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Extraction of bixin from annatto seeds using combined technologies

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ABSTRACT

Bixin is the most used carothenoid in food industry. It is conventionally extracted from annatto seeds (*Bixa orellana* L.) by alkaline solutions or organic solvents, producing bixin with low purity and generating toxic waste. This study aimed to compare different clean technologies for extraction of nutraceuticals (bixin and tocols) from annatto seeds, using a fixed bed extractor. The solvents used were supercritical CO_2, ethanol and a mixture of ethanol and water. Conventional extractions using water and chloroform were also conducted, for comparison. Scanning electron microscopy was used to evaluate the surface of seeds and confirm experimental results. The best extraction efficiencies (for bixin) were using ethanol (ambient pressure) and a sequential extraction (supercritical CO_2 as pretreatment followed by ethanol). This last result has also shown a good recovery of δ-tocotrienol, a powerful natural antioxidant. Higher concentrations of bixin in extracts were obtained using pure ethanol at ambient and high pressures.

1. Introduction

Annatto is a natural colorant that imparts colors ranging from yellow to red due to the concentration of color compounds in the solution [1]. This pigment is obtained from the seed coat of the tropical shrub *Bixa orellana* L. This tree is native to tropical South America, where it has been a traditional ingredient of some foods for centuries [2].

The main pigments of annatto seeds are bixin and norbixin, whose structures are shown in Fig. 1. These pigments are carotenoids whose colors vary between yellow and red, colors of huge importance in the food, pharmacological and cosmetic industries. In food industries these natural pigments are used in cheeses, sausages, meats and candies [3]. Annatto seems to be an important natural colorant for food and drug industries owing to its potential uses as a substitute for Tartrazine which is a synthetic colorant that is prohibited in many countries [4]. Later annatto is classified as a 'color additive exempt of certification' by FDA of United States of America [5].

Studies have shown that other peculiarity of annatto is that its lipid fraction contains a large amount of δ-tocotrienol [7,8]. Tocotrienols have been associated with hypocholesterolemic effects and are believed to be useful in the treatment of cardiovascular diseases and cancer [8,9]. The lipid-rich fraction of annatto can also be effective in the prevention of lipid peroxidation [10]; probably, the tocotrienols combined with bixin act synergistically to protect the unsaturated lipids from oxidation [11].

Annatto pigment can be separated from annatto seeds by many ways, including immersion of seeds in hot vegetable oil, dilute alkaline aqueous solutions and solvents. In the first case, the pigment is obtained by abrasion of the exocarp submerged in warm vegetable oil (70 °C). This process produces more concentrated suspensions pigment but it may contains degradation products, considering that, at elevated temperatures, annatto pigment gets degraded and form several products [12,13]. Industrially, the most used process is the extraction with an alkaline solution, usually potassium or sodium hydroxide [14]. This process is known to transform bixin (present in the seeds as an ester) into a diacid salt 'norbixate' that is soluble in water [15,16]. The removal of alkaline solution from the bixin-rich extract, as well as from the seeds, requires several subsequent unit operations, elevating the energy costs of the process [17]. Many solvents such as acetone, chloroform, ethanol, ethyl acetate and hexane are used for extraction of annatto pigment due to the strong solubility of this pigment. Solvent extraction results in the purest form of bixin pigment [18]. The commercial preparations of annatto colors with organic solvents have the disadvantage of small concentrations of pigments and a residual toxic solvent in the product. Solvents can also be removed from these high color-concentrated solutions to give bixin crystals, elevating, however, the energy costs of the process [2,19].

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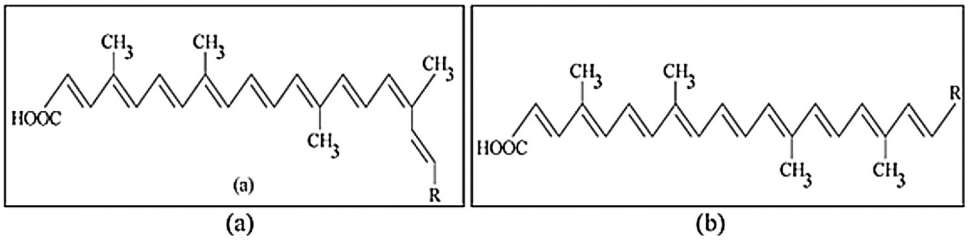

Fig. 1. Molecular structures of bixin (R=$COOCH_3$) and norbixin (R=COOH): (a) α-cis and (b) β-trans.
Source: [6].

Various techniques have been studied to develop clean extraction technologies with environmental benefits [20]. These techniques are used to extract bioactive substances, to shorten the processing time, reduce or eliminate solvent consumption, using mechanical extraction [21,22], increase the extraction yield and improve the quality of the extracts. These studies are needed because of the general trend of the market to identify products that generate economic, social and environmental advantages [2,23]. Supercritical extraction with CO_2 could be a good alternative in these cases avoiding environmental problems.

