

# High-pressure phase equilibria of the pseudo-ternary system carbon dioxide + ethanol + fish oil

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## ABSTRACT

This work provides experimental phase equilibrium data of the pseudo-ternary system CO<sub>2</sub> + ethanol + fish oil, a system of interest in pharmaceutical and food-industry applications such as the enzymatic production of fatty acid ethyl esters at mild, non-oxidative conditions. Measurements were carried out in a high-pressure variable-volume view cell in the temperature range 323.15-343.15 K and at pressures from 10 to 30 MPa. The Peng-Robinson equation of state was used to satisfactorily correlate experimental data.

## INTRODUCTION

Dense carbon dioxide as a reaction medium is expected to play an important role in ecologically friendly processing. Enzyme-catalysed ethanolysis of lipid sources in supercritical carbon dioxide (SC-CO<sub>2</sub>) or CO<sub>2</sub>-expanded media can be used to improve the production of lipid derivatives, including n-3 PUFA concentrates from fish oil [1]. However, the relatively low solubility of the reactants in SC-CO<sub>2</sub> limits the reaction performance in this medium; thus, a CO<sub>2</sub>-expanded media is preferred to minimise mass transfer limitations [2]. This way, understanding the phase behaviour of the ethanol + fish oil reactive mixture with CO<sub>2</sub> as a solvent would help in the selection of adequate ethanolysis conditions (pressure, temperature, ethanol-to-oil ratio, and amount of dissolved CO<sub>2</sub> in the reaction mixture). This knowledge can be also extended to other potential applications, such as extraction of fish oil, separation of liquid mixtures, or particle formation and formulation of fish oil and its derivatives using SC-CO<sub>2</sub>.

Experimental data related with high-pressure phase equilibria of the binary system CO<sub>2</sub> + ethanol are extensively reported in the literature [3]–[8]. Besides, the pseudo-binary systems comprising CO<sub>2</sub> and triglycerides, edible oils, other lipid derivatives or its mixtures are also widely investigated [9]. However, less data regarding the phase equilibria of the pseudo-ternary mixtures of CO<sub>2</sub> + ethanol + lipid derivatives can be found in the literature, and these data are usually related to the solubility of the lipid compounds in CO<sub>2</sub> with ethanol as a co-solvent [10].

Geana and Steiner [11] presented phase equilibrium data for the pseudo-ternary system CO<sub>2</sub> + ethanol + rapeseed oil in the temperature range 313.15-353.15 K and at pressures from 6 to 12 MPa, satisfactorily correlating the phase behaviour with the Peng-Robinson equation of state (PR EoS) [12] coupled with the conventional van der Waals mixing rules with two adjustable parameters (vdW2). Ndiaye *et al.* [13] reported phase equilibrium data of binary and ternary mixtures involving CO<sub>2</sub>, ethanol, soybean oil, castor oil, and their fatty acid ethyl esters. The pseudo-ternary system CO<sub>2</sub> + ethanol + castor oil was studied at fixed ethanol-to-oil ratios,

temperatures ranging from 313.15 to 343.15 K and pressures from 2.13 to 27.13 MPa. Experimental data were correlated both with PR EoS vdW2 and the Statistical Associating Fluid Theory (SAFT EoS) [14] with one binary interaction parameter. Among these two models, the authors considered that SAFT EoS described better the phase behaviour of the pseudo-ternary system, yet they also pointed out at some deviations from their experimental results, such as the over-prediction of the cloud point pressure at high ethanol ratios. Hernandez *et al.* [15] investigated the phase equilibrium behaviour of the pseudo-ternary mixture CO<sub>2</sub> + ethanol + sunflower oil at two different conditions of temperature and pressure (313.15 K and 13 MPa; 333.15 K and 20 MPa). A group contribution equation of state (GC EoS) [16] was employed to correlate the experimental data. Two different sets of parameters were adopted for the interaction between the triglyceride (TG) and the alcohol (CH<sub>2</sub>OH) groups, one of them corresponding to the liquid-liquid (L+L) 2-phase region, and the other to the liquid-vapour (L+V) 2-phase region. More recently, Dalmolin *et al.* [17] also studied the phase transitions in the system CO<sub>2</sub> + ethanol + rapeseed oil, at temperatures in the range 313.15-343.15 K and pressures up to 22.53 MPa. They found a 3-phase region with a vapour-liquid-liquid (VLLE) phase transition that occurred at higher pressures when increasing temperature, and satisfactorily explained the experimental results with the PR EoS vdW2 model.

