PHASE EQUILIBRIUM DATA OF A MULTICOMPONENT SYSTEM (PINK SHRIMP RESIDUE EXTRACT + ACETONE + CO$_2$)

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Abstract. Pink shrimp (*P. brasiliensis* and *P. paulensis*) residue, composed by shrimp head, cephalothorax and tail, is a source of substances useful as food and pharmaceutical ingredients. The main byproducts obtained from the pink shrimp residue are ω-3 fatty acids, carotenoid pigments, chitin and flavor compounds. Carotenoids and ω-3 fatty acids are the most interesting substances for the food industry due to their nutritional importance, beyond the antioxidant and hypolipidemic activities, although they are highly unstable. In order to preserve the cited important biological activities, the shrimp residue extract can be encapsulated by high pressure techniques, such as SAS (Supercritical Anti-Solvent process). The selection of SAS operational conditions (pressure, temperature, extract and polymer concentrations, etc.) depends on the knowledge of the equilibrium data of the involved compounds. Therefore, this work aimed to investigate the phase equilibrium behavior of the multicomponent system: shrimp residue extract, acetone and carbon dioxide (CO$_2$) in order to provide information for encapsulation processes. The shrimp residue extract was obtained by cold maceration with acetone. The phase equilibrium data were acquired using the synthetic static method. A solution of 1:128, w/w, of extract:acetone was used in the phase equilibrium experiments. Phase equilibrium data was obtained for CO$_2$ mass contents from 49.93 to 94.48% at temperatures of 308K, 313K, 318K and 333K. The system exhibits liquid-vapor equilibrium with transition pressures up to 8.3 MPa and a lower critical solution temperature behavior. The increase in temperature caused an increase in transition pressures.

Keywords: thermodynamics, supercritical technology, critical solution temperature behavior, encapsulation.

1. Introduction

The risks to human health attributed to synthetic food additives are causing their replacement by natural products. In this context, the use of carotenoids, natural pigments responsible for the red, orange and yellow colors of many vegetables and other foods, has an increasing industrial interest. Some of the most abundant carotenoids present in the human organism are β-carotene, lycopene, astaxanthin and lutein. These substances are not produced by the human body, and therefore must be supplied by the human diet [1]. Besides their use as pigments, carotenoids have several pharmaceutical and nutritional applications due to their positive effect on health such as the antioxidant potential [2, 3].

The use of pink shrimp residue, obtained from the processing of the shrimp species such as *P. brasiliensis* and *P. paulensis*, is an alternative for the recovery of the carotenoid fraction present in the industrial waste. Therefore, carotenoids enriched extracts can be obtained by several extraction procedures and different pre-treatments applied to the raw material, as extensively studied by Mezzomo et al. [4]. The authors compared the efficiency of different extraction methods and evaluated the technical viability of the process according to the content of astaxanthin, the most representative carotenoid from crustaceans like shrimp.

Because astaxanthins are easily altered in the presence of light, oxygen and/or high temperatures [5], it is important to define adequate procedures or formulations that preserve the quality of carotenoid enriched

extracts against their degradation. Different procedures are applied to chemical formulations in order to protect their characteristics, such as microencapsulation. The encapsulation consists of coating the active components, like carotenoid enriched extract, by means of a protective film, such as a polymer cover. Therefore, the main advantages of the encapsulation are: reduction of the evaporation of the active extract, increase of its shelf live and control of the release through the coating film, which may increase its biological efficiency [6].

Knowledge of the phase behavior (equilibrium data) presents considerable importance for the optimization and design of processes such as microparticle formation techniques like rapid expansion of supercritical solution (RESS), gas anti-solvent process (GAS), and supercritical anti-solvent (SAS), among others [7-12]. The phase equilibrium indicates the limits of the mass transfer between the phases involved in the process, because it reveals the composition and quantities of the phases in equilibrium, the distribution of the compounds between the phases and the variation of these quantities with temperature, pressure and concentration of the compounds [13].

