SUPERCRITICAL FLUID EXTRACTION OF CAROTENOIDS FROM TOMATO INDUSTRIAL WASTES

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Supercritical CO_2 extraction of carotenoids from tomato industrial wastes (mixture of skins and seeds) was carried out in a flow apparatus at the temperature of 60 ºC and the pressure of 300 bar. The effect of the moisture content (4.6, 23 and 58%), particle size (0.15, 0.35 and 0.72 mm) and solvent flow-rate was studied.

The carotenoids recovery (mass of extracted compounds by supercritical CO_2/mass of extracted compounds by Soxhlet) at 60 ºC and 300 bar for samples with 4.6 % moisture was higher for the lowest tomato particle size and decreased with the increase of the flow-rate.

Extraction of tomato paste wastes with organic solvents showed that the carotenoids concentration in the tomato paste was similar in the fresh tomato wastes (83% of moisture content) and in the samples dried up to a moisture content of 58% and 23%, but decreased to half of the value when samples were dried up to a moisture content of 4.6%. Supercritical CO_2 extraction of carotenoids at 60 ºC and 300 bar showed that the increase in the moisture content of the samples, from 4.6% to 23%, led to an increase in the yield (mass of extract/mass dry matter) of the extraction and to a decrease in the recovery of the carotenoids. For higher moisture degrees (58%), both the yield and the recovery decreased.

INTRODUCTION

Carotenoids are a group of pigments, yellow to red in colour, which are widely distributed in the vegetal and animal kingdoms, namely in fungi, algae, carrots, tomatoes, crustaceans, insects and fishes. The importance of these natural pigments has increased as colouring and antioxidant agents in the food, and pharmaceutical industries, due to governmental restrictions in the use of the synthetic ones.

Beta-carotene and lycopene are well known carotenoids with important nutritional and biological properties. Lycopene consumption is strongly recommended, because this compound has high antioxidant activity, reducing the risk of atherosclerosis and coronary heart disease. Moreover, epidemiological studies have related the intake of lycopene with a lower risk of the incidence of certain types of cancers [1]. Beta-carotene has an important role in human dietary, because of its pro-vitamin A activity.

Lycopene is the major carotenoid in tomato and it can represent approximately 80-85% of the carotenoids content, which include beta-carotene and some xanthophylls [2]. Tomato skins contain about five times more lycopene than the tomato pulp [3]. Since the tomato processing companies produce large amounts of solid wastes (mainly skins and seeds) [4] the recovery of carotenoids (namely lycopene) could be considered a good alternative for the valorisation of these by-products [5-11].

The objective of this work was to carry out supercritical fluid extraction of carotenoids from tomato industrial wastes (mixture of skins and seeds) with CO_2 and assess the influence of the moisture content of the tomato paste on the extraction yield and recovery. Also, the influence of the particle size and the flow-rate was evaluated.

MATERIALS AND METHODS

Materials

Carbon dioxide (99.998% purity) was purchased from Air Liquide (Portugal). Tomato industrial wastes (a mixture of skins and seeds) were obtained from a local tomato processing company: FIT - Fomento da Industria do Tomate. Standards of lycopene (90-95% purity) and all-*trans*-beta-carotene (type I, 95% purity) were purchased from Sigma. Acetone (p.a.), methanol (HPLC grade), acetonitrile (HPLC grade), and n-hexane (p.a) were purchased from Merck.

Sample preparation

Tomato paste waste, a mixture of skins and seeds, with a moisture content of 83% was dried in an oven at the temperature of 40 ºC. Different times of drying were used and so three batches with different moisture content were obtained: 4.6%, 23% and 58%. The dried tomato pastes were then packed under nitrogen and stored at -20 ºC.

The product was ground prior to the supercritical fluid extraction measurements using a cutting mill. For the experiments concerning the effect of the particle size, three samples of tomato paste with moisture content of 4.6% and mean particle size of 0.15 mm, 0.35 mm, and 0.72 mm were obtained. For the experiments concerning the effect of the flow-rate samples of tomato paste with a moisture content of 4.6% and an average particle size of 0.49 mm were used.

The amount of total extractable lipids and carotenoids of the tomato paste was determined by Soxhlet extraction with acetone:hexane (1:1) for 6 hours. The carotenoids content was determined spectrophotometrically using the Lambert-Beer law, measuring the absorbance of the obtained solutions at 470 nm. Lycopene and beta-carotene content of the tomato paste was quantified by HPLC (see below), and the amount of extractable lipids of the tomato paste was determined gravimetrically, concentrating the Soxhlet extract and weighing the obtained residue.

