High Pressure Phase Equilibria of Binary System CO_2-Oleoresin Capsicum

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ABSTRACT

Oleoresin is the viscous red liquid obtained after the extraction of red peppers with organic solvents such as hexane. Depending on the raw material and the solvent used to carry out the extraction, the oleoresin obtained will have different characteristics.

In this work, high pressure vapour-liquid equilibria for binary systems including carbon dioxide and oleoresin Capsicum were measured at different temperature conditions (313-338K) with the aim of determining the solubility and the distribution between phases of the carotenoids and capsaicinoids pigments present in this type of oleoresin. The experimental measurements were carried out by a recirculation method using a high-pressure variable-volume view cell with a capacity of 50 cm^3 and fractions collected were determined gravimetrically. The amount of carotenoids and capsaicinoids in both phases was analyzed and separation factor calculated.

Experimental results showed that solubility enhances when high pressures are employed and an increase of the inmiscibility of this system takes place at lower pressures when temperature increases. Besides carotenoids pigments can be selectively separated from capsaicinoids and lipid matter using supercritical carbon dioxide as solvent and low conditions of pressure and temperature.

INTRODUCTION

Pungent spice paprika (*Capsicum annuum L.*) is used worldwide as a natural flavour and colorant in food and as well as raw material for the pharmaceutical industries [1]. The traditional method to obtain oleoresin from pungent or non-pungent paprika is the same to produce oils and fats and consists in extraction using organic solvents such as acetone or hexane [2]. The importance of Capsicum oleoresin and in general the importance of conventional vegetable oils is because they are valuable natural products, containing triglycerides, diglycerides, free fatty acids and a number of minor components (sterols, tocopherols, phospholipids, etc.) which are important as pharmaceutical and food additives [3]. Particularly, the commercial quality of Capsicum oleoresin depends on the amount of colorant pigments, which are responsible for the colour of the oleoresins and on the amount of pungent pigments, which are responsible for the hot and spicy flavour imparted by many oleoresins.

According to colorant pigments, the colour of the oleoresin is due mainly to nine major carotenoid pigments, naturally present in multiple forms of esterification [4]. The importance of this type of compounds is due to their provitamin A activity and also because they play an important role in human health by acting as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and single oxygen [5] and inhibiting the development of certain types of cancers [6]. Figure 1 shows the structure of the main carotenoids present in oleoresin.