Therefore, the objective of the present work was to investigate the phase equilibrium behavior of the multicomponent system: shrimp residue extract, acetone and carbon dioxide (CO_2) in order to provide information for the encapsulation processes.

2. Material and Methods

2.1 Raw material preparation and extraction procedure

The raw material consists of pink shrimp (*P. brasiliensis* and *P. paulensis*) residue (waste from shrimp processing), composed essentially by head, carapace, and tail. The residue was provided by **Peixaria Nelson Santos** (Florianópolis, Santa Catarina, Brazil). The raw material was obtained from the unpeeling of the cultivated shrimp, collected in May 2010, representing the high production season of the region. The shrimp residue was pre-treated by combining the process of cooking, drying and milling. After the pre-treatment, the raw material was submitted to the extraction of the carotenoid enriched fraction, which consists in a cold maceration in acetone (P.A., Nuclear, CAQ Ind. E Com. LTDA., Brazil) for 5 days at room temperature. Details of the raw material pre-treatment and extraction procedure are presented at Mezzomo et al. [4]. According to the literature data, the cold maceration in acetone of the pink shrimp residue provides an extraction yield of 4 ± 1 % (w/w, d.b.) and the resulting extract contains an astaxanthin amount of 252 ± 6 $\mu g/g_{extract}$ [4].

2.2 Phase equilibrium apparatus and procedure

Phase equilibrium experiments were conducted by employing the static synthetic method in a high-pressure variable-volume view cell. The experimental apparatus and procedure have been used and described in a variety of studies [14-17]. Briefly, the experimental set-up consists of a variable-volume view cell, with a maximum internal volume of 27 cm^3, with two sapphire windows for visual observation, an absolute pressure transducer (Model 511, Huba Control, Würenlos/Dinamarca) and a syringe pump (260 D, ISCO, Lincon/NE/E.U.A). The equilibrium cell contains a movable piston, which permits the pressure control inside the cell. Phase transitions were recorded visually through manipulation of the pressure using the syringe pump and the solvent as pneumatic fluid. The pink shrimp extract was solubilized in acetone at a mass ratio of (1:128/ extract:acetone). This solution was kept at 2°C. A precise amount of the pink shrimp extract solution (extract + acetone) was weighed on a precision scale balance (Ohaus Analytical Standard with 0.0001 g accuracy) and loaded into the equilibrium cell. A known amount of solvent was loaded into the equilibrium cell using the syringe pump, resulting in an accuracy of ±0.005 g in carbon dioxide loadings until a desired global composition was achieved. Then, the cell content was kept at continuous agitation with the help of a magnetic stirrer and a Teflon-coated stirring bar. The temperature control was then turned on, and once the desired temperature was reached and controlled within 0.1 K, the pressure system was increased until the visualization of a one-phase system in the cell. At this point the system was kept still for at least 30 min to allow the stabilization, then, the pressure was slowly decreased (typically at a rate of 0.1 to 0.3 Mpa.min^{-1}) until initial formation of a new phase. This procedure was repeated at least two times for each temperature and global composition evaluated. After measurement achievement at a given temperature, the cell temperature was established at a new value and the experimental procedure was repeated. Acetone was chosen for being a good solvent for both the shrimp extract and the polymer for further extract encapsulation,

such as Pluronic F127[TM], a common polymer used for natural extract encapsulation [18]. The proportion of 1:128 (extract:acetone) used for the extract solution preparation was chosen taking into account the extract concentration that promoted the best encapsulation condition accordingly to Mezzomo et al [18].

3. Results and Discussion

Phase equilibrium data obtained for the system studied [CO_2 (1) + pink shrimp extract/acetone (2)] are presented in Table 1 for the temperatures (T) 308K, 313K, 318K e 333K and CO_2 mass compositions (w_1) from 49,93% a 94,48%. Table 1 shows the equilibrium results in terms of type of transition, transition pressure (P) and present the experimental error for each pressure represented by the standard deviation of replicate measurements (σ).

