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Precipitation of β-carotene microparticles from SEDS technique using supercritical CO_2

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ABSTRACT

This work investigates the application of the Solution-Enhanced Dispersion by supercritical fluids technique for the precipitation of β-carotene. The effect of pressure (8.0–12.0 MPa), temperature (293–313 K) anti-solvent flow rate (20–40 mL/min), solution flow rate (1–4 mL/min) and concentration of β-carotene in the dichloromethane solution (4 and 8 mg/mL) on the precipitation yield, particle morphology and particle size and size distribution was examined. Precipitated powders presented mean particle size varying from 3.2 μm to 96.8 μm with morphology of β-carotene microparticles changing from plate-like to leaf-like particles. The statistical analysis of the experimental results revealed that pressure, organic solution concentration and CO_2 flow rate had a significant effect on particle size. The precipitation yield was observed to be within the range of 71–94% and was statistically influenced by system temperature and pressure, and anti-solvent flow rate.

1. Introduction

Carotenoids, considered nutraceutical compounds, are the most common group of pigments in nature. The presence of small amounts of carotenoids in foods can help to prevent fast oxidation of food constituents due to the singlet oxygen quenching activity in addition to the color enhancement of foods (Schroeder and Johnston, 1995), but it is known that the application properties and the color strength of pigments are strongly dependent on their physical properties such as particle size, particle size distribution and morphology (Suo et al., 2005). In particular, β-carotene can be obtained from different natural sources (Barba et al., 2006; Maldonade et al., 2008). The isolation of β-carotene from these sources is usually carried out by solid–liquid extraction by using considerable amounts of organic solvents. As a result, other post-extraction techniques are needed for organic solvent removal and β-carotene purification and/or crystallization, such as freeze-drying and salting-out. However, these conventional techniques suffer from some drawbacks like the poor control of particle size and morphology, degradation of thermo sensitive compounds, as well as the presence of relevant amounts of organic solvent residues in the resulting product.

The use of supercritical or near critical fluids as solvents or anti-solvents for particles precipitation was demonstrated by several researchers as a useful means to the modification of material properties such as particle size, size distribution and morphology (Reverchon et al., 2003). Another advantage of such techniques is the efficient separation of the solvent and anti-solvent of the particles after precipitation, preventing organic solvent residues in the final product and permitting reutilization of solvent and anti-solvent (Rantakylä et al., 2002). Precipitation of β-carotene has been carried out employing carbon dioxide as anti-solvent due to its very low solubility such a compressed fluid in the temperature and pressure ranges commonly investigated (Mendes et al., 1999; Hansen et al., 2001; Sovová et al., 2001; Kraska et al., 2002; Saldaña et al., 2006). For example, Chang et al. (1991) employed the GAS (Gas Anti-Solvent) technique to separate isomers of β-carotene. The same technique was also used by Cocero and Ferrero (2002) to recrystallize β-carotene from ethyl acetate and dichloromethane solutions using carbon dioxide as anti-solvent. The Solution-Enhanced Dispersion by supercritical fluids (SEDS) technique was employed by He et al. (2006) for the precipitation of natural β-carotene using a pre-filming atomization system. These authors have focused on the efficiency of pre-filming atomization, studying the effect of several parameters on the particle size, size distribution and purity of precipitated carotene.

Recently, we have reported the precipitation of β-carotene using the SEDS technique (Franceschi et al., 2008a), evaluating the influence of pressure, organic solution concentration and anti-solvent flow rate on particle size, size distribution and morphology of the precipitated particles. In that work, particles with relatively large size were obtained in all the experimental conditions tested, a fact that was attributed to the size and geometry characteristics of the precipitation chamber (4 cm of inner diameter and 60 mL volume).

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In this context, the objective of this work is to perform a detailed investigation about the effects of processing parameters (temperature, pressure, organic solution flow rate, anti-solvent flow rate and initial concentration of β-carotene in the organic solution) on the precipitation of β-carotene using the SEDS technique, employing a precipitation chamber with 600 mL volume, larger than the one used in the previous work (Franceschi et al., 2008a). The effect of parameters was focused on particle size, particle size distribution, morphology and yield of precipitated powder. The morphology of precipitated β-carotene was characterized by scanning electronic microscopy (SEM), while particle size was measured employing the software Size Meter (version 1.1), and mean particle size (evaluated by the largest characteristic dimension of the particle) and size distribution was calculated using the commercial software Statistica 6.0®.

2. Experimental

2.1. Materials

Trans-β-carotene, with a purity of 95%, was purchased from Sigma–Aldrich (USA). Dichloromethane (DCM – 99.5%) was purchased from Merck (Germany) and carbon dioxide (99.9% in liquid phase) was supplied by White Martins S.A. All materials were used as received.