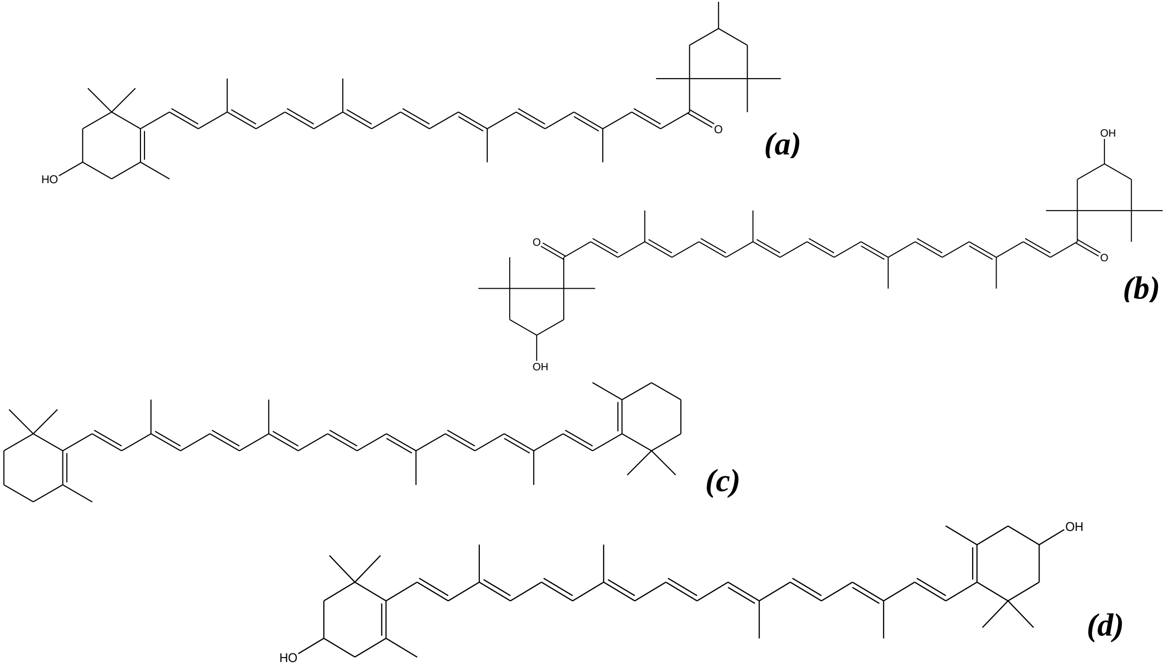

Figure 1. Main carotenoids present in oleoresin. *(a)* Capsanthin; *(b)* Capsorubin; *(c)* β-carotene; *(d)* Zeaxanthin.

About pungent pigments, a group of alkaloids called capsaicinoids (see Figure 2) are the compounds responsible for the pungency of some types of oleoresin. They are derivatives of phenylpropanoid compounds and characterized by a high biological activity and their pharmacological, neurological and dietetic effectiveness as well [7].

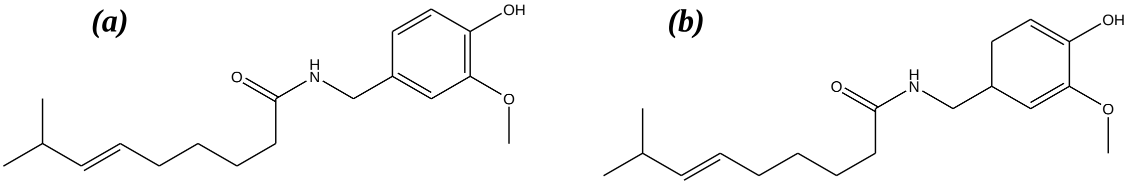

Figure 2. Main capsaicinoids present in oleoresin. *(a)* Capsaicin; *(b)* Dihydrocapsaicin.

Nowadays, the growing interest in produce better quality and healthier foods has inspired to change the conventional extraction process for an alternative one which can minimize the residues and make better use of products obtained. Supercritical Fluid Technology results in a good alternative to produce completely new products with special characteristics [8] and for that reason there are a great variety of potential applications of supercritical fluids in the industrial processing of fatty oils and derivates. In that way solubilities of various vegetable oils in CO_2 have been reported in the literature [9-11] and the study to get the fractionation and deacidification of oils have been also reported [12].

The aim of this work has been to study the vapour-liquid equilibria of the system CO_2-oleoresin in order to acquire the enough knowledge of the thermodynamic behaviour which allows us to distinguish if separation of different pigments present in oleoresin is possible and economical feasible applying high pressures.

MATERIALS AND METHODS

Chemicals

A commercial oleoresin Capsicum (OR_{Cap}) was provided by R. Sabater S.A (Murcia, Spain) with a pungency capacity of $\sim 1.4 \cdot 10^6$ Scoville Heat Units (SHU) and a colour capacity of 2500 Standard Colour Units (SCU). CO_2 with a purity of 99.8% was supply by Carburos Metálicos (Madrid, Spain).

