SEMI-BATCH FRACTIONATION OF FATTY ACIDS ETHYL ESTERS BY MEANS OF SUPERCRITICAL CARBON DIOXIDE

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A semi-batch fractionation of a synthetic mixture of fatty acids ethyl esters was carried out by means of supercritical carbon dioxide. The feed was composed of five ethyl esters of fatty acids of different chain length (from C14 to C22), chosen in order to represent a typical product derived from esterification of sardine oil. C20 and C22 classes were represented by EPA and DHA, which are the two major ϖ-3 fatty acids. Experimental temperature ranged from 42 to 60 °C, while pressure ranged from 9 to 16 MPa. The results obtained show that the process is capable of producing a raffinate rich in EPA and DHA. A modellization of the process based on the Peng-Robinson equation of state was developed. Two binary parameters related to interactions between CO_2 and ethyl esters were used.

INTRODUCTION

Production of long chain polyunsaturated fatty acids (PUFA) of the ϖ-3 series has become a very important issue because of their increasing commercial interest in recent years. In fact, since many researches have pointed out that the typical Western diet is characterised by a high ratio ϖ-6/ϖ-3 that leads to several health diseases [1], production of foods artificially enriched in ϖ-3 and dietary supplements based on ϖ-3 have concerned food and nutraceutical industries. Furthermore, ϖ-3 PUFA are under investigations for their potential application in the pharmaceutical industry because they have been proved to have a pharmacological action with respect to several health diseases. From this point of view, the eicosapentaenoic acid (EPA) and the docosahexaenoic acid (DHA) are the focus of many clinical and biochemical researches. One of their most promising fields of application is the treatment of some cardiovascular diseases, as it was recently confirmed by a clinical study on this subject [2].
The main source of EPA and DHA are mixtures of ethyl esters derived from transesterification of fish oils. EPA and DHA enrichment of these mixtures can be obtained by conventional processes (such as vacuum distillation, urea crystallisation and hexane extraction) but they are liable to produce low quality products because of PUFA thermal degradation [3] or because of the presence of some residual solvent in the final product. On the other hand, it has been shown that supercritical carbon dioxide is an effective solvent for ethyl esters, showing a selectivity that mainly depends on the length of the carbon chain [3,4]. Due to the demand of high quality products, fractionation by means of supercritical carbon dioxide appears to be more suitable than conventional processes because of the low temperatures required and the complete solvent separation from the final product that can be achieved.
In this work, a semi-batch fractionation of a synthetic mixture of fatty acids ethyl esters (FAEE) was performed by means of supercritical carbon dioxide. The data obtained were used to check the capability of the Peng and Robinson equation of state (PREOS) for simulating the supercritical process. This study, focused on the thermodynamic characterisation of a synthetic

feed and on the modellization of a single-stage process, is intended as an intermediate step in order to develop a simulation of a multistage process on a natural feed derived from fish oils.