Experimental Procedure

The supercritical measurements were carried out in a flow-type apparatus already described in a previous paper [12]. In this apparatus, 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 temperature, the fluid passes through a heat exchanger 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 extraction vessel consisted in a 5 cc pressure cell with an internal diameter of 7.9 mm, which was filled with about 1.6 g of tomato paste, packed between two layers of glass wool. About 120 g of CO_2 were used in the extractions.

Fractions of 5 to 10 L of expanded gas were collected along the time. Three flow-rates were tested: 0.29 g/min 0.59 g/min and 1.18 g/min.

The extracts were collected washing the glass wool, the inside of the three-way valve and the expansion tubing with acetone containing 0.2 wt. % of BHT.

To assess the amount of carotenoids extracted, UV-Visible spectra were run between 380 and 700 nm, and the concentration of carotenoids was determined using the Lambert-Beer law, measuring the absorbance at 470 nm.

The collected solutions were analysed by HPLC in order to quantify the amounts of lycopene and beta-carotene extracted. The system consisted of a liquid chromatograph, Hewlett Packard 1100 series, with UV/VIS detector adjusted to 470 nm. A mobile phase of methanol:acetonitrile (90:10 v/v) was used at 1 ml/min with the reversed phase column, 250x4.6 mm, Vydac 201 TP54. Lycopene and beta-carotene were identified through comparing the retention times of the two carotenoids with those of the standards compounds. In order to determine the total amount of lycopene and beta-carotene, calibrations curves were obtained using the respective standard compounds.

The amount of extracted lipids was assessed gravimetrically, concentrating each fraction collected and weighing the obtained residue.

RESULTS AND DISCUSSION

To our knowledge, all the studies dealing with supercritical fluid extraction of lycopene from tomato or tomato industrial wastes only used fresh or well dried samples (moisture content of 2.3-10%). It was verified that the supercritical extraction of compounds from fresh tomatoes or fresh tomatoes industrial waste showed low extraction recovery of lycopene [9, 10]. Also it was reported that lycopene content of the dried samples was lower than its content in fresh tomato waste samples [9].

In this work samples of fresh tomato paste waste (83% moisture content) were dried up to several moisture degrees: 58, 23 and 4.6%. Soxhlet extraction of these samples showed that the carotenoids content of tomato paste fresh and dried up to 23% of moisture was almost the same. The discrepancies observed are possibly due to some heterogeneity of the samples, because the processing waste is resulting of tomato of several origins. However, the carotenoids content decreased to half of the initial value when tomato paste was dried up to 4.6% of moisture content (see Table 1). This reduction in the carotenoid content of the dried sample possibly was not due to degradation of these compounds during the dryness process of the sample, because HPLC and spectrophotometry at 470 and 450 nm of the Soxhlet organic extracts did not detect any degradation product. Most probably, this decrease in carotenoids content of the dried sample could be due to physical changes in the structure of the skins [14], namely those related with the fact that lipid pillars of a plant cell elementary membrane change with the water content, and if there is not enough water in the system the pillar closes the membrane, making it impermeable [13].

Table 1: Composition of the fresh and dried tomato paste (Soxhlet extraction)

Moisture content (%)	Carotenoids (µg/g oil-free dry matter)	Lycopene (µg/g oil-free dry matter)	Beta-carotene (µg/g oil-free dry matter)	Lipids (mg/g oil-free dry matter)
83	849.7	576.7	93.1	305.6
58	898.5	431.2	100.2	299.6
23	923.9	477.6	61.1	225.9
4.6	470.7	291.5	47.5	226.9

Fresh tomato paste waste (83% moisture content) was submitted to supercritical CO_2 at 60ºC, 300 bar and 0.59g/min, but there was no extraction of carotenoids except in trace amounts. Supercritical fluid extraction of samples with different moisture content was carried out at 60ºC, 300 bar and 0.59 g/min. In Figures 1 and 2 are shown the yield (mass of extract/mass of oil-free dry matter) of the carotenoids and lipids supercritical CO_2 extraction and the percentage of recovery (mass of extracted compounds by supercritical CO_2/mass of extracted compounds by Soxhlet), of the same type of compounds, respectively. It was verified that the yield of extracted carotenoids increased when the moisture content of the sample increased from 4.6 to 23%, possibly due to the modifications in the skin structure before mentioned, implying less available compounds in tomato paste with lower moisture content. However, for higher values of moisture the yield decreased, in this case, probably due to the water preventing the contact between CO_2 and tomato particles. On the other hand, the recovery percentages of the carotenoids and lycopene decreased when the moisture of the samples increased (Figure 2). The sample with the lowest moisture content, which presents the lowest content in carotenoids (Table 1), showed the highest recovery percentage.