Equipment and experimental procedure

Experimental measurements were carried out by a recirculation method using a high-pressure variable-volume cell model HPPE 300-RE (Eurotechnica GmbH, Germany) (see Figure 2). The equipment consists of a variable-volume cell, supplied with a front and upper window and light for visual observation of phase separation. The cell has a maximum capacity of 50 cm^3 and contains a piston system, consisting in a manual pressure generator, a cylinder and a movable piston made of PTFE to avoid pressure drops when samples are taken. The movable piston separates the equilibrium chamber from the pressurizing circuit. To allow a smooth displacement of the piston inside the cylinder, the piston was driven by the manual pressure generator and water was used as the pressurizing fluid. All the system is heated externally by an air bath made of PMMA capable to resist temperatures up to 80ºC. A gear pump provides appropriate stirring inside the cell under constant operating conditions (pressure and temperature) until equilibrium was reached. After 1.5 h of phase separation the samples from gas phase are collected isobarically through a six port valve connected to a 20 cm^3 loop and samples from liquid phase are collected through needle valves (V-5, V-6) into a glass trap. The amount of released gas was measured by a gas meter (model Ritter TG-05), while the amount of oleoresin was determined gravimetrically (accurate to ±0.0001 g). Results were expressed as molar fraction of carbon dioxide (x_i, y_i).

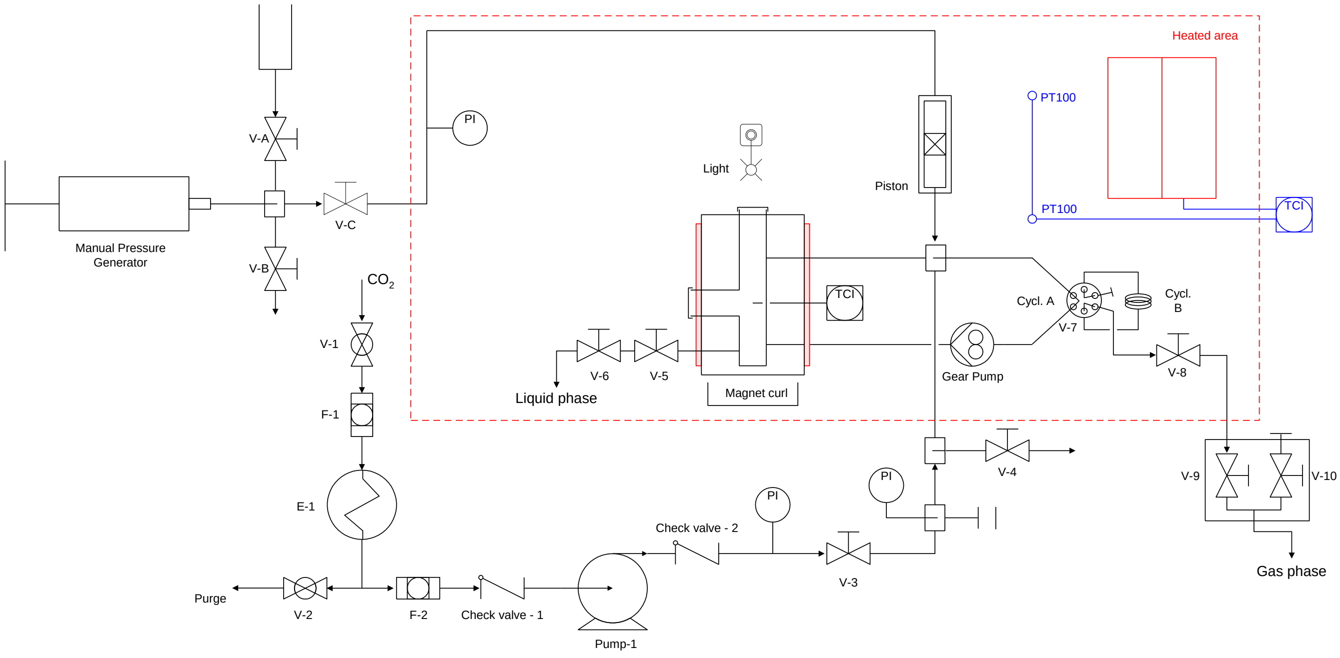

Figure 2. Experimental set-up employed to high pressure phase equilibria measurements.

Quantification of carotenoids and capsaicinoids

The concentration of carotenoids in the samples collected was carried out by spectrophotometrical methods using a UV-Vis spectrophotometer Thermo-Scientific model Helios Zeta (Madison, US). A known amount of sample was diluted in acetone and measured at two different wavelengths (472nm and 508nm). To calculate the concentration of carotenoids the equations proposed by Hornero-Méndez [13] were used.

