PRESSURIZED LIQUID EXTRACTION (PLE) OF BIXIN FROM DEFATTED ANNATTO SEEDS

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Abstract. Bixin, a diapo-carotenoid ($C_{25}H_{30}O_4$) with cis configuration and soluble in organic solvents, is the predominant pigment in annatto seeds. Extraction with pressurized liquid (PLE) is a process of extracting organic compounds that can be applied to a variety of solid and semisolid samples. Therefore, the aim of this study was to evaluate the performance of PLE for the extraction of bixin from annatto seeds previously defatted by supercritical solvent extraction. The solvents used were water, ethanol and ethyl acetate; the extractions were performed at 2 MPa and 333 K. For comparison purposes, low-pressure solvent extractions (LPSE) were performed room temperature and pressure. The quantification of the bixin in the extracts was made by a spectrophotometric method at 487 nm (extinction coefficient 3.090). The lowest and highest process yields were was obtained when water was used as solvent: 0.80% (PLE) and 20.1% (LPSE). The yields for the solvent ethanol were 17.5% (PLE) and 9.5% (LPSE). The effects of process conditions were less important for the solvent ethyl acetate: the yield was 19.65% for PLE and 14.3% for LPSE. These preliminary results indicate that for the solvent water PLE is not a recommended process.

Keywords: Bixin, defatted annatto seeds, pressurized liquid extraction, supercritical fluid extraction, annatto, colorant.

1. Introduction

Annatto (*Bixan orellana L.*) is a tropical tree whose seeds produce pigments which have a widespread use in the food industry, namely for colouring butter, margarine, cheese, oils and sauces, with hues ranging from yellow to red. The pigments are located in the pericarp of the seeds (2- 3 wt. %) and have two main components: oil-soluble bixin (80 wt. %) and water-soluble norbixin [1].

It has the natural colorant bixin, which is the most widely used colorant in food processing [2]. The pigments of the seeds, bixin and norbixin, are amongst those most used in the food, pharmacological and cosmetic industries due to the intensity of their colours, their greater stability and the wide variety of tones from yellow to red. This range of colours is an additional advantage of the annatto carotenoids over other carotenoids, such as those of the carrot and beetroot, which only show their respective colours [3].

More than 80% of the total pigments in the seeds coat of annato consists of the carotenoid bixin, 6-methyl hydrogen 9'-cis-6,6'-diapocarotene-6,6'-dioate [4]. The long system of conjugated double bonds present in the structure of bixin is responsible for the red color, but also for the poor stability of the molecule under processing and storage conditions such as high temperature, light and oxygen [5].

Nowadays, food scientists have collaborated with nutrition researchers to develop plant-based functional foods to promote healthy eating habits. In food research, carotenoids from fruits and vegetables have attracted a great deal of attention, mainly focused on the analysis of geometric carotenoid isomers. Carotenoids found in fruits and vegetables have also attracted great attention for their functional properties, health benefits and prevention of several major chronic diseases [6].

The carotenoid composition of foods of plant origin is variable and made complex by low levels of biosynthetic precursors and derivatives of the main components. Incapable of biosynthesizing carotenoids, animals depend on dietary carotenoids, which are selectively or unselectively absorbed, converted to vitamin A, deposited as such or slightly altered to form carotenoids typical of animal species. Consequently, carotenoids are not as widely distributed in animal-derived foods and are present at much lower levels [7].

An important method applied for the commercial extraction of annatto pigments from the *B. orellana* fruit consists of heating the suspension of seeds in oil to a maximum temperature of 130 °C under vacuum [8]. It is important to mention that the temperature appears as one of the main sources of the bixin molecule degradation. Near this extraction temperature, bixin undergoes a series of degradation reactions. The colorant degradation is observable by color changes, which is an important characteristic for the food industry as by controlling the temperature and duration of the heating process the red/yellow balance of this colorant can be tuned [9].

The possibility of tailoring the colorant end color opens a vast range of use. Nevertheless, the degradation or structural modification of the bixin is accompanied by the release of *m*-xylene, toluene, toluic acid, and toluic acid methyl ester, all of which are undesirable in preparations intended for food use [10]. Extraction with organic solvents and extraction into aqueous alkali are also important commercial processes used to extract the pigment from dried annatto seeds.