Table 1 – Phase equilibrium experimental data for the multicomponent system (CO_2 (1) + pink shrimp extract/acetone (2))

T/K	P/Mpa	σ/Mpa	Transition[1]
	$w_1 = 0.4993$		
308	4.1	0.02	LVE- BP
313	4.4	0.02	LVE- BP
318	4.8	0.01	LVE- BP
333	6,0	0.01	LVE- BP
	$w_1 = 0.6999$		
308	5.2	0.05	LVE- BP
313	5.7	0.01	LVE- BP
318	6.2	0.01	LVE- BP
333	8,0	0.13	LVE- BP
	$w_1 = 0.7999$		
308	5.6	0.06	LVE- BP
313	6.5	0.06	LVE- BP
318	7,0	0.01	LVE- BP
333	8.7	0.08	LVE- BP
	$w_1 = 0.8496$		
308	6.2	0.1	LVE- BP
313	7,0	0.01	LVE- BP
318	7.6	0.01	LVE- BP
333	9.4	0.03	LVE- BP
	$w_1 = 0.8996$		
308	6.8	0.07	LVE- BP
313	7.5	0.1	LVE- BP
318	8.1	0.02	LVE- BP
333	9.5	0.04	LVE- BP

Table 2 Continuation

T/K	P/Mpa	σ/Mpa	Transition[1]
		$w_1 = 0.9299$	
308	6.9	0.03	LVE- BP
313	7.6	0.01	LVE- BP
318	8.3	0.07	LVE- BP
333	10	0.07	Cloudy[2]
		$w_1 = 0.9448$	
308	6.9	0.02	LVE- BP
313	7.5	0.02	LVE- BP
318	8.2	0.04	LVE- BP
333	10.2	0.01	Cloudy[2]

[1]LVE-BP: liquid-vapor equilibrium- bubble point. [2] The system became cloudy but no separation of phases was observed.

The pressure-composition (P-w) diagram for the results showed in Table 1 is presented in Figure 1. It can be observed from Figure 1 that over the temperature range from 308 to 318 K vapor–liquid transitions with bubble points were observed. The single phase is observed in the area above the isotherms and a liquid-vapor equilibrium phase is observed in the region below the isotherms. Accordingly to Brunner [13], the addition of a co-solvent to a mixture usually increases its critical properties [19]. The presence of acetone in the mixture may explain the absence of dew points for the temperatures from 308 to 333 K. The critical temperature of the mixture acetone + carbon dioxide calculated by the Prausnitz rule [20] is 396 K (kij = 0), a value above the experimental range of temperatures studied. For the 333 K isotherm, at the pressure of 10 Mpa for CO_2 mass ratio of 92.99% and 10.2 Mpa for CO_2 mass ratio of 94.48%, the system changed the appearance form transparent to cloudy. However, no separation of phases was observed for lower pressures (down to 5 Mpa).
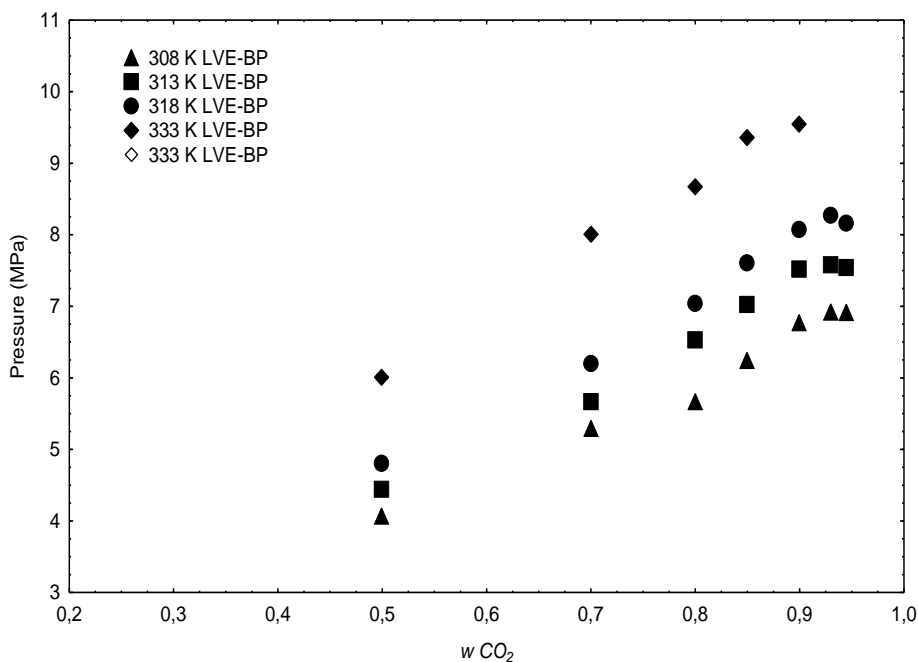