For the capsaicinoids concentration an HPLC method was applied. The chromatographical analyses were carried out using a Jasco Chromatograph (Essex, UK) equipped with a quaternary pump PU-1580, an autosampler AS-1550 and an in-line degasser DG-1580-53. For the determination of the capsaicinoids content in the samples the mobile phase used was water/acetonitrile (45:55, v/v) with a flow rate of 1 mL/min and a Discovery C18 column (15 cm × 4.6 mm, 5 μm) purchased from Analisis Vinicos (Tomelloso, Spain). A Diode Array MD-1510/1515 detector (λ = 280nm) was used and the identification and quantification of peaks was made by comparison with standards.

RESULTS

Vapour-Liquid Equilibria for CO_2-OR_{Cap}

High pressure vapour-liquid equilibria for the binary system oleoresin Capsicum-carbon dioxide was measured at different temperatures among 313-338K in order to establish the influence of this variable on solubility and the results can be seen at Figure 3.

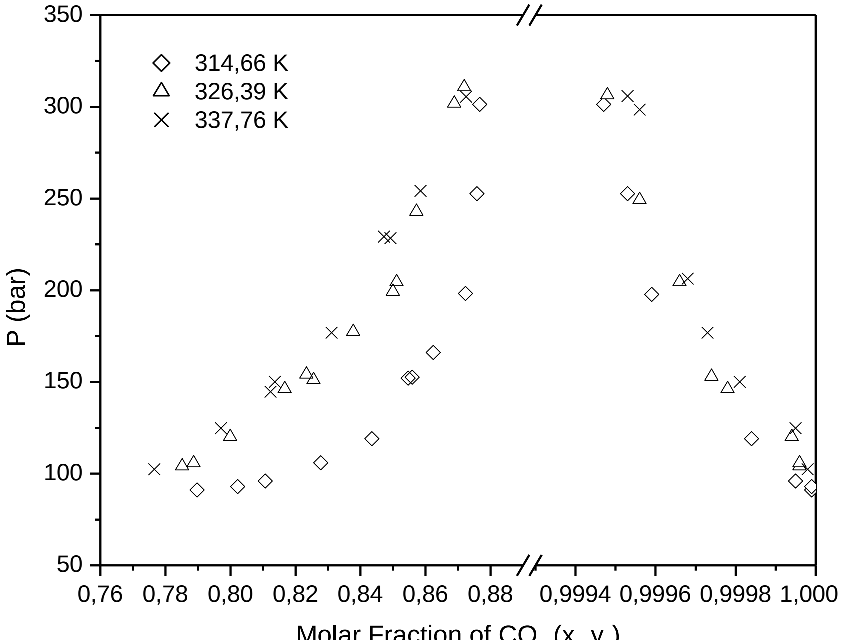

Figure 3. *P-xy* diagram for the binary system CO_2-OR_{Cap}.

As can be observed, over the whole range of pressures studied, the increase in temperature produces an increase in the size of the inmiscibility region of the binary system more noticeable when lower pressures are used. As the pressure increases, the difference in the size of the inmiscibility region at different temperatures is reduced and if pressure continues increasing over the range studied in this work it seems that at high temperatures the inmiscibility region will decrease its size and no separation will be possible.

Due to the fact that solubility of low volatile compounds depends on solvent density, a dependence with temperature and pressure exists. Usually, solubility increases when density increases. Nevertheless the solvent power of a supercritical fluid not only depends on density but also on the vapour pressure of the solute. The higher the vapour pressure of a solute at the same density, the higher the solubility reached. Thus, two opposite effects occur. The vapour pressure of a solute increases with increasing temperature, but the density of the supercritical solvent decreases. Depending on the pressure, one effect will be dominant. In general, at lower pressures the decrease in density dominates the increase in vapour pressure and a decrease in solvent capacity occurs. At higher pressures the decrease in density due to a higher temperature is usually smaller and the solvent capacity increases.

