SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF BIXIN FROM ANNATTO SEEDS

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Supercritical CO_2 extraction of the pigments from annatto seeds was carried out at a pressure of 200 bar and a temperature of 40 °C using two flow rates. The efficiency of the extraction was low for the conditions studied. However, the solubility of bixin in CO_2 modified with ethanol (5 mol %) was determined, having been verified a high increase relatively to the solubility in pure CO_2.

Therefore, supercritical fluid extraction of the pigments from annatto seeds using CO_2 containing 5 mol % of ethanol was carried out at pressures of 200 and 300 bar and temperatures of 40 and 60 °C.

The extraction efficiency using the modified supercritical fluid was higher than that obtained with pure supercritical CO_2. The extraction yield increased with pressure and temperature. On the other hand, it decreased with flow-rate.

To assess both the efficiency and selectivity of bixin extraction from a matrix with higher solute bulk concentration, an annatto preparation was obtained from annatto seeds, and submitted to supercritical fluid extraction using the same modified solvent. The efficiency of the extraction was similar to that obtained when annatto seeds were used, but the pigment concentration in the supercritical extracts was higher.

INTRODUCTION

Annatto (*Bixa orellana*) 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, 2].

Bixin ($C_{25}H_{30}O_4$) is a carotenoid with a molecular weight of 394 g/mol, and chemically is the mono-methyl ester of dicarboxylic acid norbixin [3]. This compound is one of the main natural colouring materials [4], being soluble in oils upon heating and weakly soluble in supercritical CO_2 [5]. The norbixin is soluble in water and insoluble in supercritical CO_2 [6].

Commercial extracts of oil-soluble annatto pigments are obtained from the seeds by several processes such as suspension in oil, mechanical processes and solvent extraction (with chloroform, dichloroethane and acetone) [3,6]. The commercial preparations of annatto colours with organic solvents have the inconvenient of small concentration of pigments and residual toxic solvent in the product [2,7]. Suspensions of annatto pigments in vegetable oil are more concentrated but can contain several degradation products due to the fact that elevated temperatures are applied in the extraction process (>100 °C) [2,7]. Supercritical extraction with CO_2 could be a good alternative to avoid these problems.

Studies of Annatto pigments extraction have been carried out using supercritical CO_2 [1], [6], [8], and CO_2 modified with several entrainers: methanol, chloroform and acetonitrile containing acid trifluoracetic [8], and soybean oil [6]. It was shown that the presence of the entrainers increased the extraction efficiency.

The objective of this work is to carry out supercritical fluid extraction of pigments from annatto seeds and from an annatto preparation, with CO_2 and CO_2 modified with ethanol, and to assess the influence of temperature, pressure, solvent flow-rate and matrix arrangement on the extraction yield.

MATERIALS AND METHODS

Materials

Carbon dioxide (99.998% purity) and carbon dioxide modified with 5 mol % ethanol were purchased by Air Liquide. Annatto seeds, and bixin were supplied by Universidade Federal do Rio de Janeiro - Brazil, through a joint project. Chloroform (p.a.), methanol (HPLC grade), acetic acid (p.a) and n-hexane (p.a) were purchased from Merck.

Sample preparation

For the solubility studies 4 g of *cis*-bixin were mixed with 3 mm glass beads packed between two layers of glass wool, in a 32 cc equilibrium cell. Previously to the solubility measurements, bixin was purified by passing several hundred litres (STP) of supercritical carbon dioxide and carbon dioxide modified with 5 mol % ethanol [5].

The supercritical extraction measurements were carried out in a 5 cc pressure vessel filled with about 2 g of annatto seeds, packed between two layers of glass wool.

The annatto preparation was obtained by solvent extraction of the seeds following the process suggested in [3]: ground annatto seeds, previously washed with hexane, in order to remove unwanted impurities, were submitted to extraction with chloroform and these extractions were repeated several times until a colourless residue was obtained. Then the chloroform, containing the pigment, was evaporated under vacuum. Two batches of annatto preparation were submitted to supercritical fluid extraction. In the first one 46 mg of the annatto preparation were precipitated on 3 mm glass beads and enclosed in the 5 cc cell between two layers of glass wool. In the second one a mixture of 20 mg of annatto preparation and 3 mm glass beads was obtained and put into the 5 cc cell. Clean glass wool was placed at the two ends of the extraction vessel.

The pigment content of the seeds was determined by organic extraction using the method suggested in [7]. This method consists in to carry out repeated extractions of the seeds with chloroform, until the exhaustion of the material colour. The total pigment content was determined using the Lambert-Beer law, measuring the absorbance of the solution at 501 nm (absorptivity 282.6 L/(g.cm) [7]). Whole seeds and ground seeds were analysed, being the results similar for both conditions.