MATERIALS AND ANALYSIS

The mixture used as a feed was made from myristic acid ethyl ester (EE-C14:0, Fluka Chemika), palmitic acid ethyl ester (EE-C16:0, Sigma Aldrich), stearic acid ethyl ester (EE-C18:0, Sigma Aldrich), EPA and DHA ethyl esters (K.D.-Pharma). The saturated esters had a purity higher than 99%, while EPA and DHA had a purity higher than 90% and 70% respectively. The major impurities of the two ϖ-3 products were identified, by means of mass spectrometry, and their mass fraction was evaluated by means of the GC-analysis. The feed oil was prepared in order to reproduce a typical product derived from ethyl transesterification of sardine oil. Feed composition, evaluated by GC analysis, was: EE-C14 5.3 %; EE-C16 27.6 %; EE-C18 23.0 %; EE-C20 25.9 %; EE-C22 18.2 %. Carbon dioxide used in this work had a purity higher than 99.9 % (Siad, Italy).
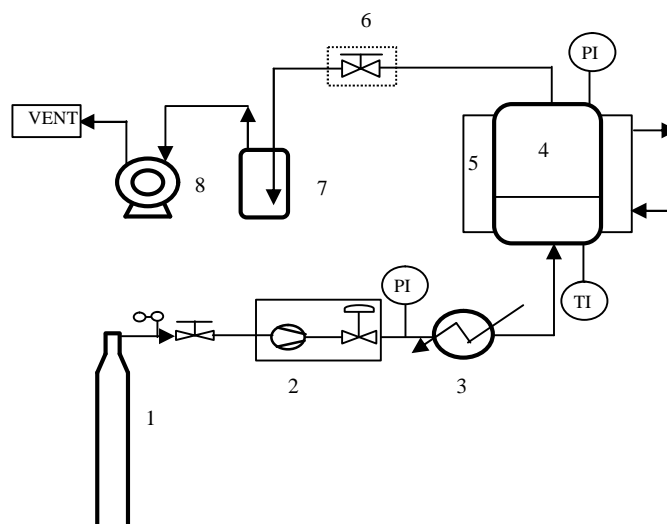
The analyses of the samples were performed by means of a Hewlett Packard Gas Chromatograph 580 equipped with a capillary column type EC5 (Alltech, 30 m length, 0,32 mm ID, 1.0 μm film thickness). The analytical procedure consisted in 1 μl injections of samples previously diluted in ethanol at a ratio of 10 g of ethanol per 1 g of sample. The split ratio was fixed at 60:1. The temperature program was: 200 °C, 4 min. constant; 6 °C/min. to 250 °C; 250 °C, 23 min. constant; 6 °C/min. to 280 °C; 280 °C, 6 min. constant. A F.I.D. detector was used at a temperature of 300 °C. Helium at a flow rate of 2 ml/min was used as a carrier gas and the injection temperature was fixed at 250 °C. The composition of the streams was calculated lumping different components of the oil in five classes, on the basis of the chain length: EE-C14, EE-C16, EE-C18, EE-C20, EE-C22.

EXPERIMENTAL APPARATUS AND RESULTS

A diagrammatic scheme of the apparatus used to perform the experimental runs is reported in Figure 1. The batch extractor (4) (volume 200 cm^3, i.d. 40 mm) was initially charged with 10 g of feed oil. In order to reach phase equilibria conditions during the fractionation, the contact time and the contact surface between the solvent and the oil were increased by filling the extractor with a glass random packing (filled volume 60 cm^3). Carbon dioxide was fed to the extractor, keeping the expansion valve (6) closed, until the operational pressure was reached. The

Figure 1: Experimental apparatus

temperature inside the extractor was maintained at the operational value through a heating jacket (5). In order to have an equilibrium state before starting the dynamic fractionation, the extractor was kept closed for about 16 h. Subsequently the expansion valve (6) was opened and the solvent started to flow through the system. Carbon dioxide, stored in the cylinder (1), was compressed by means of a volumetric compressor (2), and then was passed through a pre-heater (3), in order to reach the extractor temperature before entering it. By regulating the

opening of the valve (6) and the compressor ratio, the extractor pressure and the solvent flow rate were maintained at the desired values. Carbon dioxide flow rate was measured by the flow meter (8) and maintained at 0.4 l/min (measured at 25°C and 1 atm) for all the experimental runs. The high pressure vapour phase leaving the extractor was expanded through the valve (6) in order to release the solute, which was recovered in the separation vessel (7). The expansion valve (6) was provided with a heating system to avoid solidification. The oil collected in a certain interval of time was withdrawn from the vessel (7), weighed and analysed. Experimental runs continued until the feed was completely extracted.

Table 1: Experimental and calculated (in round brackets) average selectivity for each run.

