pressure, placing the solvent in contact with the material to be extracted. After extraction, the solvent is removed, and the extract is concentrated (Leal et al., 2010; Rodrigues, Alcázar-Alay, Petenate, & Meireles, 2014; Santos, Veggi, & Meireles, 2012).

However, to be competitive in today's market, processes to obtain pigment-rich products must be not only efficient but also relatively cheap. Factors such as performance (obtaining as much product as possible), productivity (requiring the least amount of processing time) and selectivity (obtaining a product rich in the substance of interest) should be considered when determining the economic viability of a process (Prado, Prado, & Meireles, 2011). Moreover, integration of processes could be an attractive approach for the production of valuable products of higher quality at lower costs (Cardenas-Toro et al., 2015; Fujii, 2012; Mendes et al., 2009).

Supercritical fluid extraction (SFE) removes most of the lipid fraction present in annatto seeds without extracting a significant amount of pigment (Albuquerque & Meireles, 2012; Moraes, Zabot, & Meireles, 2015). The raw material for this work comprised semi-defatted annatto seed, residue produced after the extraction of the lipid fraction from annatto seeds using SFE. Reutilization constitutes a viable alternative to revalorize industrial residues and reduce their contaminant capacity (Díaz, Alvarado, de Ory, Caro, & Blandino, 2013; Galanakis, 2013).

Food residues are considered as a cheap source of valuable components since the existent technologies allow the recovery of target compounds and their recycling inside food chain as functional additives in different products (Galanakis, 2012).Therefore, the aim of this study was to generate an alternative method for annatto pigment extraction through the use of clean technologies that can concentrate the pigment from semi-defatted annatto seeds. The pigment concentration was conducted using four phases: i) a preliminary study to establish the best sequence of operations over semi-defatted annatto seeds; from this preliminary study, the mechanical fractionation process was established; ii) a first extraction study on the fine particle fraction integrating LPSE with the mechanical fractionation to identify significant parameters in the solvent extraction process; iii) a second study of pigment extraction to optimize the integrated process; and finally (iv) an evaluation of the economic feasibility of the process, comparing mechanical fractionation method and an integrated process consisting in a mechanical fractionation and LPSE methods.

2. Material and methods

2.1. Raw material

The raw material used in this work—denoted Batch 1 and Batch 2—was produced from previous work performed in our laboratory by Albuquerque and Meireles (2012) and Silva, Gomes, Hubinger, Cunha, and Meireles (2015), respectively.

Annatto seeds from Batch 1 were donated by the Agronomic Institute of Campinas (IAC—Instituto Agronômico de Campinas, São Paulo, Brazil) in 2012, and Batch 2 was obtained from Estação dos Grãos Ltda. (São Paulo, Brazil) in 2014. Annatto seeds were partially defatted by SFE using CO_2 (99.9% purity, Gama Gases Especiais Ltda., São Bernardo do Campo, Brazil) in a commercial SFE-2x5LF-2-FMC system (Thar Technologies, Pittsburgh, USA) to yield semi-defatted annatto seeds. The conditions of SFE extraction included a temperature of 40 °C and a pressure of 200 bar; these parameters were determined by Albuquerque and Meireles (2012) for the extraction of the annatto lipid phase with a high content of γ and δ-tocotrienols.

For Batch 1, the SFE was performed using a solvent mass-to-feed mass ratio (S/F) of 3.5, which has been suggested as the best condition for annatto lipid fraction extraction, a condition in which a high content of bixin in semi-defatted annatto seeds was maintained (Albuquerque & Meireles, 2012). For Batch 2, an S/F value of 11 was used, with the objective of extracting the highest amount of lipid fraction from annatto seeds. The semi-defatted annatto seeds were stored at a freezing temperature (− 18 °C) and protected from light before use as the raw material in this work.

2.2. Raw material characterization

The annatto seeds and semi-defatted annatto seeds were characterized in terms of moisture (Sluiter et al., 2008), lipids (Thiex, Anderson, & Gildemeister, 2003) and bixin (Smith & Wallin, 2006) content. The sum of carbohydrate, protein and ash content was determined as the difference between 100 and the sum of the percentages of water, total lipid (fat) and bixin pigment. The raw material was milled using a knife mill (TECNAL, Piracicaba, Brazil). A sieve (CATEL, Piracicaba, Brazil) was used to separate the fractions according to the particle diameter (d_p). The sieves correspond to 16, 24, 32 and 48 mesh, Taylor standard (d_p of 1000, 700, 500 and 300 μm, respectively). The material was classified according to particle size. Particles with a diameter lower than 48 mesh ($d_p \leq 300$ μm) were denoted as fine particles, and the particles with a diameter larger than 48 mesh ($d_p > 300$ μm) were denoted as large particles.

2.3. Preliminary study of the raw material

A preliminary study was conducted with semi-defatted annatto seeds from Batch 1 only. The experiment runs for extraction of the pigment were performed using the LPSE technique. The paths followed for this study are shown in Fig. 1.

Path A followed the operations of mill and sieving of semi-defatted annatto seeds; paths B and C include the LPSE technique for pigment extraction. Path B proposed the LPSE process using milled semi-defatted annatto seeds as the raw material, whereas path C proposed the LPSE process over integer semi-defatted annatto seeds (without any preparation or milling), and the seeds were milled only after the extraction process.

2.4. Solvent extraction method (LPSE)

Semi-defatted annatto seeds (20 g) were placed in Erlenmeyer flasks (250 mL) and extracted with ethanol (Chemco, Hortolandia, Brazil). The Erlenmeyer flasks were placed into an incubator–shaker (MARCONI, Piracicaba, Brazil) at 295 rpm. The S/F ratio was 8, and the extraction was performed for 120 min at a temperature of 50 °C and atmospheric pressure (Rodrigues et al., 2014). Each assay was conducted in triplicate, and every flask was sealed using plastic paraffin film to restrict the loss of solvent. The extract was then filtered through a filtration system using filter paper (LABCENTER, São Paulo, Brazil), with nominal porosity of 15 μm. For quantitative determinations, the solvent was evaporated from the remaining extract using a vacuum-controlled rotary evaporator (Laborota, Vertrieb, Germany) at 35 °C and protected from light. The pigment dry extract obtained was maintained in a desiccator until achieving a constant mass. The extracts were kept at − 18 °C to determine the overall yields and quantification of the total pigment (expressed as bixin) and the total phenolic compounds (expressed as gallic acid equivalents).

2.4.1. First study of pigment extraction in fine particle fraction

In these experimental runs, fine particle fraction from Batch 1 were used as the raw material. Three temperatures (40, 50 and 60 °C), three