PHASE BEHAVIOR OF (CO_2 + DICHLOROMETHANE + PASSION FRUIT SEED OIL) AND (CO_2 + DICHLOROMETHANE + PASSION FRUIT SEED OIL + PLGA) SYSTEMS

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ABSTRACT – Large amount of passion fruit seeds are misspent after juice production. Heat and light can cause loss or reduction of seed oil antioxidant and antitumor activities. Thus, co-precipitation with polymers by Supercritical AntiSolvent process (SAS) may be used to preserve important compounds. This work aimed to study the phase behavior of the systems 1) CO_2+ dichloromethane+ seed oil and 2) CO_2+ dichloromethane+ seed oil+ PLGA (poly(lactic-co-glycolic) acid), to determine the operational conditions to be employed on future SAS assays. The static synthetic method was applied using different CO_2 compositions (60 - 98.8 wt %) and temperatures (35 and 45 °C). Liquid-vapor, liquid-liquid and liquid-liquid-vapor phase transitions were observed. Conditions suggested to proceed the SAS encapsulation study are: 35 °C to preserve extract quality, CO_2 concentration between 92.5 and 97.0 %, ensuring its action as anti-solvent, and pressure above 75 bar to guarantee a single phase equilibrium.

1. INTRODUCTION

Brazil is the world´s largest producer and consumer of fresh and processed yellow passion fruit, accounting for 50-60 % of the total world production. Today passion fruit grows nearly everywhere in the tropical belt, but South America is the main exporter of yellow passion fruit concentrated juice as its demand is growing worldwide (von der Linden, 2007). The juice production engenders a large amount of residues such as seeds and rind.

Despite the current efforts of companies to reuse process residues, large amounts of passion fruit seeds are still underutilized by the industries. The literature evinces its high content of unsaturated fatty acids, especially linoleic acid (up to 70 %), and the presence of phenolic compounds in the seeds (Malacrida and Jorge, 2012; Ferreira *et al*., 2011). Phenolic compounds are known to present antioxidant activity that inhibits oxidative damage and may consequently prevent inflammatory conditions, ageing and neurodegenerative diseases. Some studies reported antioxidant, antitumor and other biological activities of the passion fruit seed oil or from specific compounds present in the oil (Sano *et al*., 2011; Kim *et al*., 2009; Lee *et al*., 2009; Kuo e Hsu, 2008). So its reuse to obtain functional ingredients could find diverse applications in the food,

pharmaceutical and cosmetic industries (Malacrida and Jorge, 2012; Piombo *et al.*, 2006) enabling the conversion of considerable amount of this residue generated by juice industries into added value products.

Exposure to heat, light and oxygen can cause loss or reduction of biological activities of interest compounds, such as phenolics. With the intent of preserving important compounds for possible future applications in commercial products, the co-precipitation with biopolymers processes can be of interest (Higuera-Ciapara *et al.*, 2004), such as the Supercritical AntiSolvent process (SAS). In the SAS process, the rapid mutual diffusion at the interface of the supercritical antisolvent and the liquid phase containing the substances for co-precipitation induces phase separation and super saturations of the polymer/solute thus leading to smaller particle diameters. The supercritical fluid is used both as an anti-solvent for its chemical properties and as a 'spray enhancer' by mechanical effects. The morphology and particle size of the product can be controlled by employing optimum process parameters (Jung and Perrut, 2001; Adami *et al.*, 2008).

Poly-d,l-lactide-co-glycolide (PLGA) is a biocompatible and biodegradable polymer widely used in the pharmaceutical industry to protect active ingredients from harsh environments and improve their delivery and uptake. PLGA can be used to encapsulate hydrophobic active materials through a relatively simple process, so its application could also be useful in the food industry to disperse hydrophobic compounds in hydrophilic compositions (Hill *et al.*, 2013).