T [°C]	P [MPa]						
	9.0	10.0	11.0	12.0	13.0	14.0	16.0
42	2.1 (2.9)	1.4 (1.5)	1.0 (1.0)	-	-	-	-
50	-	-	2.1 (2.3)	1.5 (1.7)	-	1.1 (1.1)	-
60	-	-	-	-	2.1 (2.3)	1.5 (1.9)	1.2 (1.3)

Figure 2: Mass of oil extracted as a function of the solvent passed through the extractor.
50°C (A):*11.0 MPa;? 12.0 MPa;o14.0 MPa. **60°C (B)**: *13.0 MPa;? 14.0 MPa; o16.0 MPa.
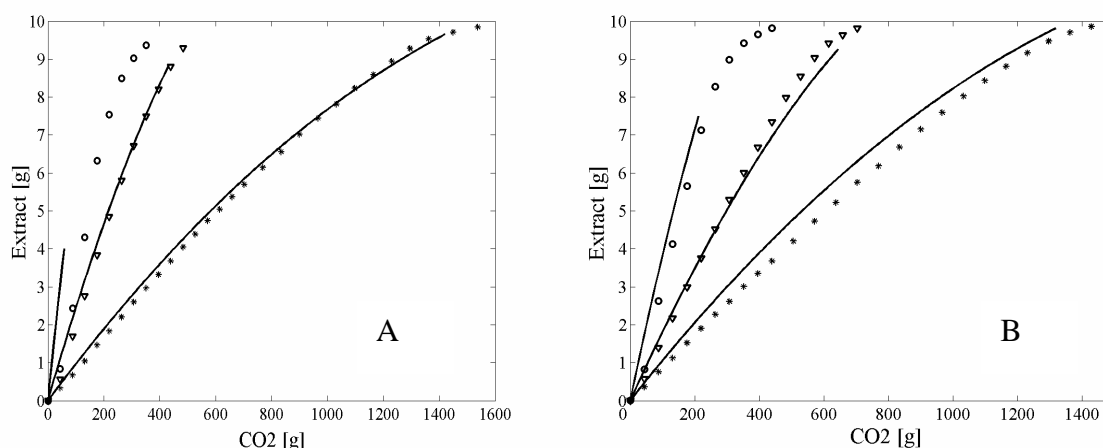

Table 1 reports the operational parameters of the experimental runs that were performed. Figure 2, referred to the experimental runs at 50 °C and 60 °C, shows the mass of oil extracted as a function of the total amount of solvent passed through the extractor. The effect of pressure and temperature is clearly showed: at fixed temperature, when pressure increases, oil solubility increases. On the contrary, at fixed pressure, when temperature increases, oil solubility decreases. The typical behaviour of vapour composition as a function of the extraction degree *A* is shown in figure 3A. Lighter components, such as EE-C14 and EE-C16, are preferentially extracted, whereas EE-C20 and EE-C22 are concentrated in the raffinate. As for EE-C18, its separation from heavier components is more difficult since this component tends to be distributed between the two phases.

From the experimental determinations of extracted mass and extract composition, it was possible to evaluate, by means of a material balance, the mass of oil components inside the extractor as a function of time. The typical behaviour of their mass decrease inside the extractor is shown in figure 3B. In order to select optimal operational conditions to perform ethyl esters fractionation by means of carbon dioxide, a selectivity was defined as:

$$S(A) = \frac{Y_l(A)/X_l(A)}{Y_h(A)/X_h(A)} \qquad (1)$$

Figures 3: (A) vapour mass fraction (on solvent free basis) as a function of the extraction degree at 60 °C and P = 13 MPa. (B) ethyl esters mass inside the extractor as a function of time at 50 °C and P = 11 MPa. (?: EE-C14; ? : EE-C16; ? : EE-C18; o : EE-C20; *: EE-C22)
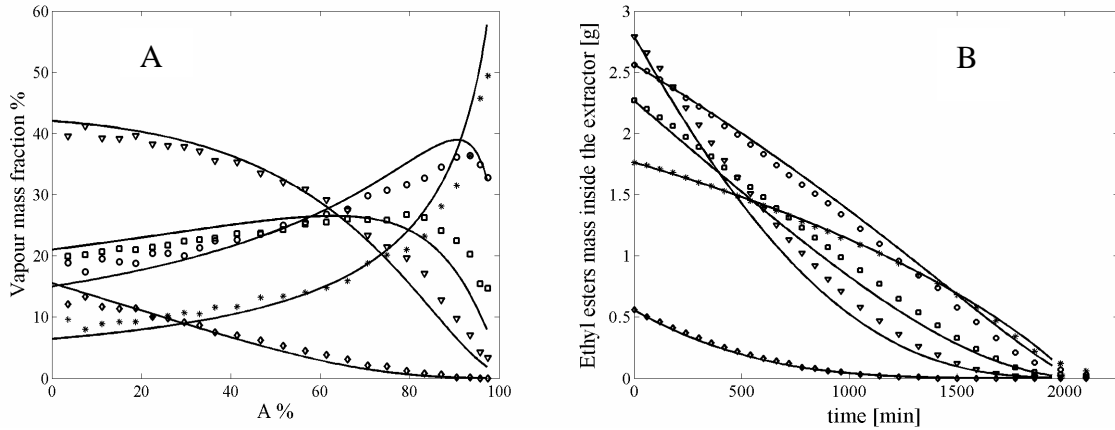
were Y_l, X_l are the vapour and liquid mass fraction of light components (here defined as EE-C14 + EE-C16 + EE-C18) while Y_h, X_h are the analogous quantities for heavy components (EE-C20 + EE-C22). Average selectivities were calculated for each experimental run and reported in Table 1. For each temperature, a selectivity very close to 1 was obtained at the highest pressure, corresponding to an absence of enrichment in the raffinate. It is likely that in these conditions an homogeneous phase is formed for some value of *A* during the extraction process. At these pressures or above no separation can be obtained. For each temperature, best selectivities are obtained at the lowest pressure. However, to obtain high selectivities at 42 °C it is necessary to perform the process at a pressure that gives lower solubilities. It is apparent that the best compromise between solubility and selectivity can be found at 50 °C and 60 °C.

MODELLIZATION
The dynamic behaviour of the semicontinuous operation was simulated assuming the system composed of six components (CO_2 – EEC14:0 – EEC16:0 – EEC18:0 – EE-EPA – EE-DHA) and assuming equilibrium conditions between the liquid and the vapour phases. The mass decrease of oil components inside the extractor was calculated from the balance differential equation:

$$\frac{dN_i}{dt} = -F_{out} y_i \qquad (2)$$

where F_{out} is the outlet mass flow rate, y_i represents the mass fraction in the outlet stream and N_i stands for the mass of component *i* inside the extractor. N_i is given by:

$$N_i = L_i + V_i \qquad (3)$$

where L_i and V_i stands for the mass of component *i* in the liquid and the vapour phase respectively. Since it was assumed the perfect mixing of the vapour phase inside the extractor,

the composition of this phase is equal to that one in the outlet stream. Vapour mass fractions were linked to the liquid mass fraction from the equilibrium condition:

$$y_i = \frac{\bar{\boldsymbol{f}}_i^L}{\bar{\boldsymbol{f}}_i^V} x_i \tag{4}$$

Furthermore, the condition about the extractor fixed volume was imposed:

$$V_{extr} = \left(\sum_{i=1}^{6} V_i \right) \cdot \hat{V}_V + \left(\sum_{i=1}^{6} L_i \right) \cdot \hat{V}_L \tag{5}$$

Fugacity coefficients and specific volumes were calculated by means of PREOS with van der Waals mixing rules for the parameters a_m and b_m. In the mixing rules it was assumed:

$$a_{ij} = a_i \quad \text{for } i = j; \qquad a_{ij} = \sqrt{a_i a_j}(1 - k_{ij}) \quad \text{for } i \neq j \tag{6}$$

$$b_{ij} = b_i \quad \text{for } i = j; \qquad b_{ij} = \frac{b_i + b_j}{2}(1 - \boldsymbol{h}_{ij}) \quad \text{for } i \neq j \tag{7}$$

