SEPARATION OF NATURAL COLORANTS USING A COMBINED HIGH PRESSURE EXTRACTION-ADSORPTION PROCESS

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***Abstract*— The goal of this work was to find a process to extract and separate carotene from natural sources using supercritical carbon dioxide. A high pressure extraction process was compared with a combined extraction-adsorption process. The experiments were performed with a pilot plant which can be operated up to 50 MPa and 100°C. A technique using an on-line photometer, was used to estimate the concentration of carotenoids in the fluid phase, to monitor the different steps of the process. During the extraction of paprika powder, a time fractionation of pigments was observed: a low concentration fraction of free carotenoids at the beginning of the process and an enriched esterified carotenoid fraction at the end. With the combined extraction-adsorption process, using silica gel as adsorbent, a selective adsorption occurred. Red pigments (mainly esterified carotenoid) were adsorbed but the yellow ones (mainly free carotenoid) remained in supercritical phase which were collected in a separation step at lower pressure.**

***Keywords*—, Fractionation, Supercritical Fluids Carotenoids, Paprika**

I. INTRODUCTION

Carotenoids of, yellow, orange and red color are among the most wide spread and important natural pigments. Carotenoids are used as natural colorants for foods, animal feeding and cosmetic products (Palace *et al.*, 1999). They are also the main source of vitamin A for humans. Free carotenoid pigments together with xanthophyll esters and other lipophylic substances (mainly triglycerides) are extracted at industrial scale using organic solvents to produce extracts from seeds, fruits or flower petals which are used directly or partially saponified in food, cosmetic and pharmaceutical industry.

Carbon dioxide, a non-toxic, inexpensive and easily separable solvent, has been used during the last three decades to extract lipid compounds from natural matrices, in order to replace traditional solvent extraction (Eggers, 1996). Many potential applications of Supercritical Fluid Extraction (SFE) for recovery and fractionation of spices and its extracts have been investigated. Extraction of carotenoids from natural products, such as ß-carotene from carrots, algae and palm-oil, lycopene from tomatoes, lutein from flowers, capsanthin from paprika, has been extracted with supercritical fluids. In all cases, not only the extraction kinetics but also the total extracted carotenoids are strongly depending on the extraction operating conditions, such as temperature, pressure and specific solvent flow.

Many experiments were carried out in order to find the optimal conditions (e.g. extraction-separation pressure and temperature) for carotenoids fractionation (Jarén *et al.*, 1999). However, no carotenoid concentration profiles in supercritical fluids were measured so far. In this work, a combined extraction-adsorption process to obtain fractions of different pigment composition is proposed. It is helpful to understand the complete extraction-adsorption process to optimize the operating conditions in order to reach high carotenoid concentration.

II. METHODS

A. Supercritical fluid extraction-adsorption plant

The experiments with supercritical carbon dioxide (SC-CO_2) were performed in an extraction-adsorption plant which was designed and build up at the Technical University Hamburg-Harburg. The plant was sent to Argentine to be used in the Univesidad Nacional de Rio Cuarto. A schematic diagram of the plant is shown in Fig. 1 and the main characteristics of the equipment are summarized in Table 1.

Both, extractor and adsorption column, are provided with internal sample holder (300x70 mm and 600x16 mm respectively), in order to keep the solids in a fixed bed. A view cell (Sitec) connected to a single channel photometer (Optek 116/AF16) is placed in the CO_2 circuit (behind the extractor or adsorption column) which allows online measurements of the pigment concentration.

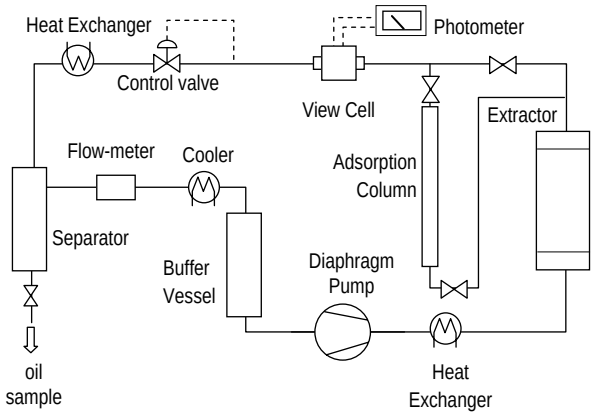

Fig. 1. High Pressure Extraction-Adsorption Plant

Table 1. Main characteristics of the high pressure plant

Extractor	Separator	Diaphragm Pump (Lewa)	Adsorption Column
P_{max} = 50 MPa	P_{max} = 50 MPa	P_{max} = 50 MPa	P_{max} = 30 MPa
T_{max} = 100 °C	T_{max} = 80 °C	T_{max} = 80 °C	T_{max} = 80 °C
Volume = 2.6 l	Volume = 0.5 l	Flow = 30 kg/h	Volume = 0.5 l

