PRESSURIZED WATER EXTRACTION (PWE) OF ANNATTO PIGMENT

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Abstract. Annatto (*Bixa orellana L.*) contains the natural pigment bixin which is not soluble in water under ambient conditions. The industrial process used to obtain bixin from annatto seeds is the extraction with alkaline solution; this process is known to transform bixin (ester) into norbixin (dicarboxylic acid) that is soluble in water. The overall aim of this work was to use pressured water extraction (PWE) technique to increase the efficiency of the extraction process. For this purpose PWE was conducted on defatted annatto seeds using pure water and alkaline solutions (NaOH) of pH 10 and 14. Temperatures of 303, 353 and 393 K and pressures of 2, 4 and 20 MPa were used. A low-pressure solvent extraction (LPSE) was also performed. Bixin and norbixin on extracts were determined by UV at 470 and 482 nm; the molar extinction coefficient used was $E_{1cm}^{1\%}$= 2826 and 2870, respectively. The largest yields in bixin were obtained for the LPSE process: 20.9% (pure water), 10.5% (pH = 10) and 2.95% (pH = 14). For pure water at 303 K, the yields were 0.13% and 0.08% for 2 and 4 MPa, respectively. For pure water at 393 K, the yields were 0.37% and 0.20% for 2 and 20 MPa, respectively. At 353 K and 2 MPa, the yields were 0.22%, 0.58% and 0.40% for pure water, water at pH = 10 and water at pH = 14, respectively. Therefore, this work shows that PWE is not an efficient process for the extraction bixin from defatted annatto seeds.

Keywords: *Bixa orellana L.*, bixin, pressured liquid extraction, pressured water extraction.

1. Introduction

Over the last decade, the technology of pressurized fluid extraction (PFE) using water (or subcritical water) has emerged as a sustainable alternative for the extraction of antioxidants from natural sources. Subcritical water extraction (PWE) is an environmentally friend technology that is fast, selective can be automated and uses none or small amounts of organic solvents [1, 2].

There are many important studies of the extraordinary properties of subcritical and supercritical water for chemical reactions, the properties of superheated water, subcritical water, and supercritical water change with temperature and density [3].

Many of the anomalous properties of water are due to the very strong hydrogen bonding. Over the superheated temperature range the extensive hydrogen bonds break down, changing the properties more than usually expected by increasing temperature alone [3].

Water has several distinguishing properties that make it one of the most ideal solvents in natural products studies. First, water is the greenest, cheapest and most easily available solvent. Secondly, by adjusting pH or adding certain salts water can provide very selective extraction, finally, the polarity of water can vary significantly with temperature changes so that water may be used to extract a variety of compounds and behaves more like an organic solvent, such as methanol or ethanol with different polarities. Water can be used as a solvent, reagent, and catalyst in industrial and analytical applications, including extraction, chemical reactions, and cleaning [3, 4].

However, high temperature is not always desirable for natural products studies because unwanted reactions such as oxidation, decomposition, degradation or rearrangement reactions may occur at elevated temperatures [4].

The main pigments present in the seeds of annatto (*Bixa orellana L.*) are bixin and norbixin, whose structures are shown in Figure 1. These pigments are carotenoids of coloration yellow and red, colors of importance in the food, pharmacological and cosmetic industries [5, 6]. In food industries these natural pigments are often used in cheeses, sausages, meats and candies.

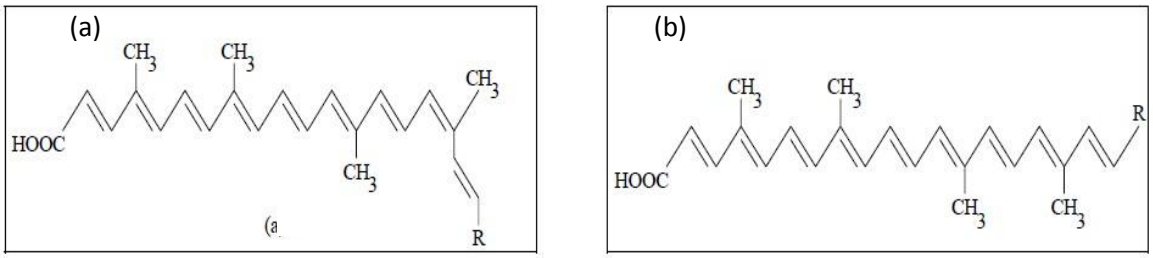

Figure 1. Molecular structures of bixin (R=COOCH3) and norbixin (R=COOH): (a) α.-(cis) and (b) ß-(trans).