In this work, the phase behaviour of the pseudo-ternary mixture CO<sub>2</sub> + ethanol + fish oil in the temperature range from 323.15 to 343.15 K and pressures from 10 to 30 MPa has been studied by means of an analytical isothermal method with recirculation of the vapour phase (AnTVcir), as described by Dohrn and Brunner [18]. The knowledge obtained will be useful in the development of applications involving this pseudo-ternary mixture, such as the previously mentioned enzymatic reactions, supercritical extraction, and particle formation techniques.

## **MATERIALS AND METHODS**

Refined fish oil was kindly provided by AFAMSA S.A. (Pontevedra, Spain) being a mixture of tuna (*Thunnus* sp.) and sardine (*Sardina pilchardus*) oil. The fatty acid profile of the fish oil has been previously reported [19]. Absolute ethanol (99.9 %) was purchased from Merck KGaA. Carbon dioxide (99.9%) was supplied by Air Liquide S.A. (Spain). All other chemicals used in different analyses were of analytical or HPLC grade.

The high-pressure apparatus used for the phase equilibrium measurements was designed and built by Eurotechnica GmbH (Germany) and consists of an equilibrium cell made of stainless steel (SS-316) and equipped with a sapphire window for observing the content of the cell during measurements. Internal volume of the cell ranges from 40 to 70 mL, adjustable through a manual screw piston. The cell includes a pressure transducer and an immersed thermocouple connected to a Data Acquisition System (DAS). Mixing of the components of the system was achieved by recirculating the vapour phase into the liquid phase by means of a gear pump (Micropump IDEX). For sample withdrawal without disturbing the equilibrium too much, the apparatus is equipped with a 6-way valve (VICI) and a sampling loop for the vapour phase. A micro-metering valve is also connected to the bottom of the equilibrium cell through a 1/16" capillary for the heavy phase. Pressure drop occurring when sampling the liquid phase was compensated by actuating the manual screw piston and reducing the volume of the cell. Maximum specifications of the apparatus are 32 MPa and 393 K.

In a typical experiment, the equilibrium cell was first gently purged with low pressure CO<sub>2</sub> to eliminate the residual air inside the cell. Immediately afterwards, known volumes of fish oil and ethanol were introduced into the cell by means of a binary HPLC pump (Agilent) and liquid CO<sub>2</sub> was charged into and pressurized with a high-pressure syringe pump (ISCO). The masses of fish oil and ethanol were then calculated using their respective densities at room temperature. The amount of CO<sub>2</sub> charged into the cell was measured by a Coriolis mass flow meter (Rheonik). Once the cell was charged, the gear pump was connected and recirculation of the vapour phase was performed for at least 2 h to facilitate the mixing of the components and its distribution in the different phases of the system. The system was then let to repose for another 2 h. Phase separation was visually verified through the sapphire window and samples from the vapour and liquid phases were taken by the 6-way and the micro-metering valve, respectively. Pressure variations up to ±0.1 MPa were observed during sampling, while temperature change was not detected.

Samples were decompressed to atmospheric pressure and released CO<sub>2</sub> was measured by means of a thermal mass flow meter (Bronkhorst). Ethanol and fish oil were separated from CO<sub>2</sub> and collected in an ice-cooled glass trap. The amount of each component was determined by weighing the vials in a precision analytical balance (accurate to ± 0.0001 g) before and after evaporation of ethanol in an oven at 373.15 K.

Measured phase equilibrium data were correlated with the Peng-Robinson equation of state [12] (PR EoS) in combination with the conventional van der Waals mixing rules with two adjustable parameters (vdW2) (Table 1).