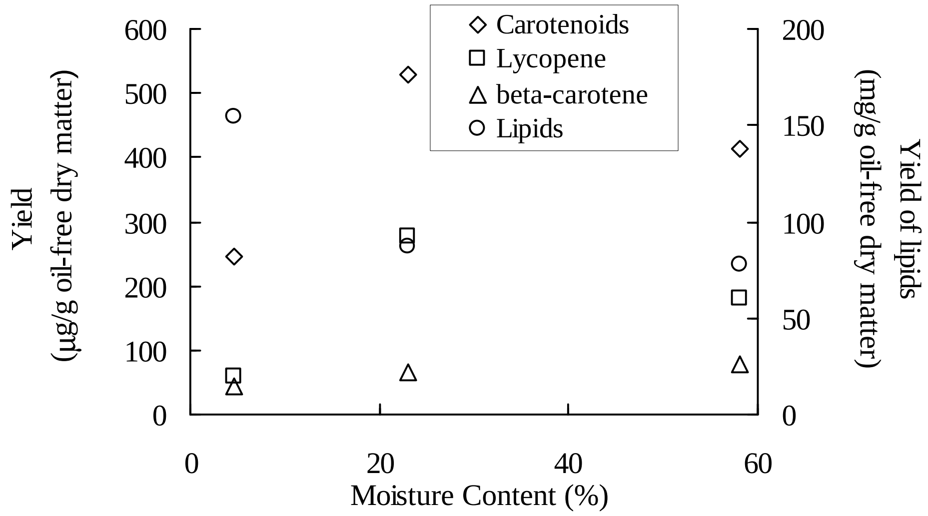

Figure 1: Yield of extracted carotenoids, lycopene, beta-carotene and lipids, as a function of the moisture content of the samples, with supercritical CO_2 at 60ºC, 300bar and 0.59g/min.

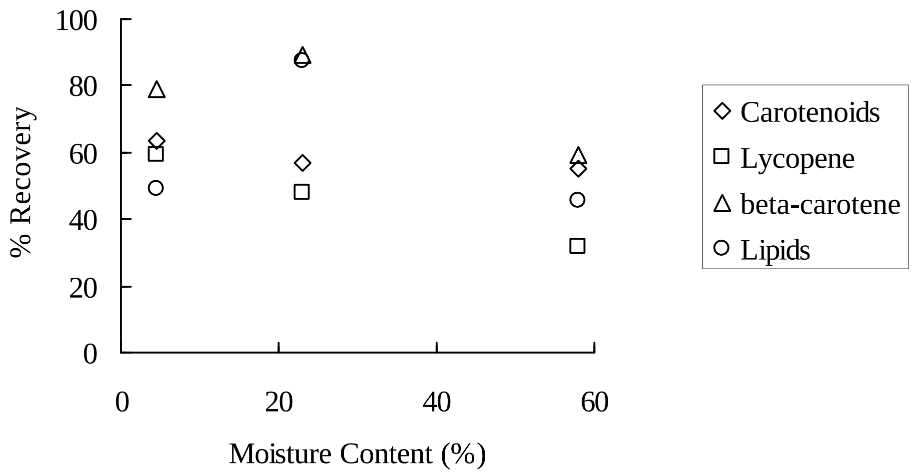

Figure 2: Recovery of carotenoids, lycopene, beta-carotene and lipids, as a function of the moisture content of the samples, using supercritical CO_2 at 60ºC, 300bar and 0.59g/min.

The effect of the particle size on the supercritical extraction behaviour was also studied. Three different mean particle size samples of the tomato paste waste (0.15, 0.35 and 0.72 mm) with moisture content of 4.6%, were submitted to supercritical CO_2 at 60ºC, 300 bar and 0.59 g/min. In Figure 3 are shown the percentages of lipids, carotenoids and lycopene recovery as a function of the particle size of the tomato paste. As it can be seen, the recovery of carotenoids and lycopene increased with the decreasing of the particle size, specially when mean particle size decreases from 0.35 mm to 0.15 mm, reaching values of 78% and 83% for the recovery of lycopene and total carotenoids, respectively. The percentage of lipids recovery was nearly the same for particle sizes of 0.15 and 0.35 mm. Particle size of 0.72 mm showed the lowest recovery for all compounds. The obtained results are as expected, since the particle size reduction process of plant material increases the lipids recovery, because it increases the surface area and may cause ruptures of the cell walls.