The seed oil content was quantified by gravimetric analysis, evaporating to dryness portions of 5 ml of the total volume of the organic extraction solution. On the other hand, the bixin concentration in the seeds was determined analysing the solution obtained with organic extraction of the seeds by HPLC (see below). Moreover, the annatto preparation was also submitted to spectrophotometric and HPLC analysis in order to determine pigment and bixin content, respectively.

The composition of annatto seeds and the annatto preparation is shown in table 1.

Experimental Procedure

The supercritical measurements were carried out in a flow-type apparatus thoroughly described in a previously paper [9]. In this apparatus, which was used to measure the solubility of β-carotene in supercritical carbon dioxide and in supercritical ethane[10] and also the solubility of bixin in carbon dioxide[5], the metering pump compresses the liquid solvent to the desired pressure, which is controlled by a back-pressure regulator. In order to guarantee that the fluid reaches the extraction vessel at the desired conditions of temperature and pressure, the fluid passes through a coil immersed in a temperature-controlled water bath. After passing the extraction vessel the fluid is expanded to atmospheric pressure through a three-way valve, and the extract precipitates in the glass wool placed inside the cooled glass U-tube. Gas flow-rate is monitored by a rotameter and total volume of gas is measured with a wet test meter.

The extracts were collected washing the glass wool, the inside of the three way valve and the expansion tubing with chloroform containing 0.2 wt. % of BHT. The total pigment content was quantified by spectrophotometry.

For the solubility measurements at a given pressure and temperature, the system was allowed to equilibrate for one hour after which a purge was carried out passing 10 L (STP) of gas. The gas flow-rate was of 0.2 L/min.

For the supercritical extraction measurements fractions of 10 to 20 L, of expanded gas, were collected along the time. Two flow-rates were tested: 0.34 L/min and 0.57 L/min.

To assess the amount of pigment extracted UV-Visible spectra were run between 300 and 700 nm, and the concentration of pigment was determined using the Lambert-Beer law, measuring the absorbance at 501 nm [7]. A value of 282.6 L/(g.cm) was used for the absorptivity [7].

The collected solutions were also analysed by HPLC. The method described in [6] was used to separate *cis*-bixin from other carotenoids (*trans*-bixin and some degradation compounds) The system consisted of a liquid chromatograph, Hewlett Packard 1100 series, with UV/VIS detector adjusted to 501 nm. A mobile phase of methanol:2% acetic acid (87:13 v/v) was used at 1 ml/min with the stationary phase Nova Pak C_{18} column.

The quantification of the total supercritical extract (oils) for each seed batch was determined by gravimetric analysis, weighing the seeds or annatto preparation before and after each set of runs.

Table 1: Composition of annatto seeds and annatto preparation (wt. %)

	pigments	bixin	oil
annatto seeds	1.2	0.8	9.5
annatto preparation	23.0	16.1	100.0

RESULTS AND DISCUSSION

The solubility of bixin in supercritical CO_2 modified with 5 mol % of ethanol was determined at 40 °C and pressures up to 300 bar. Figure 1 shows the mole fraction of bixin in this modified solvent and in pure supercritical CO_2 [5], as a function of pressure. As it can be seen from this figure, there was a great increase in the solubility of bixin when CO_2 modified with ethanol was used. This increase in the solubility was expected [5] since bixin is a polar compound (presence of a carboxylic group), therefore by the same reason weakly soluble in pure supercritical CO_2.

Supercritical CO_2 extraction of pigments from annatto seeds was carried out at 40 °C, 200 bar and solvent flow rates of 0.34 L(STP)/min and 0.57 L(STP)/min. The results are shown in figure 2. The yield of the extraction increased with the decreasing of the flow-rate but it was low for both conditions. The concentration of the pigments in the supercritical extracts (0.4 wt. %) was much lower than that obtained by organic extraction of the seeds.

Also, the concentration of bixin in the pigments (60 wt. %) was slightly lower than that obtained with organic extraction of the seeds.

In order to increase the efficiency of the extraction, CO_2 modified with 5 mol % ethanol was used, and it was shown (fig.3), similarly to the solubility results, that there was a great increase in the extraction yield when this solvent was used.

Whole seeds were submitted to extraction with carbon dioxide modified with ethanol, at temperatures of 40 °C and 60 °C, pressures of 200 bar and 300 bar, flow-rates of 0.34 L/min and 0.57 L/min. Ground seeds were extracted at a temperature of 40 °C, a pressure of 200 bar and a solvent flow rate of 0.34 L/min. (fig. 3).