2.2. Experimental design

A full factorial 2^5 experimental design was adopted to organize the experiments and to evaluate the main effects of process parameters on the precipitation of β-carotene from dichloromethane by the SEDS technique. In this work, it was investigated the influence of precipitation pressure (P), precipitation temperature (T), concentration of β-carotene in the organic solution containing β-carotene and dichloromethane (C), organic solution flow rate ($\dot{q}_S$), and anti-solvent flow rate ($\dot{q}_{AS}$). The nozzle diameter was maintained constant at 100 μm, following the work of Franceschi et al. (2008a). The process variables ranges were selected based on previous results of the group, and on the fluid phase behavior of the ternary system CO_2 + dichloromethane + β-carotene (Franceschi et al., 2008a). The pressure and temperature ranges adopted were 8–12 MPa and 293–313 K, respectively, which means pressure and temperature values near and above the mixture critical point and in the region of compressed liquid (Franceschi et al., 2008a). The values adopted for the solute concentration in the organic solution were from 4 to 8 mg/mL at room temperature, allowing the operation with diluted and concentrated solutions (Tres et al., 2007). Regarding the anti-solvent flow rate and solution flow rate, the selected range was from 20 mL/min to 40 mL/min and 1 mL/min to 4 mL/min, respectively, with the aim of increasing the turbulence inside the precipitation chamber promoting a more intense mixing between the solution and anti-solvent and working with different anti-solvent to solution flow rate ratios.

The main effects of above-mentioned variables on mean particle size, particle size distribution and on the precipitation yield were evaluated using the commercial software Statistica® 6.0, adopting a confidence level of 95% ($p < 0.05$). Due to the visual observation of the morphology of precipitated powder, the particle length was defined as the proper parameter to compute the characteristic size of the precipitated β-carotene.

2.3. Precipitation apparatus and procedure

Fig. 1 presents a schematic diagram of the experimental apparatus employed for β-carotene precipitation. Briefly, it consists of a home-made cylindrical vessel, with an internal volume of 600 mL and internal diameter of 8 cm, which was used as precipitation chamber (PC); two syringe pumps for CO_2 displacement (ISCO, Model 500D), operated independently by a set of ball valves – V1 to V4 (Swagelok, Model SS-83KS4), and a digital HPLC liquid pump (Acuflow, Series III) used for organic solution delivery.

The organic solution was sprayed into the precipitation chamber through a silica capillary fusing tube, with an internal diameter of 100 μm, connected to a polyetheretherketone tubing (Peek Tubing, Upchurch Scientific). This arrangement was linked to a BPR (back pressure regulator, GO-Regulator, Series BP-66, Model 1A11QEQ151) and to a tee (T) connector (Swagelok), to link the anti-solvent and the solution flows.

During the experiments, the temperature into the precipitation chamber was controlled by an ultrathermostatic bath (Nova Etica, Model 521/2D), while the pressure was controlled by two needle valves (HIP, Model 15-11AF1). The first valve (V5) controlled the anti-solvent flow rate, while the other one (V6) controlled the depressurization. A second vessel (SC) connected after valve V6 was used to keep the stream that leaves the precipitation chamber at a relatively low pressure (about 4 MPa) to prevent the blockage of valve V6. A system for powder collection was located in the bottom of the precipitation chamber, and was composed by sintherized metal filter (superficial porosity of 1.0 μm) as a support to the polytetrafluorethylene membrane filter linked to a high density polyethylene support (Millipore, model FGLP with a porosity of 0.22 μm).

The experimental procedure started with CO_2 filling the precipitation chamber up to the desired pressure. The anti-solvent flow rate was controlled by setting V5 and V6 valves, and monitored by the syringe pump. When the temperature, pressure and anti-solvent flow rate were stabilized, the organic solution was added through the capillary tubing. The pressure for solution spray into the precipitator was controlled by BPR manipulation and monitored by the liquid pump. The solution volume added to the chamber was 30 mL, which enabled the collection of sufficient amount of precipitated powder for analysis. After the solution addition, CO_2 was continuous flowed at least 2 h in order to dry the precipitated particles inside the precipitation chamber. The precipitation chamber was slowly depressurized to atmospheric pressure and particles were collected and stored at appropriate conditions for subsequent analysis and characterization. A more detailed description of the apparatus and experimental procedure can be found elsewhere (Franceschi et al., 2008a,b).

2.4. Precipitation yield

The precipitation yield was evaluated considering the amount of precipitated powder collected in the precipitation chamber. The percentage of precipitation yield was calculated by the ratio between the mass of β-carotene collected in the precipitation chamber after each assay and the mass of β-carotene present in the organic solution added to the precipitation chamber at each experiment. It may be convenient to elucidate that the volume of solution added to the precipitation chamber was quantified with a precision of ±0.1 mL.

2.5. Analysis and characterization

Precipitated particles were analyzed by a Shimadzu model SS-550 Superscan scanning electron microscope (SEM) to determine particle morphology and shape. Particle size and size distribution were measured by the Size Meter software (version 1.1) (Carlson, 2001), using at least 500 particles collected at each experiment.