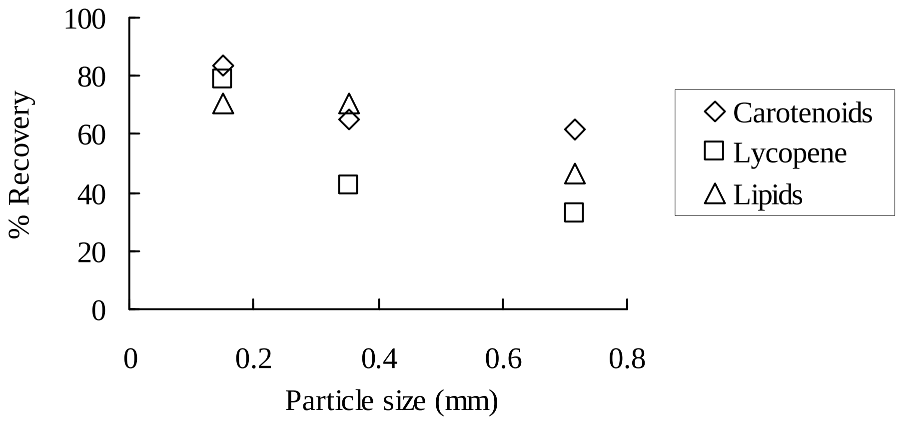

Figure 3: Recovery of lipids, carotenoids and lycopene, as a function of the particle size of the tomato paste, with supercritical CO_2 at 300 bar, 60 ºC, 4.6 % of moisture content and 0.59 g/min.

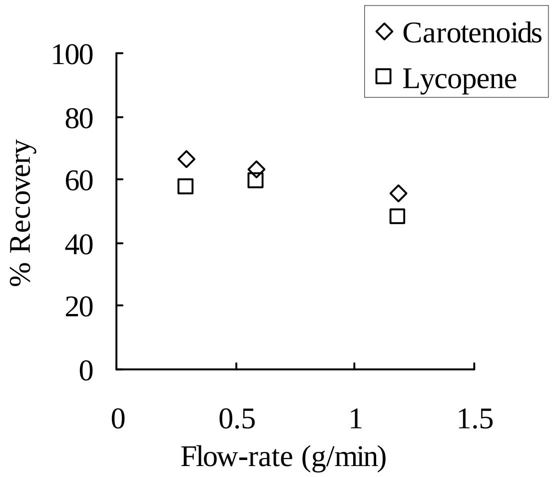

Figure 4: Recovery of carotenoids and lycopene, as a function of the solvent flow-rate, with supercritical CO_2 at 300 bar, 60 ºC, 0.49 mm average particle size and 4.6% moisture content

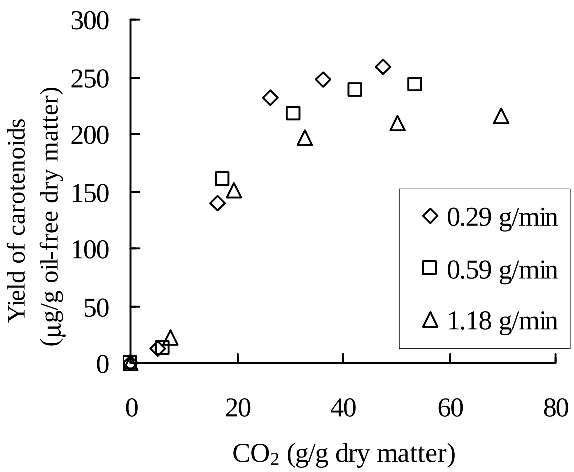

Figure 5: Yield of carotenoids as a function of CO_2 amount at 60ºC, 300 bar, 0.49 mm average particle size and 4.6% moisture content.

In what concerns the effect of flow-rate, supercritical CO_2 extraction experiments were carried out at the temperature of 60ºC, pressure of 300 bar, mean particle size of 0.49mm, moisture content of 4.6% and three different flow-rates: 0.29, 0.59 and 1.18 g/min. In Figures 4 and 5 are shown the results for the percentage of recovery of carotenoids and lycopene and the yield of extracted carotenoids, respectively. It was verified that the yield of the extracted carotenoids increased with the decreasing of the flow-rate. The recovery percentage of extracted lycopene increased when the flow rate decreased from 1.18 to 0.59 g/min, but remain practically the same for lower flow-rates (0.29 g/min).

CONCLUSIONS

Tomato industrial wastes (mixture of skins and seeds) well dried (4.6 % moisture content) were submitted to supercritical CO_2 at 60ºC and 300 bar in order to extract carotenoids. The recovery of the carotenoids, namely lycopene, increased when the particle size decreased, reaching a value of almost 80% of the lycopene present initially, for the lowest particle used (0.15 mm).

The moisture content of the sample is also an important parameter to optimise. Samples with moisture content of 4.6% suffer strong reduction of the carotenoids content in the drying process. It was shown that samples dried up to 58 and 23% of moisture content, remained with about the same carotenoid content of the fresh material. Moreover, when these samples were submitted to supercritical CO_2 extraction at 60ºC and 300 bar the highest yield of carotenoids extraction was obtained for the sample with 23% of moisture content.

Acknowledgments: This work was supported by FCT (Portugal) through the project POCTI/MAR/15237/1999. Beatriz P. Nobre thanks FCT for a research grant.

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