When comparing the extraction of the whole seeds with the milled ones, at temperature of 40 °C, pressure of 200 bar and flow rate of 0.34 L/min, it can be seen that the total efficiency was almost the same for both conditions, although there was a slight increase in the initial concentration of the pigments in the supercritical fluid, when milled seeds were used (1.5 times higher), possibly due to a higher entrainment effect of the intracellular compounds, such as β-carotene [5].

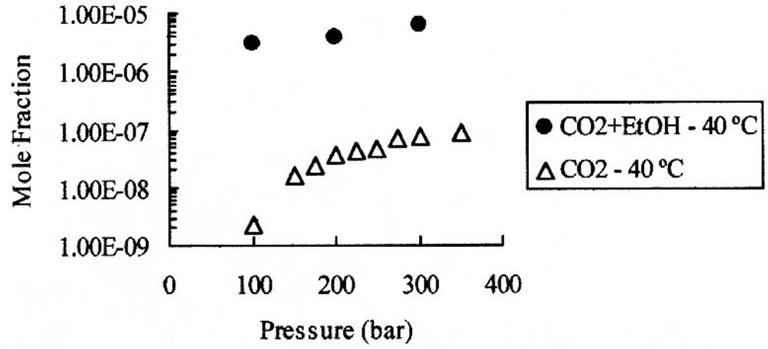

Figure 1: Solubility of bixin in supercritical CO_2 [5] and CO_2 modified with 5 mol % ethanol, at 40 °C.

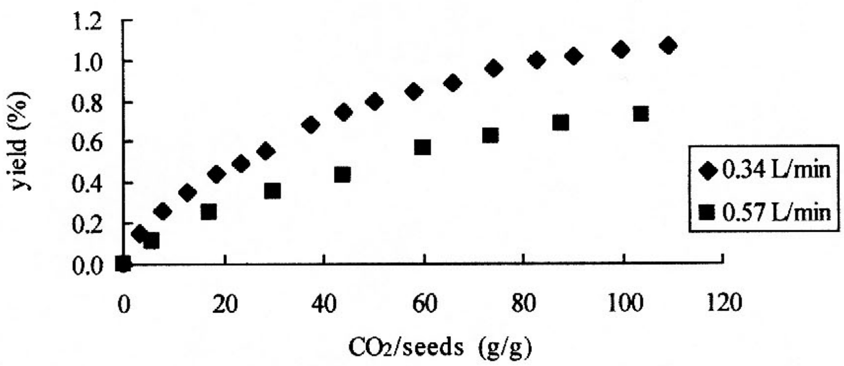

Figure 2: Yield in extracted pigments from annatto seeds as a function of CO_2 amount, at 40 °C and 200 bar.

Moreover, it was verified that the yield of the extraction increased with the temperature and with the pressure for the same flow-rate. However, at constant pressure and temperature it decreased with the flow-rate, although, in this case, the curves overlap until about 24% of the total amount of pigments was extracted.

The major component of the pigments in the seeds is *cis*-bixin (about 69 wt. %). The composition of the pigments in the supercritical extracts was near this value, although for the first fractions collected there was an increase in the concentration of bixin (about 75 wt. %), for the several extraction conditions.

The concentration of the pigments in the supercritical extracts varied from 9 wt. % (at 200 bar, 40 °C and 0.34 L/min) to 19.9 wt. % (at 300 bar, 40 °C and 0.34 L/min). These values are much higher than those obtained with pure CO_2 (0.4 wt. %).

The increase in the concentration of pigments in the supercritical extracts and the concentration of bixin in the pigments, shows that a higher selectivity for pigments is possible using the modified fluid instead of the pure CO_2, in the same conditions of temperature and pressure.