Normally, three main methods can be used to extract the pigment from the annatto seeds: vegetable oil extraction, alkaline solution extraction and organic solvent extraction. In the first case, the pigment is obtained by abrasion of the exocarp submerged in warm vegetable oil (340 K). When extracted by organic solvent, such as acetone and methanol, a product with higher pigment concentrations, can be obtained. In this case, after extraction, the solvent is removed and then the powder pigment is dissolved in vegetable oil. The water-soluble form of this pigment is produced by abrasion of the annatto seed exocarp in alkaline solution, and the resultant product is the salt of norbixin (cis and trans) [5, 7, 8, 9]. Therefore, bixin is easily converted into norbixin by dissolving the bixin in an alkaline medium.

The chemical, toxicological, and antioxidant properties, and degradations of bixin and norbixin have been extensively studied. Considering the restrictions placed on the use of synthetic pigments by the World Health Organization, interest in natural pigments is increasing. The annatto seed pigments, bixin and norbixin, are amongst those most used in the food, pharmacological and cosmetic industries due to the intensity of their colors, their greater stability and the wide variety of tones from yellow to red. This range of colors is an additional advantage of the annatto carotenoids over other carotenoids, such as those of the carrot and beetroot, which only show their respective color [9].

The objectives of this work was to study the effects of temperature (303–393 K) and pressures (2–20 MPa) on the extraction of bixin and norbixin from defatted annatto seeds using PWE; and, to compare the extraction efficiency of PWE with that of conventional extraction methods.

2. Materials and methods

2.1 Materials

Sample preparation. Annatto seeds (*Bixa orellana L.*) was obtained from Institute Agronomic of Campinas (IAC, Brazil); the whole seeds were subjected to supercritical fluid extraction (SFE) with supercritical CO_2 (99.9% CO_2, Gama Gases Especiais Ltd., São Bernardo do Campo, Brazil) in a commercial extraction system (Spe-ed SFE Laboratory System, 7071, Applied Separations, Allentown, USA), equipped with an electric oven and a pneumatic pump [10]. SFE was conducted at conditions of 313 K and 20 MPa, and resulted in the extraction of a lipidic fraction, with high content of γ e δ-tocotrienol [9, 10, 11, 12]. The residue from this process consisted of seeds with a small lipid content [10, 14]; the amount of bixin and norbixin removed from the seeds were very small. The defatted seeds, which were the raw material for this study, were maintained at 255 K and protected from light.

2.2 Extraction procedures

Pressurized water extraction (PWE). The pressurized liquid extraction unit is shown in Figure 2. The solvent was pumped by a HPLC pump (Thermoseparation Products, Model ConstaMetric 3200 P/F, Fremoni, USA) into the extraction cell, which was placed in an electrical heating jacket at a desired temperature, until the required pressure was obtained. All connections within the system were made using stainless steel tubes [13].

Defatted annatto seeds (4.0 g) were placed in a 6.57-cm^3 extraction cell (Thar Designs, Pittsburg, USA) containing a sintered metal filter at the bottom and upper parts. The cell containing the sample was heated, filled with extraction solvent and then pressurized.

The sample was placed in the heating system for 5 min to ensure that the extraction cell would be at the desired temperature (303–393 K) during the filling and pressurization procedure.

Thereafter, the blocking and micrometric valves 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 30 min (dynamic extraction time).

After PWE, the extracts were rapidly cooled and maintained at 255 K and protected from light to prevent pigment degradation prior to analysis.

The extraction solvents were: pure water and alkaline solutions (NaOH) at pH 10 and 14.