B. Materials

A variety of paprika powder, *"Capsicum annuum"*, was tested. Fat content in raw material, determined with Soxhlet method using either acetone or petrol ether as solvent, was 17.1 % w/w. Only 2 % of starting material had a particle size greater than 2 mm. Previous to supercritical extraction, samples were dried overnight under vacuum at 60 °C to prevent major thermal decomposition of carotenoids. Moisture content was lower than 0.5 %. Silica gel (SIGMA, 75-230 mesh, 60Å pore diameter) and a bleaching earth (TONSIL® CO 614 G, granulated) utilized in oil industry, were used as adsorbent.

C. Carotenoind determination

Carotenoids can be divided into two main groups a) hydrocarbons, which are termed "carotenes" and b) oxygen containing derivatives, which are termed "xanthopylls". The hydroxy derivatives can exist in the free state or esterified with fatty acids such as linolenic acid. Carotenoids such as capsanthin (red), capsorubin (red), ß-carotene (yellow-orange), zeaxanthin (light orange) and violaxanthin (yellow) are the most important carotenoids in paprika (Govindarajan, 1985). Identification and quantification of carotenoids and its esters in recovered extracts samples were carried out with a coupled HPLC/MS method. The results were compared to UV reference spectra as well as the retention times for each carotenoid.

In the SC-CO_2 phase, a qualitative behavior of pigment concentration was followed with the online photometer. Due to the diverse composition of pigments in paprika powder, synthetic ß-carotene (BASF) was adopted as a reference pigment to calibrates the detector device. An extinction value of 2.4 in concentration units (C.U.) at a wave length of 450 nm (optical path length: 9.1mm), corresponded to a saturated value of 12 mg/kg of carotene in CO_2 (30 MPa, 60 °C) according to literature (Johannsen and Brunner, 1997).

III. RESULTS

A. Extraction process

Samples of paprika (approx. 0.5 kg) were extracted at 60°C y 30 MPa using a CO_2 / paprika mass ratio of 80:1. A mass flow of 10 kg/h of CO_2 was used which represents a residence time of ~2 min in the extractor unit. In Fig. 2, the extract yield related to total extract in starting material (curve A) and qualitative carotenoid concentration in SC-CO_2 phase, expressed in concentration units (curve B), are shown. At the beginning of the process, extract yield shows a linear behavior corresponding to a constant extraction rate. From this curve, near 75 % of total extract is recovered in this step involving only 15 % of the total CO_2 necessary to complete the extraction. This linearity indicates that the recovering is limited by the extract solubility in SC-CO_2. During this stage, the concentration of carotenoids in both, SC-CO_2 and recovered extract, remains practically constant. At the end of this period the extraction rate is more slowly, but the content of carotenoids increases sharply in both, extract and SC-CO_2 phase (curve B).

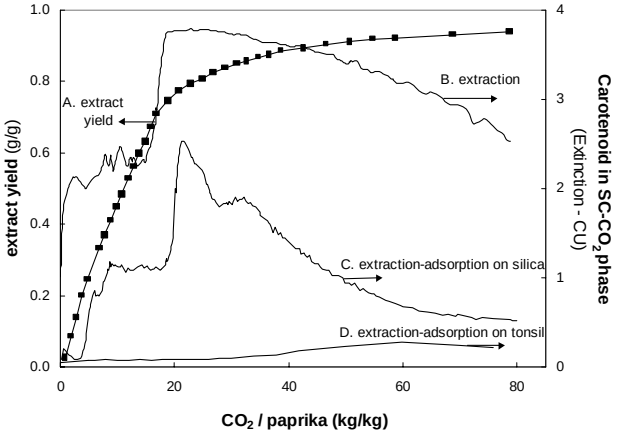

Fig. 2. Extract yield and concentration of total carotenoids of paprika in SC-CO_2 extraction. Comparison with a combined extraction-adsorption process (30 MPa, 60°C).

This behavior is attributed to the presence of oil which affects the solubility of carotenoids in SC-CO_2. Solubility investigations of ß-carotene dispersions in edible oil has been reported (Pietsch, 2000). A lower oil concentration in oil-carotene dispersion favors ß-

carotene solubility in SC-CO_2. Thereby, at the beginning of the extraction, oil recovery is high but the concentration of carotenoid in carbon dioxide remains low. Capsaicinoids, compounds responsible of pungent properties of paprika is also extracted in this stage due to its high solubility in SC-CO_2 (Škerget and Knez, 1997). At the end of linear stage, the concentration of carotenoids increases due to the lower oil content. When the extract yield is almost spent, the concentration of carotenoids in recovered extract is 50 times greater than in linear stage in spite of the lower content in SC-CO_2.