The knowledge of the phase behavior (equilibrium data) presents considerable importance for the modeling, optimization and design of several high pressure separation processes such as co-precipitation/micro and nanoparticle formation techniques like rapid expansion of supercritical solution (RESS), gas anti-solvent process (GAS), and supercritical anti-solvent (SAS), among others (Reverchon *et al.*, 2000; Jung and Perrut, 2001; Diefenbacher and Türk, 2002). The type of phase transition along with the range of conditions where this system is a single phase are the most important aspects related to the processes of micronisation or encapsulation (Kalogiannis and Panayiotou, 2005). Therefore, this work aimed to study the phase behavior of the systems 1) CO_2 + dichloromethane + passion fruit seed oil and 2) CO_2 + dichloromethane + passion fruit seed oil + PLGA (poly(lactic-co-glycolic) acid), evaluating the influence on phase transitions of the PLGA addition to system 1 and determining the operational conditions to be employed on future SAS assays.

2. MATERIALS AND METHODS

2.1. Materials

The passion fruit seed was supplied, cleaned and dried, by the company Extrair Óleos Naturais located in Rio de Janeiro state, Brazil, presenting on arrival a moisture content of 8.50 ± 0.09 % w/w, determined according to 940.26 method from A.O.A.C. The raw material was ground in a domestic blender (Black & Decker, SP, Brazil) prior to supercritical fluid extraction. The extraction was performed at pressure of 150 bar and temperature of 40 °C, at constant

solvent flow rate of 0.5 kg CO_2/h, for 2.5 h. The experimental procedure for the high-pressure operation and the unit components were described by Michielin *et al.* (2005). The process used pure CO_2 99.9 % delivered at pressures up to 60 bar (White Martins, Brazil). The fixed bed of particles was formed with 10.0 g of ground seed, placed slowly inside the extractor to obtain a uniform bed and avoid wall effects and channeling.

The phase equilibrium assays were performed with two different solutions: a) passion fruit extract + dichloromethane (P.A., Nuclear, CAQ Ind. e Com. LTDA., Brazil) in a concentration of 1:125 (w:v) and b) passion fruit extract + copolymer poly(lactic-co-glycolic acid) (PLGA) (Resomer RG 503, Evonik) + dichloromethane in a proportion of 1:3:125 (w:w:v). The dichloromethane (DCM) was selected to be used as cosolvent due to the good solubilization of the PLGA in this solvent which does not occur in most of organic solvents. The solution ratio was defined based on the experience of other phase equilibrium and co-precipitation studies on the group, in order to guarantee adequate solubilization of solutes, provide proper visualization of the phase transition as well as further co-precipitation.

2.2. Phase equilibrium apparatus

Phase equilibrium experiments were performed using the static method in a high-pressure variable-volume view cell which the apparatus and the experimental procedure are well described in a variety of studies (Michielin *et al.*, 2008; Rosso *et al.*, 2009, Comim *et al.*, 2010), based on the work of Oliveira *et al.* (2000). Briefly, the experimental set-up consists of a variable-volume view cell, with a maximum internal volume of 27 mL, with two sapphire windows for visual observation, a thermocouple, an absolute pressure transducer (Model 511, Huba Control, Würenlos/Denmark), with a precision of ±0.03 MPa, a portable programmer (HT 201, Smar, Sertãozinho, SP, Brazil) for the pressure data acquisition and a syringe pump (260HP Teledyne ISCO, Lincon, NE, USA) with pressure range from 0.7 to 655.2 ± 0.5 bar. The equilibrium cell contains a movable piston, which permits the pressure control inside the cell. The phase transitions were visually observed by pressure manipulation using the syringe pump and the solvent (carbon dioxide) as pneumatic fluid.