**Table 1.** Peng-Robinson equation of state (PR EoS) [12] and conventional van der Waals mixing rules with two adjustable parameters (vdW2).

<b>PR EoS [12]</b>	$P = \frac{RT}{V-b} - \frac{a(T)}{V(V+b)+b(V-b)}$		(1)
<b>Conventional vdW2 mixing rules</b>	<b>Pure-fluid constants</b>		
$a = \sum_i \sum_j z_i z_j (a_i a_j)^{0.5} (1 - k_{ij})$	(2)	$b = 0.07780 \frac{RT_c}{P_c}$	(4)
		$a(T) = 0.45724 \frac{(RT_c)^2}{P_c} \alpha(T)$	(5)
$b = \sum_i \sum_j z_i z_j \frac{(b_i + b_j)}{2} (1 - l_{ij})$	(3)	$\alpha(T) = [1 + \beta(1 - \sqrt{T/T_c})]$	(6)
		$\beta = 0.37464 + 1.54226\omega - 0.26992\omega^2$	(7)

The critical properties ( $T_c$  and  $P_c$ ) and acentric factors ( $\omega$ ) of the components of the system are listed in Table 2. Values for CO<sub>2</sub> and ethanol were obtained from the literature [20] whereas group contribution methods were used to estimate the critical properties and the acentric factor of the pseudo-component fish oil [21], [22].

**Table 2.** Critical temperature ( $T_c$ ) and pressure ( $P_c$ ) and acentric factor ( $\omega$ ) of the components of the pseudo-ternary system CO<sub>2</sub> + ethanol + fish oil.

(pseudo-)component	$T_c$ (K)	$P_c$ (MPa)	$\omega$	Ref.
CO <sub>2</sub>	304.1	7.38	0.225	[20]
Ethanol	513.9	6.14	0.644	[20]
Fish Oil	945.3	0.643	1.906	[21], [22]

Binary interaction parameters  $k_{ij}$ ,  $l_{ij}$  were estimated and fugacity coefficients were calculated for each data set according to PR EoS model. The tie-line compositions that simultaneously minimized the objective function (Equation 8), satisfied the isofugacity criterion ( $\varphi_k^V y_k = \varphi_k^L x_k$  for every component  $k$ ), and the mass balances in every phase were calculated by the Newton-Raphson method by fixing pressure, temperature, and composition of fish oil in the liquid phase.

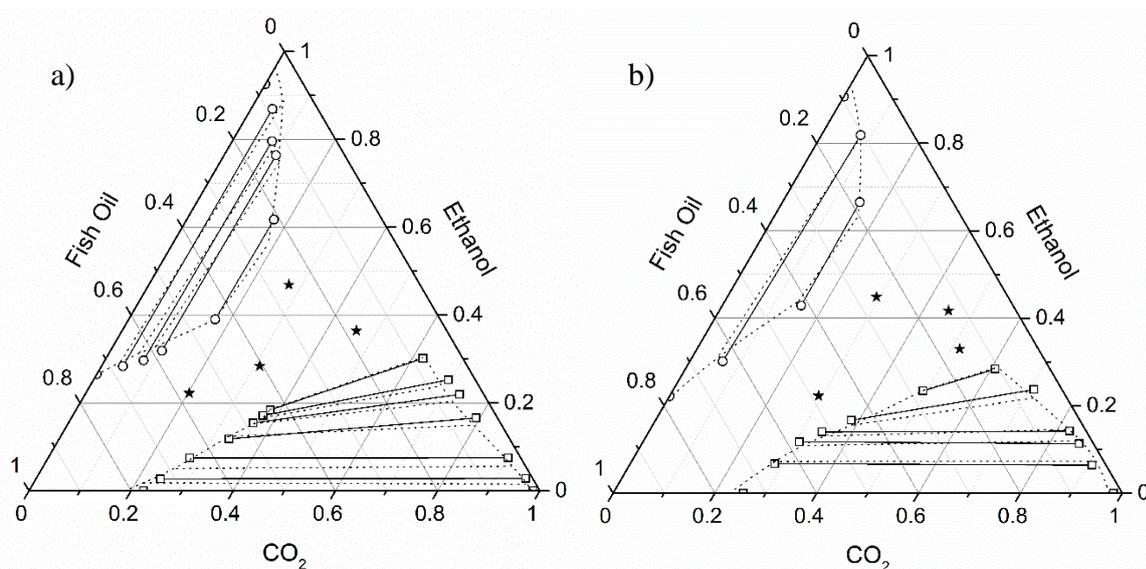
$$OF = \sum_{i=1}^C \sum_{j=1}^{TL} (z_{ij}^{exp} - z_{ij}^{calc})^2 \quad (8)$$