B. Combined extraction-adsorption process

The composition of carotenoids in both, extract and SC-CO_2 phase, is significantly affected by the presence of an adsorbent after the extraction unit. In Fig. 2, the concentration of carotenoids in SC-CO2 of an extraction process followed by an adsorption stage, filled with silica (curve C) or tonsil (curve D), are compared with extraction one. A significant delay is observed in both cases due to adsorption phenomena. In the case of tonsil, an extend of 40% of the total extraction time can be recognized. In the linear stage, the concentration of carotenoids in SC-CO_2 exhibits also a plateau, but at a lower level compared to the extraction process, 50 % for silica and only 4% for tonsil. These results are in accordance with concentration of carotenoids in recovered extract. In the linear stage only 50 % of total pigments were retained by silica while at the end of the process the retention increased until 90%. In the non-linear stage, the concentration of carotenoids decreases faster than in normal extraction.

Adsorption on tonsil was significantly higher than on silica, indicated by the lower the concentration of carotenoids in SC-CO_2. This effect could be due to the presence of metallic ions such as Fe^{3+} on the surface which catalyzes a reaction or served as active sites for chemisorptions of carotenoids responsible of its decomposition (Khoo *et al.*, 1979). With silica as adsorbent, the properties of carotenoids remained unchanged after adsorption, that indicates a physical adsorption process.

C. Fractionated extraction and selective adsorption

Capsanthin, cryptoxanthin, and *ß*-carotene were adopted as reference component for diester, monoester and free compounds respectively. The contents of these pigment in paprika powder are *ß*-carotene 12%, cryptoxanthin 13%, capsanthin 32% (Govindarajan, 1985). In Fig. 3, percentage content of these carotenoids in extract, taken at different times, were plotted. Full and empty symbols represent extraction and coupled adsorption-extraction process respectively.

During the first stage in extraction process, *ß*-carotene is preferably extracted than esterified carotenoids and represents up to 60% of whole pigment. Mono and diester carotenoids are less soluble in SC-CO_2, so they are later extracted. At the end of the extraction, *ß*-carotene concentration decreases and consequently diester increases. In coupled extraction-adsorption process with silica as adsorbent, *ß*-carotene (free) and cryptoxanthin (monoester) was poorly adsorbed while capsanthin (diester) was efficiently retained.

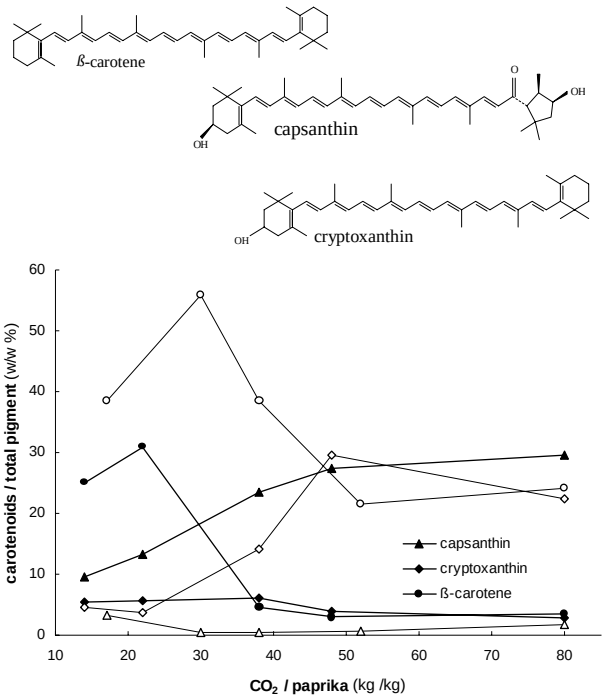

Fig. 3. Fractionated extraction (full symbols) and selective adsorption (empty symbols) of carotenoids

IV. CONCLUSIONS

A time fractionation of paprika carotenoids with supercritical carbon dioxide extraction was observed. In a first stage, content of total carotenoids in extract remains constant with time and CO_2 extract preferably free carotenoids like *ß*-carotene. In a second stage total the concentration of carotenoid reached a value 50 times higher and practically only esterified carotenoids were extracted. A selective adsorption of free and esterified carotenoids was also observed when silica was used. Diester carotenoids were preferably adsorbed in comparison to free or monoester ones. Recovery of substances from adsorbent using SC-CO_2 technique is not always an efficient process. For that reason, organics adsorbents that could be used together with colorants, such as cellulose and cyclodextrins, are now being considered

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