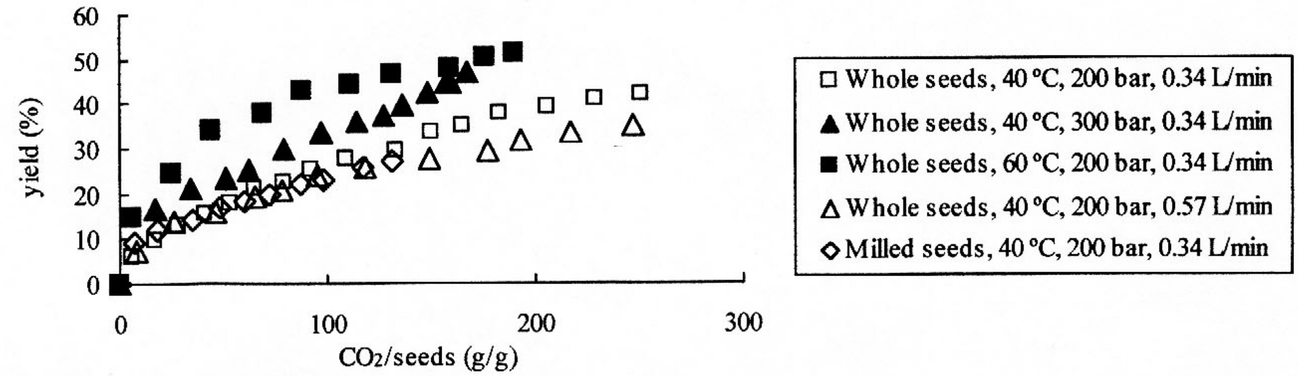

Figure 3: Yield in extracted pigments from annatto seeds, as a function of the amount of modified CO_2.

To assess the efficiency of the extraction, an annatto preparation with a higher bulk concentration of pigment (23 wt. %) was submitted to supercritical fluid extraction with carbon dioxide modified with 5 mol % ethanol, at temperature of 40 °C, pressure of 200 bar and flow-rate of 0.34 L/min (fig. 4).

Two extraction curves were obtained. In the first one, the annatto preparation used was precipitated on glass beads. In the second one, it was used a mixture of annatto preparation and glass beads. In what concerns the first method, the total efficiency of the extraction was low, probably due to strong adsorption of the annatto preparation to the glass beads. The second method, revealed to be a better alternative and the obtained yield was slightly above that achieved for the same conditions with the annatto seeds. Although the concentration of the pigments in the supercritical extract was higher (1.3 times) than that reached when annatto seeds were used, the bixin concentration in the extracted pigments had the same value for these three different conditions.

CONCLUSIONS

The solubility of bixin in supercritical CO_2 modified with 5 mol % of ethanol was determined at 40 °C and pressures of 100, 200 and 300 bar. It was shown that the obtained values are several times higher than those obtained for pure CO_2.

Supercritical fluid extraction of pigments from annatto seeds was carried out with the same solvent mixture and also with pure CO_2. When pure CO_2 was used, the extraction yield was very low, as well as the concentration of the pigments in the extract and that of bixin in the extracted pigments. Annatto pigments concentration was 4 times lower than that obtained by organic extraction of the seeds, showing that pure supercritical CO_2 presents a low efficiency for the extraction of pigments from annatto seeds.

Supercritical extraction of pigments from annatto seeds with CO_2 modified with ethanol led to higher values of the yield. The efficiency of the extraction increased with temperature and pressure but decreased with the flow-rate. The highest yield, 51%, was obtained at 60 °C, 300 bar and 0.34 L/min. Also, the selectivity of the extraction increased when this modified solvent was used. In fact, the concentration of the pigments in the supercritical extracts increased 1.5 times, at 40 °C, 300 bar and 0.34 L/min, relatively to the values obtained with organic extraction of the seeds. Moreover, there was also an increase in the concentration of bixin in the pigments.

The use of annatto preparations instead of seeds did not lead to much improvement in both efficiency and selectivity of the supercritical extraction, at the studied conditions. However, further work should be performed on annatto preparations not bound to any matrix.

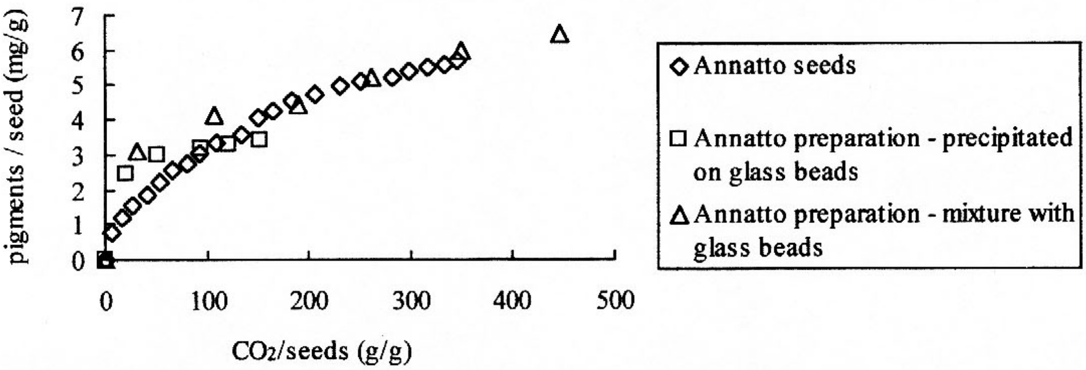

Figure 4: Yield in extracted pigments from annatto seeds and from the annatto preparation, as a function of the amount of modified CO_2, at 40 °C, 200 bar and 0.34 L/min.

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