where  $C$  = number of components of the system,  $TL$  = number of experimental tie-lines, and  $z$  is either  $x$  or  $y$ . The superscripts *exp* and *calc* refer to experimental and calculated values.

## RESULTS

Measured high-pressure phase equilibria data of the pseudo-ternary system  $\text{CO}_2$  + ethanol + fish oil showed two different 2-phase regions at 323.15 K for both 10 and 30 MPa. At these conditions, an ethanol + oil region (L1+L2) and an oil +  $\text{CO}_2$  region (L2+V) can be distinguished. Homogeneous monophasic mixtures were visually and analytically verified. In the case of the phase equilibria at 343.15 K and 10 MPa, three 2-phase regions (L1+L2, L1+V, and L2+V) and a 3-phase region (L1+L2+V) were observed. The appearance of the L1+V region is consistent with the published phase equilibrium data of the binary system  $\text{CO}_2$  + ethanol [3]–[8]. Performance of PR EoS in combination with vdW2 mixing rule can be observed in Figs. 1-2.  $OF$  values (Equation 8) are reported in Table 3.

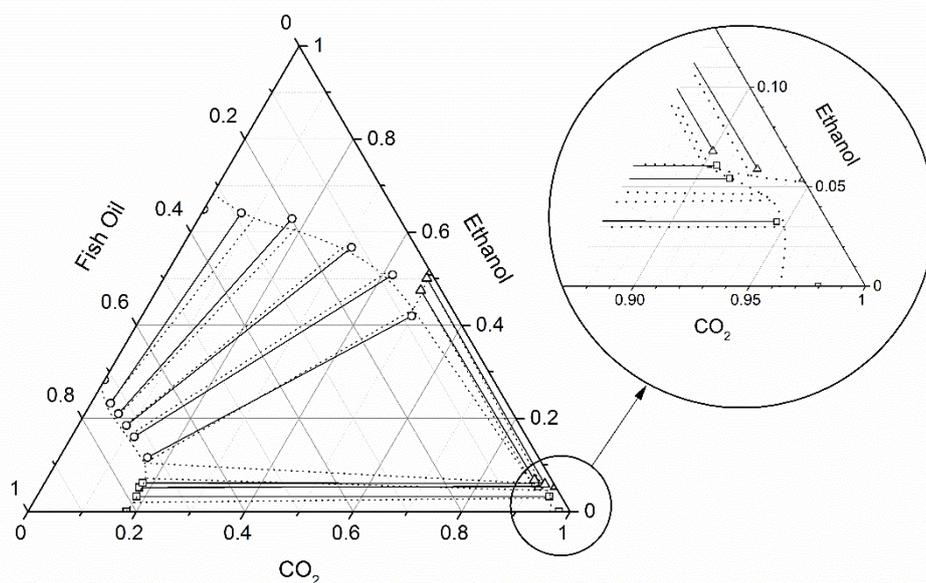
Results obtained for the binary ethanol + fish oil tie-line at 323.15 K (Figure 1) are similar to those obtained by Bucio *et al.* in previous works at the same temperature and atmospheric pressure [19]. The composition of the two liquid phases (L1+L2) became more similar as more  $\text{CO}_2$  is dissolved. The same was true for the L2+V region, where the compositions of the liquid and vapour phases in equilibrium tend to merge with increasing amounts of dissolved ethanol. A similar trend has been found for other pseudo-ternary mixtures of  $\text{CO}_2$  + ethanol + natural lipids, such as castor oil [13] and sunflower oil [15].