2.3. Phase equilibrium experimental procedure

Initially, a precise amount of one of the solutions was weighed in an analytical balance (Ohaus, Model AS200S, NJ, USA) with ± 0.0001 g of precision and loaded into the equilibrium cell. A known amount of carbon dioxide at 5 °C and 100 bar was loaded into the equilibrium cell using the syringe pump, resulting in an accuracy of ± 0.005 g in CO_2 loadings until a desired mass fraction was achieved (from 60 to 98.8 % of CO_2). The mass of carbon dioxide loaded is determined by volume dislocation in the syringe pump and volume-mass conversion through the CO_2 density at the fixed conditions of temperature and pressure obtained from NIST Chemistry Webbook (NIST, 2014). The cell content was kept at continuous agitation with a magnetic stirrer and a Teflon-coated stirring bar. Once the desired temperature was reached, the pressure system was increased up to the visualization of one-phase system. At this point, the pressure was slowly decreased (at a rate of 3.5 bar/min) until incipient formation of a new phase. This procedure was

repeated at least two times for each temperature and global composition tested. The temperatures tested were 35 and 45 °C as above that the PLGA melts and remains attached to the cell wall. The liquid-vapor (bubble point) (LVE-BP), liquid-liquid (LLE), liquid-liquid-vapor (LLVE) and liquid-fluid equilibria curves are represented in a P–w diagram (pressure versus solvent mass fraction).

3. RESULTS AND DISCUSSION

Experimental data for the systems 1 and 2 (presented previously) were measured for mass fraction of CO_2 from 60 to 98.8 %, temperatures of 35 and 45 °C and pressures from 51.7 bar to 99.2 bar. The equilibrium results are presented in a P–w diagram in Figure 1, which shows the phase equilibrium data for both systems and isotherms. The results indicate the occurrence of different phase behavior such as vapor–liquid equilibrium (VLE) (represented by the solid markers), liquid–liquid equilibrium (LLE) (upper void markers curves), vapor–liquid-liquid equilibrium (VLLE) (lower void markers curves). In each isotherm it can be observed the one phase region above the upper curves; the two liquid phase area (between void markers), one rich in CO_2 and the other rich in the organic solvent; and the three phase transition, represented by the lower void markers curves.

It is also observed from Figure 1, for both systems, an increase in the transition pressures with the increase in temperature and in CO_2 content, as the internal energy of the system raises a higher pressure is required to reach the homogeneous system (one phase), characterizing a *Lower Critical Solution Temperature* (LCST) behavior - when a homogeneous system becomes a two-phase system when the temperature is increased (van Konynenburg e Scott, 1980). An increase in the CO_2 concentration causes a correspondent decrease in solvent polarity, diminishing the so-called solvent power of the mixture, which leads to higher pressure liquid-liquid phase transition values. In other words, at low CO_2 contents, the system is far more miscible and only vapor–liquid transitions are observed (Bender *et al*., 2010). Also, for a system exhibiting LCST behavior, an increase in CO_2 content or temperature will result in the expansion of the system. In simple terms, the anti-solvent agent of the polymer, in this case CO_2, tends to swell and dissolve the organic solvent, reducing its solvent strength and inducing the precipitation of solutes. As a consequence, greater pressures are necessary to attain the one-phase system region (Kalogiannis and Panayiotou, 2005).