Supercritical CO_2 with different pressures and temperatures proved to be advantageous for removing compounds from complex food systems than conventional methods [24] because of least thermal effects on products, high quality of recovered products, low energy requirement for solvent recovery and high selectivity in the separation process. Recently, many attempts have been carried out to use supercritical carbon dioxide fluid for the extraction of pigment from annatto seed. However, the results of these studies seemed to be unsatisfactory both in terms of economics and efficiency [1,6,24–27]. These studies showed that the efficiency of the extraction was improved with increasing temperature and pressure, the seed oil acted as a co-solvent in the extraction of bixin from the seeds and the yield of bixin was significantly increased with the use of organic solvents as co-solvents [1,24,26,27]. Supercritical CO_2 method has been studied, also, as a pretreatment for defatting of annatto seeds [19,20]. These studies aimed to remove a lipid layer strongly associated to the bixin in the seed; the removal of the lipidic layer could increase bixin recovery.

Based on the improvement of the clean technologies, the objective of this work was to carry out supercritical fluid extraction from annatto seeds with CO_2 combined with other extraction methods which have low environmental impact, applying both high and low pressure, in order to increase the results and make the process more economically attractive.

2. Materials and methods

2.1. Materials

Annatto seeds were obtained from Rio Vermelho – MG and stored at −18 °C, protected from light in dark bags of polyethylene until further analysis and first tests. An 18 kg cylinder of liquid CO_2 was provided by White Martins (Praxair Inc., Campinas, Brazil). The solvents used in analyses were: chloroform, hexane, acetone and ethanol. All of them were of analytical-grade.

2.2. Seeds characterization

A batch of the seeds was characterized for moisture, protein, ash, total lipids and bixin content. Moisture, protein and ash contents were determined according to the official methods published by the Association of Official Analytical Chemists (AOAC) [28]. The seed oil content was quantified by gravimetric analysis, evaporating until dry the total volume of an extraction in a Soxhlet apparatus with hexane (P.A.). The carbohydrate content was determined as the difference, experiments were carried out in duplicate.

The annatto oil extracted was also characterized in term of its fatty acids profile using capillary gas chromatography (GC) according to AOCS official method Ce 1-62 [29]. The analysis was performed on a CGC AGILENT 68650 SERIES GC SYSTEM, column DB-23 AGILENT (50% cyanopropil) – methylpolysiloxane, 60 m × 0.25 mm × 0.25 μm, column flow rate of 1.0 mL/min, carrier gas: Helium and volume injected 1.0 μL.

The tocols content (tocopherol and tocotrienol) was determinated by high performance liquid chromatography (HPLC) according AOCS official method Ce 8-89 [29]. The isomers (alpha, beta, gamma, and delta tocotrienols and tocopherols) were quantified using a standard curve prepared under the same conditions as that of the analysis. The analysis was performed on a Hibar RT Column (250 mm × 4 mm Li Chrosorb Si 60, 5 mm). The mobile phase, hexane/isopropanol (99:1) was used under isocratic conditions at a flow rate of 1.0 mL/min.

The pigment content of the seeds was determined by a method using organic extraction [12], which consists in repeated extraction of the seeds with chloroform, until the exhaustion of pigment contained in the seeds. This extraction was conducted at 50 °C using a balance cell coupled to a reflux condenser, whose purpose was to prevent the loss of desirable compounds (volatiles). The temperature of the bath connected to the Soxhlet was 5 °C. These conditions were chosen based in the work performed by Silva et al. [30] whose results show that, besides the good performance of chloroform as solvent, temperatures below 80 °C cause minimum degradation in bixin.

For results comparison, the same extraction was performed with water, also at 50 °C. The bixin content of the seeds was determinate, then, using spectrophotometric method according to Joint FAO/WHO Expert Committee on Food Additives Monographs [31]. Sample Absorbance was measured in a 1 cm quartz cuvette at 487 nm, using a UV–vis spectrophotometer. The percentage of bixin in the annatto seeds was calculated, then, according to Lambert–Beer law, using $E^{1\%}_{1\,cm} = 3090$ [31], in Eq. (1):

$$\text{Bixin}\,(\%) = \frac{A * V_1 * \ldots * V_n}{E * m_{\text{sample}} * V_1' * \ldots * V_n'} \quad (1)$$

In which:

A = average absorbance of the samples;
V_i = dilution volume (i = 1, 2,. . ., n);
E = absorptivity coefficient E (equal to 3090 for bixin);
M_{sample} = sample mass, in grams; and
V_i' = volume of aliquot for dilution (i = 1, 2,. . ., n).

2.3. Fixed bed extraction

The fixed bed experiments were conducted in an experimental unit bench, in the Laboratory of Extraction, Applied