Figure 1 Plot of pressure against mass fraction of carbon dioxide for the multicomponent system (CO_2 (1) + pink shrimp extract/acetone (2))

Presented in Figure 2 is the pressure-temperature diagram for the system studied (CO_2 (1) + pink shrimp extract/acetone (2)) that indicates a LCST (*lower critical solution temperature*) behavior [21]. The area above the transition region is a single phase while below it is a heterogeneous system (biphasic). For a constant pressure value, considering a fixed composition (represented by each of the transition curves), the system becomes biphasic with increasing temperature [21].
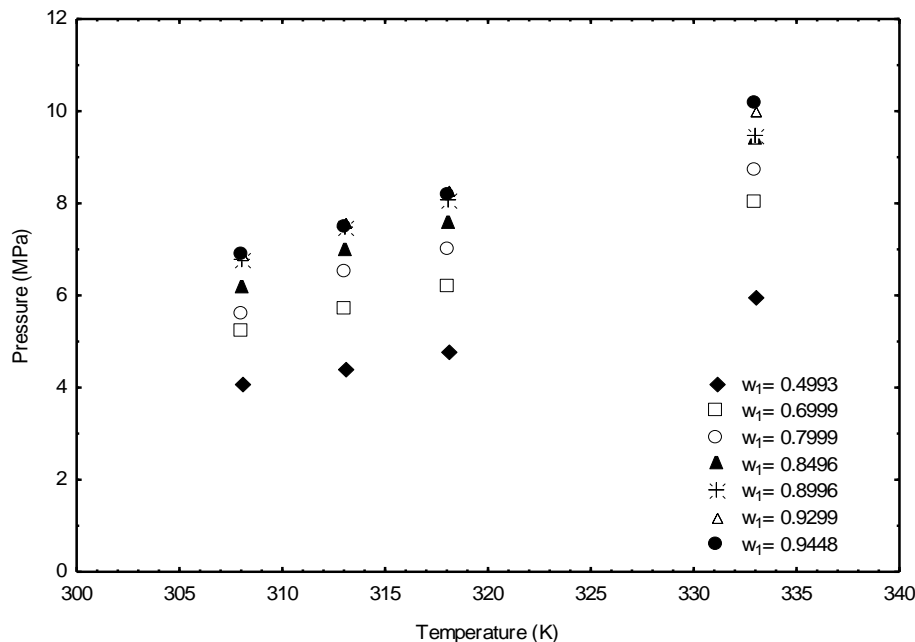

Figure 2 Plot of pressure against temperature for the for the multicomponent system [CO_2 (1) + pink shrimp extract/acetone (2)]

4. Conclusions

Phase behavior of the system (CO_2 (1) + pink shrimp extract/acetone (2) was investigated over the temperature range of 308 to 333 K and covering a wide overall composition range of carbon dioxide from 0.4993 to 0.9448 in mass fraction Phase transition points were recorded for pressures up to 8.3 MPa. Vapour–liquid transitions were found at all the measured points. The system presents a LCST behavior. One should emphasize that phase equilibrium experimental results presented in this work will be of relevance for the understanding and design of the precipitation and/or encapsulation process of pink shrimp extract using the supercritical technology, such as the SAS method. The phase equilibrium data reported in this work will help to define the operational conditions for the SAS process.

Acknowledgements

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