At the so called liquid-fluid transition, represented by the star points in Figure 1, it was observed a less intense cloud in the system not followed by phase separation, i.e., only one phase remained after the cloud was dispelled. This behavior probably indicates that the system reached the vicinity of the critical point, where small changes in some thermodynamic variable (such as temperature or pressure) result in substantial changes of the thermodynamic properties of the system (such as density and viscosity) however not related to volumetric variations inherent to phase separation.
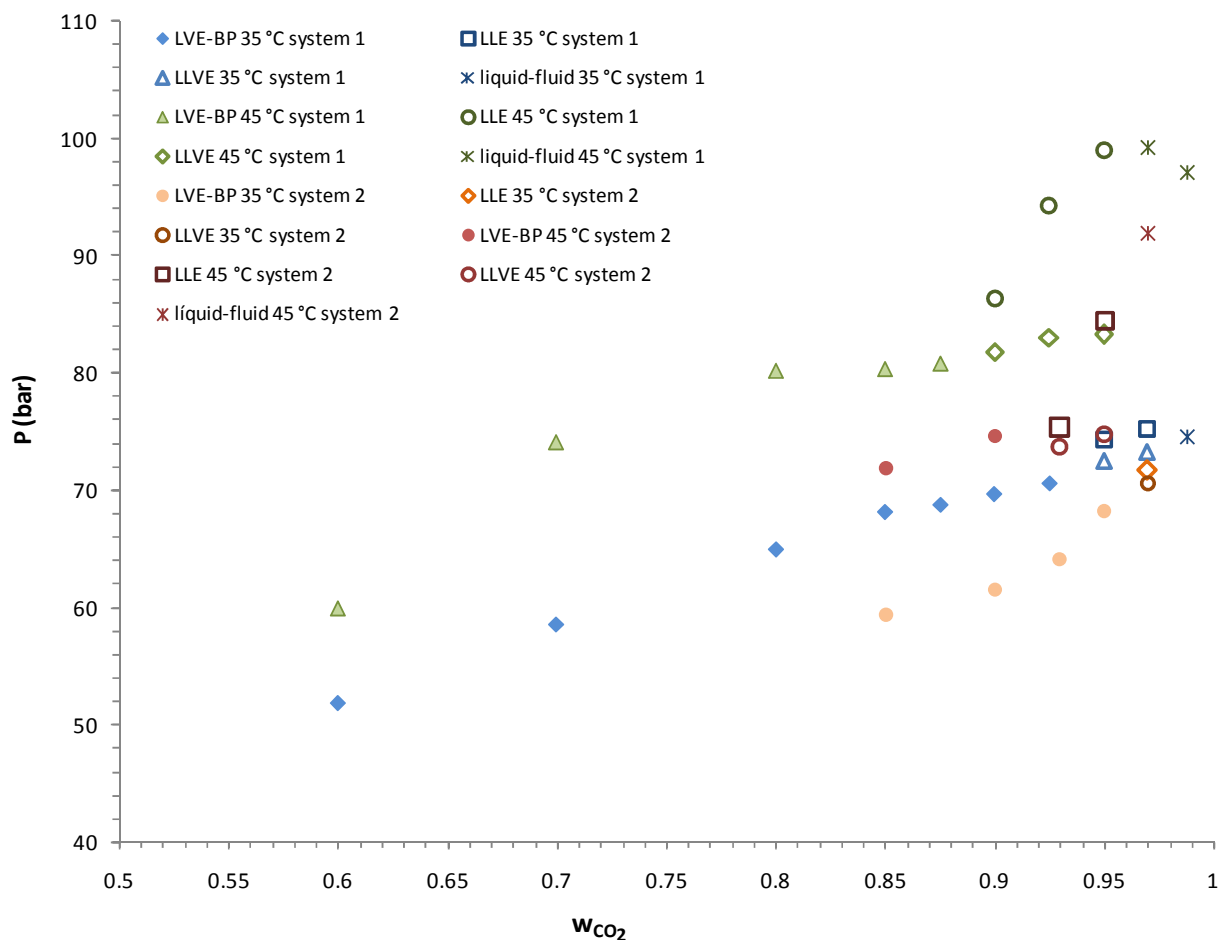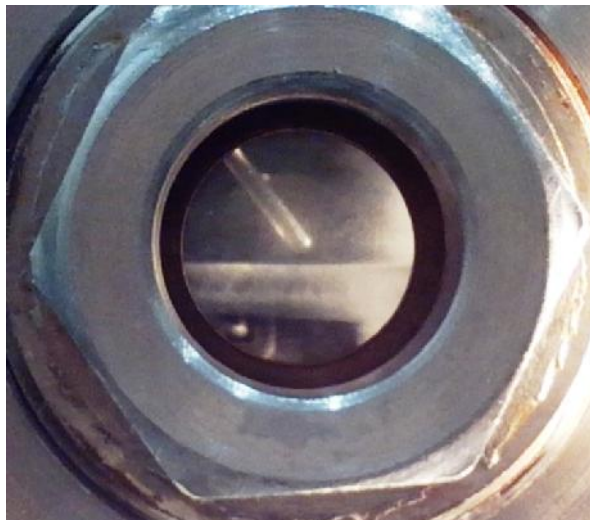
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