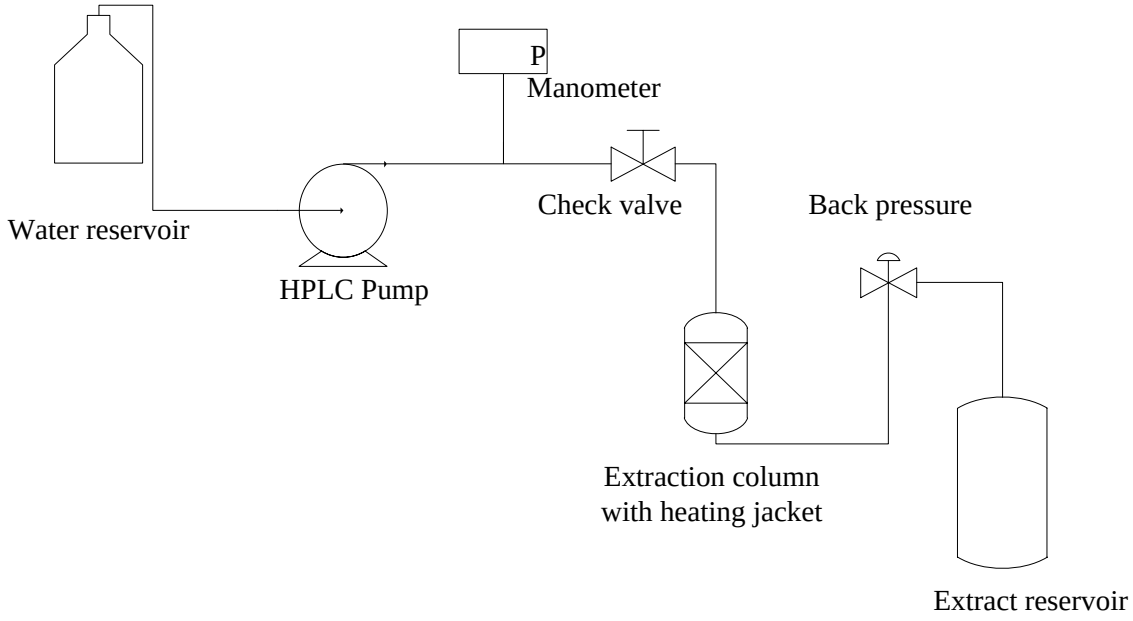

Figure 2. Pressured water extraction unit.

Conventional low-pressure solvent extraction (LPSE). Conventional solid–liquid extraction was performed at room temperature and pressure, immersing 4.0 g of defatted annatto seeds into Erlenmeyer flasks containing 30 cm^3 of solvent (pure water and alkaline solutions (NaOH) of pH 10 and 14). Extractions were carried out with agitation for 30 min. After extraction, the solvent was separated from the annatto seeds and the extracts were rapidly cooled and maintained at 255 K temperature and protected from light.

2.3 Analysis of the extract

The bixin and norbixin in the extracts were determined according to the method issued by Joint FAO / WHO Expert Committee on Food Additives Monographs [5]. The bixin and norbixin were exhaustively extracted from the seeds by maceration and with acetone (Merck, Darmstadt, Germany) until discoloration of seed at room temperature.

The liquid extracts of PWE and LPSE were lyophilized and after dilute in acetone to yield suitable concentrations of bixin or norbixin for analysis.

The yield bixin and norbixin extracts (%) of the defatted annatto seeds and of the extracts were determined by UV (Spectrophotometer Hitachi U 3010) at 470 and 482 nm (A); the molar extinction coefficient used was $E_{1cm}^{1\%}$= 2826 and 2870, respectively with Equation 1. V_1 and V_2 are volumes of dissolution, v_a is an aliquot volume and m_{EXT} is the extract weight (μg).

$$\text{Content of pigment (\%)} = \frac{A \times 10^4}{E_{1cm}^{1\%}} \times \frac{V_1 \times V_2}{m_{EXT[\mu g]} \times v_a} \quad (1)$$

3. Results and discussion

3.1 Bixin content

The results for PWE are shown in Table 1. The highest yield of 0.37% bixin extract was showed at temperature of 393 K and pressure of 2 MPa. At 393 K, the content of bixin was much higher for the extraction done at 2 MPa (0.37%) than that done 20 MPa (0.20%). The final extracts under these conditions (393 K and 20 MPa) showed a brownish coloration, in contrast with the coloration of bixin characteristic of yellow-red. The extracts also had a strong odor of caramel due at hydrolysis reaction and degradation of the sugars present in annatto seeds. At 303 K, the bixin content varied from 0.13% (2 MPa) to 0.08% (4 MPa). However in this case, both extracts showed extracts a characteristic color (yellow). In this study the increase of pressure decreased the yield, thus, further experimental work is needed to understand the role of pressure over the system pressurized water + defatted annatto seeds. At 2 MPa, bixin contents were 0.13% (303 K), 0.22% (353 K) and 0.37% (393 K) indicating a favorable effect of temperature. Higher temperatures were not tested considering the unwanted reactions and degradation of pigment.

Table 1. Global yield and Bixin content (%)

	PWE extract (K/MPa)					LPSE extract (K/MPa)
	303/2	353/2	393/2	303/4	393/20	303/0.1
Global yield	2.61	5.41	11.86	2.62	12.59	2.90
Bixin content	0.13	0.22	0.37	0.08	0.20	20.90

The largest bixin content in the extract was obtained by technique LPSE (20.90%), therefore, this work shows that PWE is not an efficient process for the extraction bixin from defatted annatto seeds.

3.2 Norbixin content

Norbixin was quantified in the process performed using alkaline solutions (pH 10 and pH 14) for PWE done at 353 K/2 MPa and the LPSE (Table 2).

Table 2. Global yield and norbixin content (%)

	PWE extract (353K/2MPa)		LPSE extract (303K/0.1MPa)	
	Global yield	Norbixin content	Global yield	Norbixin content
Alkaline Solution (pH 10)	5.65	0.58	2.28	10.50
Alkaline Solution (pH 14)	15.60	0.40	13.25	2.95

The largest content of norbixin (10.50%) was obtained by LPSE using the alkaline solution at pH 10; increasing the alkaline solution pH to 14 decreased the yield. For the PWE process the same trend with respect to the effect of pH was observed, nonetheless, the yields were two orders of magnitude smaller.

4. Conclusion

This study demonstrated that PWE is not an adequate method for the extraction of pigments from defatted annatto seeds as compared to LPSE.

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