In particular, the stability of bixin was evaluated under the effect of several factors, such as light, oxygen concentration, temperature, and solvent [11]. Bixin showed great stability in the dark, in the presence or absence of oxygen, but under direct illumination was degraded. The thermo- and photostability of the 9'-cis double bond in methyl bixin was reported as being so great that the molecule underwent a second trans-cis isomerization given considerable amounts of the 9, 9'-di-cis isomer, but in the presence of iodine and light, 9'cis-trans isomerization occurred [12]. Therefore, it seems that the fade of bixin could be strongly dependent on the experimental conditions (e.g., direct illumination, heating, photosensitization, etc.) [13].

There are several techniques that can be used to extract bixin from annatto seed. Among them, PLE has been reported to be highly efficient for the extraction of bixin while presenting several advantages over conventional methods.

The main purpose of extraction is to transfer the analyte(s) from the matrix to a suitable medium for introduction into the analytical instrument for analysis. There are four major factors governing the extraction efficacy: solute-matrix interactions, solute solubility and diffusion, and viscosity of the solvent.

Usually extraction conventional methods have time and temperature high and may promote the degradation of thermolabile compounds, like the bixin.

In order to overcome these drawbacks short time extraction conditions using pressurized solvent methods, such as Supercritical Fluid Extraction (SFE) and Pressurized Liquid Extraction (PLE) methods have been used successfully to obtain antioxidant pigments-rich extracts [14].

The supercritical fluid state is characterized by fluid being above its critical temperature (Tc) and critical pressure (Pc). Manipulating the temperature and pressure of the fluid can solubilize the material of interest and selectively extract it [15].

A major advantage of PLE over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents remain in a liquid state well above their boiling points, allowing for high-temperature extraction. Higher extraction temperature can improve analyte solubility in the solvent and improve its desorption from the raw material matrix [16].

The method of extraction with pressurized fluid uses lower volumes of solvent to conventional extraction (Soxhlet, etc.) and is faster (extraction time varies between 10 and 20 minutes) [17]; thus, decreasing degradation of thermolabile compounds.

Usually, for both extraction processes with pressurized fluids, as for the precipitation processes, solvents GRAS ("Generally Recognized As Safe") are preferred, like carbon dioxide, water and ethanol, more commonly used.

In this context, the aim of this study was to evaluate the performance of PLE using different solvents for the extraction of bixin from annatto seeds previously defatted by supercritical solvent extraction.

2. Materials and Methods

2.1 Plant material

Annatto (*Bixa Orellana*) seeds variety Piave was obtained from the Instituto Agronômico de Campinas – IAC (Agronomic Institute of Campinas), Department of Agriculture and Supply of the State of São Paulo. Sample were identified and stored at 275K until being used as raw-material for the extractions.

2.2 Pressurized liquid extraction

Supercritical fluid extraction was used as a pretreatment for defatting annatto seeds. This process was performed at 333k and 40 MPa. More details about this process can be found in Albuquerque and Meireles [19].

The diagram of the PLE system is shown in Figure 2. Some solvents were evaluated for the extraction of bixin: Ethyl acetate PA (Merck, Darmstadt, Germany); Ethanol PA (Santo Amaro, Brazil) and distilled water. Four grams of defatted annato seeds was placed in the extraction cell (6.57 mL, Thar Designs, CL 1373, Pittsburg, USA) containing a synthesized metal filter at the bottom and upper parts. The cell containing the sample was heated at 333K by an electrical heating jacket for 5 min to ensure that the sample reaches thermal equilibrium, and then filled with desired extraction solvent and pressurized. The extraction solvent was pumped by a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, San Jose, USA) into the extraction cell until the pressure of 2 MPa was reached. The pressure was maintained constant (2 MPa) for the duration of static extraction time (10 min). Thereafter, the check valve and back pressure valve were carefully opened, keeping the pressure at an appropriate level for the desired flow (1.67 cm^3/min), to rinse the extraction cell with fresh extraction solvent for 18 min (dynamic extraction time).