**Figure 1.** Phase diagram of the pseudo-ternary system  $\text{CO}_2$  + ethanol + fish oil.

a) 323.15 K and 10 MPa; b) 323.15 K and 30 MPa. -○-: experimental L1+L2 tie-line; -□-: experimental L2+V tie-line; ★: experimental monophasic mixture; ···: PR EoS model with vdW2 mixing rules.

From the phase diagram at 343.15 K and 10 MPa (Figure 2), it can be observed that, starting from the binary sides of the phase diagram, increasing amounts of the third component made the tie-lines of the 2-phase regions approach the sides of the 3-phase region triangle. Inside this 3-phase region, it was experimentally verified that theoretical mixtures split in a L1+L2+V system, each phase with a composition defined by the vertices of the triangle.



**Figure 2.** Phase diagram of the pseudo-ternary system CO<sub>2</sub> + ethanol + fish oil at 343.15 K and 10 MPa.  
 -○-: experimental L1+L2 tie-line; Δ experimental L1+V tie-line; -□-: experimental L2+V tie-line;  
 ⋯: PR EoS model with vdW2 mixing rules.

The binary interaction parameters for the PR EoS vdW2 model, which were estimated from the best fit of calculated and experimental isothermal-isobaric data, are shown in Table 3. The interaction parameters for the mixture CO<sub>2</sub> + ethanol ( $k_{12}$ ,  $l_{12}$ ) were obtained from binary experimental and literature data [3]–[8] at both isotherms (323.15 and 343.15 K), and were fixed in the calculations of the phase equilibria of the pseudo-ternary system. Up to 3 different sets of binary interaction parameters were adopted for the ethanol + fish oil mixture ( $k_{23}$ ,  $l_{23}$ ). One set for each of the 2-phase regions observed. This procedure was also adopted by Hernandez *et al.* [15] in the correlation of CO<sub>2</sub> + ethanol + sunflower oil phase equilibrium data with GC EoS [16]. For the L1+L2 and L2+V regions at 343.15 K and 10 MPa, only the first binary interaction parameter ( $k_{23}$ ) was necessary to describe the phase behaviour of the pseudo-ternary system, thus  $l_{23}$  was not considered being set to 0.

**Table 3.** Estimated binary interaction parameters of the PR EoS vdW2 model for the pseudo-ternary system CO<sub>2</sub> (1) + ethanol (2) + fish oil (3) at different temperature and pressure conditions in the liquid-liquid (L1+L2) and liquid-vapour (L1+V, L2+V) 2-phase regions.

		$k_{12}$	$k_{13}$	$k_{23}$	$l_{12}$	$l_{13}$	$l_{23}$	<i>O. F.</i>
323.15 K 10 MPa	L1+L2			-0.1448			-0.0181	0.0207
	L2+V	0.0961	0.0888	-0.0472	-0.0228	0.0342	0.0288	
323.15 K 30 MPa	L1+L2			-0.1508			-0.0312	0.0248
	L2+V	0.0961	0.0749	-0.0631	-0.0228	0.1016	-0.0721	
343.15 K 10 MPa	L1+L2			-0.119			-*	0.0417
	L1+V	0.0913	0.2427	0.0741	-0.0103	-0.1107	-0.0129	
	L2+V			-0.0628			-*	

\* not considered ( $l_{23} = 0$ )

## CONCLUSION

Experimental phase equilibrium data of the pseudo-ternary system CO<sub>2</sub> + ethanol + fish oil is presented in this work at 3 P-T combinations. Results showed an ethanol + oil region (L1+L2) and an oil+CO<sub>2</sub> region (L2+V) at 323.15 K in the range 10-30 MPa. An additional CO<sub>2</sub> + ethanol (L1+V) and a 3-phase region (L1+L2+V) were observed at 343.15 K and 10 MPa. Experimental data were satisfactorily correlated with the Peng Robinson equation of state with conventional van der Waals mixing rules with two binary interaction parameters.

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