Therefore, two binary interaction parameters (k_{ij} and $\boldsymbol{h}_{ij}$) were included in the mixing rules. Pure component parameters (a_i and b_i) were calculated from critical properties and acentric factors. Critical values of fatty acids ethyl esters were estimated by a group contribution method [5] while acentric factors were calculated by the Lee-Kesler method, reported in [6]. Since it was also necessary to estimate normal boiling temperatures of FAEE, because no experimental data are available for most of them, a new group contribution method was adopted [7]. These data are reported in Table 2. Binary interaction parameters (k_{ij} and $\boldsymbol{h}_{ij}$) between CO_2 and ethyl ester j were obtained from correlation of experimental data reported in the literature [8-10] and are listed in table 3. On the contrary, binary parameters of interaction between ethyl ester i and ethyl ester j were neglected because these components show similar thermodynamic behaviour.

Table 2: Normal boiling point temperatures, critical properties and acentric factors used in the modellization.

Compound	T_b [K]	T_c [K]	P_c [MPa]	ϖ
EE-C14:0	577.4	735.8	1.454	0.855
EE-C16:0	603.8	757.9	1.315	0.928
EE-C18:0	628.4	778.2	1.200	0.998
EE-EPA	659.8	817.1	1.269	1.046
EE-DHA	682.7	837.3	1.194	1.108
CO_2	194.7	304.2	7.376	0.225

DISCUSSION

Process simulation, developed with interaction parameters obtained from literature binary data fitting, gave good results at 50 °C, but failed to represent the process at different temperatures. In order to improve the modellization, binary interaction parameters k_{ij} were allowed to vary with temperature while $\boldsymbol{h}_{ij}$ were kept constant. The necessity to introduce temperature

dependent interaction parameters was also pointed out in another work on this subject [11]. Therefore, interaction parameters $k_{ij}'(T)$ were introduced and it was assumed:

$$k_{ij}' = k_{ij} + q_{ij}(T - 323.15) \qquad (8)$$

where k_{ij} are determined as explained above, whereas q_{ij} were calculated by experimental data obtained in this work. Table 3 shows values of k_{ij}, h_{ij} and q_{ij} used to perform the modellization.

Table 3: Values of the binary parameters k_{ij}, h_{ij} and q_{ij} used for the modellization.

Parameter	EE-C14	EE-C16	EE-C18	EE-EPA	EE-DHA
k_{ij}	0.0572	0.0560	0.0510	0.0510	0.0550
h_{ij}	0.0000	0.0300	0.0670	0.0200	0.0120
q_{ij}	0.00000	0.00185	0.00177	0.00127	0.00186

Figures 2-3 show some simulation results, obtained with parameters reported in Table 3, together with the corresponding experimental data. The continuous lines, referred to simulations, stop when an homogeneous phase is predicted. The behaviour of extract composition as a function of A is well represented. Furthermore, the model correctly predicts the absence of separation at higher pressures: in these cases an homogeneous phase is predicted at low values of A, which is consistent with selectivity values very close to 1 that were found in the corresponding experimental runs. Table 1 also reports average selectivity data calculated by the model: as it can be seen, they are in good agreement with the experimental data. The oil solubility, shown in figure 2 for the runs at 50 and 60 °C, and the decrease of oil components inside the extractor, shown as an example in figure 3B, were also well predicted except for the simulation at 42 °C and 9.0 MPa, which gave approximately half solubility than the experimental one. Since vapour composition and selectivities are well represented by the model, the relative solubility of different ethyl esters is well predicted. This means that neglecting binary parameters of interaction between ethyl ester i and ethyl ester j is not an oversimplification. On the contrary, further improvements could be obtained for the prediction of the oil solubility at different temperatures by means of a thermodynamic model that requires only parameters referred to pure components and binary mixtures.

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