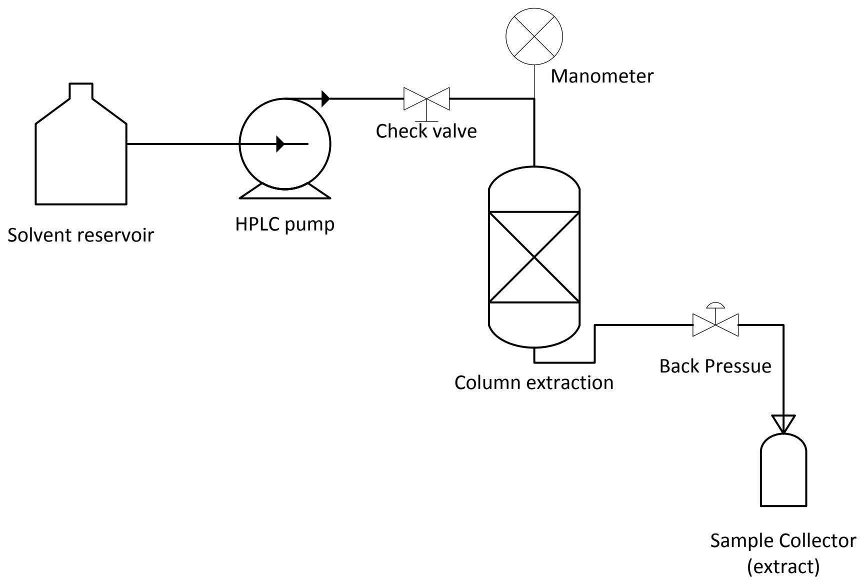

Figure 2. A simple flow schematic of the PLE process.

3.2 Low-pressure solvent extraction (LPSE)

Conventional solid–liquid extraction was performed at room temperature and pressure. Four grams of defatted annatto seeds was placed Erlenmeyer flasks containing 30 cm^3 of solvent (ethanol, ethyl acetate and water). Extractions were carried out with agitation for 30 min. After extraction, the solvent was separated of the annatto seeds and the extracts were rapidly cooled and maintained at 255 K temperature and protected from light.

3.3 Bixin analyses

The bixin contents of the seeds and of the extracts were determined according to Joint FAO/WHO Expert Committee on Food Additives Monographs [2]. Bixin was exhaustively extracted from the defatted seeds with acetone (Merck, Darmstadt, Gemany), i.e. until the seeds were colorless [19], by agitation at room temperature (293 K). The PLE extracts were diluted in acetone to yield suitable concentrations of bixin for analysis. Sample absorbance was measured at 487 nm with a UV–vis spectrophotometer (U-3010, HITACHI, Tokyo, Japan), and the bixin content was calculated according to the Lambert–Beer law (Equation 1), using

E1% 1cm = 3.090 [19]. V1 and V2 are volumes of dissolution, va is an aliquot volume and mEXT is the extract weight (µg).

$$Bixin\ \% = \frac{A \times 10^4}{E_{1\ cm}^{1\%}} \times \frac{V_1 \times V_2}{m_{EXT[\mu g]} \times v_a} \qquad (1)$$

3. Results and Discussion

3.1 Defatting of annatto seeds

The efficiency of SFE for obtaining the extract was 85%, and the seeds were almost completely degreased. The results of this process conditions (333 K and 40 MPa) are low yields of bixin so as to obtain the maximum amount of extract with the lowest level of pigment possible, enabling subsequent reextraction of bixin from the seeds with a generally recognized as safe (GRAS) solvent.

3.2 Reextraction of annatto seeds: extracting bixin

In this research, the reextraction was done with PLE and LPSE using ethyl acetate, ethanol and water. The extraction conditions were kept constant in 333 K temperature and 2 MPa pressure. The overall yield (X_0) at the end of each extraction is calculated according to Equation 2, as the ratio of the total mass of extract and of initial sample weight on a dry basis.

$$X_0 = \frac{m_{extract}}{m_{sample}} \times 100 \qquad (2)$$

For comparison purposes, low-pressure solvent extractions (LPSE) were performed room temperature and pressure. The overall yields (%) and yields bixin of the extractions are showed in Table 1.

Table 1. Overall yields and yields of bixin of the extractions.

Solvent	Overall yield		Yield of bixin	
	PLE	LPSE	PLE	LPSE
Ethanol	7.04%	3.15%	17.54%	9.51%
Ethyl acetate	5.65%	3.97%	19.65%	14.34%
Water	17.10%	2.90%	0.80%	20.87%

Comparing both extraction methods, the one which resulted in a higher overall yield was that using the PLE method, where overall yield is the ratio between the final extract mass (after extraction and solvent separation) and the initial sample mass. The largest overall yield observed was for water extraction (17.10 %). The overall and bixin yields are shown in Figure 2 and Figure 3, respectively.

The yield of bixin was calculated relative to the total amount of bixin found in the defatted seed that was 1.93%. In PLE method, ethanol and the ethyl acetate presented a smaller overall yield than water, but the percentage of bixin extracted by water was the lowest of the solvents. The LPSE method had yield of bixin lower than the method by PLE when using ethanol and acetate solvents. Just when water was used as solvent in the LPSE method we have a better yield of bixin.