In order to allow for these density dependencies, Figure 4 reports the OR_{Cap} solubility in relation to pure solvent density, which was calculated using the equation proposed by Bender [14].

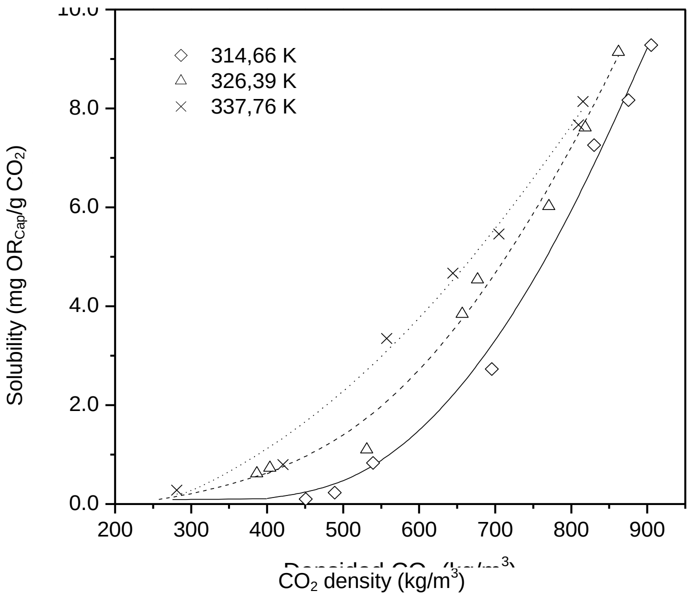

Figure 4. Influence of solvent density on OR_{Cap} solubility.

Figure 4 shows that at the same CO_2 density, the solubility of OR_{Cap} increases with temperature supporting the above-mentioned effect that at higher pressures the increase in the vapour pressure of the solute dominates the decrease of the solvent density.

Separation of carotenoids and capsaicinoids

In order to study the possibility to separate the different pigments (carotenoids and capsaicinoids) present in this type of oleoresin, the distribution coefficients (k_{ij}) of each type of pigments were calculated as in equation (1). Table 1 reports the values obtained.

$$k_{ij} = \frac{y_i}{x_i} \qquad (1)$$

Table 1. *k values* for capsaicinoids and carotenoids present in OR_{Cap}.

P (bar)	*T (K)*	$k_{capsaicinoids}$	$k_{carotenoids}$
105.9	314.66	1.081	0.083
152.0	314.66	1.051	0.090
198.3	314.66	0.924	0.108
252.7	314.66	0.849	0.077
146.1	326.39	1.102	0.051
177.3	326.39	1.208	0.056
242.8	326.39	1.064	0.028
205.1	337.76	1.147	0.043
298.9	337.76	1.044	0.028

As can be seen, the *k values* for capsaicinoids are close to one. This means that it is relatively difficult to get the separation of this kind of pigments between different phases, especially at 314K and pressures lower than 150bar. Nevertheless, at this temperature but applying higher pressures separation improves. According to the *k values* of carotenoids the proximity of this coefficient to a zero value implies that the separation of carotenoids between liquid and vapour phase can be achieved easily. In that way carotenoids stay at the liquid phase when extraction takes place.

Comparing now the *k-values* of both types of pigments and calculating the separation factor (α_{ij}) between them it can be concluded that the separation between capsaicinoids and carotenoids is possible in all the conditions studied but is favoured at pressures near to 250bar and temperatures of 326K.

CONCLUSION

The *P-xy* diagram of the vapour-liquid equilibria for the system CO_2-OR_{Cap} was experimentally obtained at three different temperatures and the effect of this variable studied. Over the whole range of pressure and temperature, an increase in temperature increases the size of the two-phase region even more noticeable when lower pressures are used. Distribution coefficients were calculated for carotenoids and capsaicinoids and in that way separation factor could be obtained. Carotenoids are enriched in the liquid phase while capsaicinoids neither are enriched easily in the liquid nor in the gaseous phase under the conditions studied. However, the separation between the two types of pigments is possible as can be concluded for the separation factors calculated.

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