Ethanol pressurized (PLE) showed improved yields of extraction of bixin than ambient conditions (LPSE). Therefore the use of ethanol by PLE for extraction of bixin is a viable alternative, since it could be easily separated from the extract, which would represent a lower energy expenditure in its obtaining. Therefore, it is suggested to use ethanol in the extraction of bixin by PLE in defatted seed.

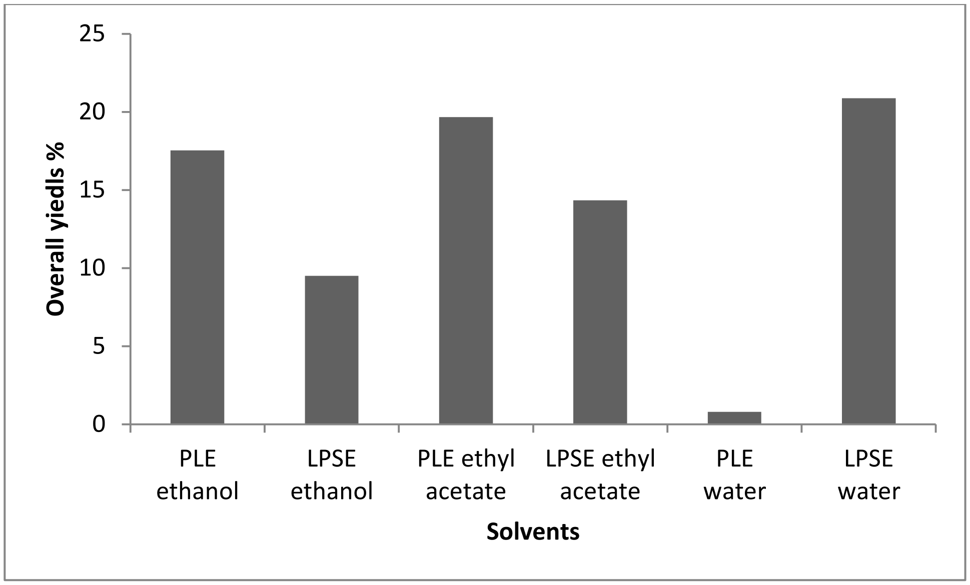

Figure 2. Overall yield.

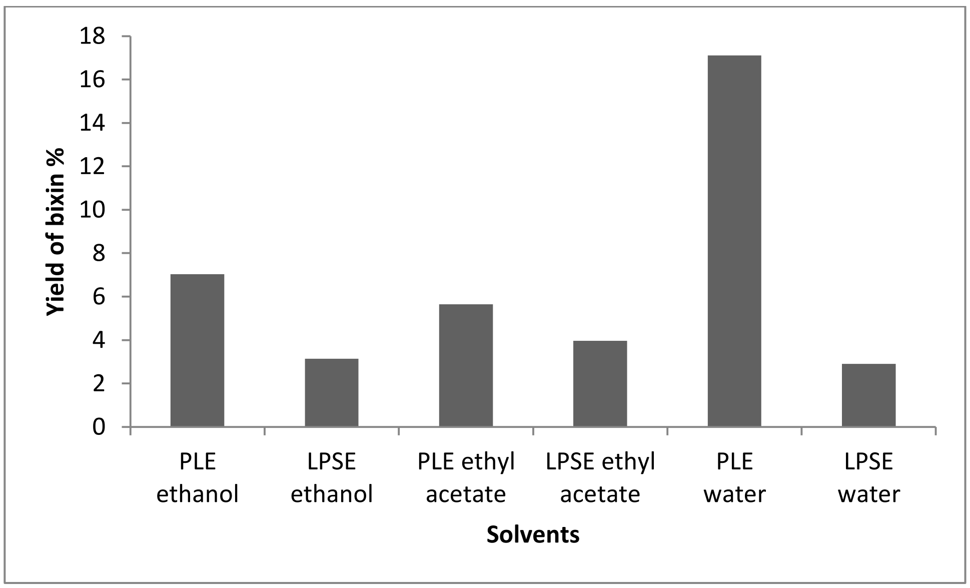

Figure 3. Yeld of bixin.

4. Conclusions

In this study, PLE and LPSE were used to extract bixin from deffated annatto seeds. The highest yield of bixin was obtained using ethyl acetate for PLE and water for LPSE. The results indicate that for the solvent water PLE is not a recommended process. Reduced solvent consumption and shorter time extraction has been the main advantages of PLE over LPSE. Although the ethyl acetate presented the best yield of bixin by PLE, ethanol is the best alternative because is generally recognized as safe (GRAS) solvent. Therefore, it is suggested to use ethanol in the extraction of bixin by PLE in defatted seed.

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