J Amer Oil Chem Soc (2007) 84:597–601
DOI 10.1007/s11746-007-1071-3

ORIGINAL PAPER

Selectivity of Supercritical CO_2 in the Fractionation of Hake Liver Oil Ethyl Esters

Iván Jachmanián · Lucía Margenat · Ana I. Torres · Maria A. Grompone

Received: 11 December 2006 / Revised: 19 March 2007 / Accepted: 1 April 2007 / Published online: 15 May 2007

Abstract The solubility of different ethyl esters derivatized from hake liver oil in supercritical carbon dioxide was studied. A selectivity factor was used to determine optimal conditions to fractionate the ethyl ester mixture. A strong influence of solvent pressure and temperature was observed within8.63–18.04 MPa and 40–70 °C. The lowest total solubility of the ethyl ester mixture was obtained when using supercritical carbon dioxide at the lowest density (the lowest pressure and the highest temperatures value tested). The highest discrimination against long-chain polyunsaturated fatty acids (e.g. EPA and DHA) was also obtained at these above conditions. Conversely, higher solubility and lower selectivity were obtained when solvent density increased. Considering this inverse correlation between selectivity and solubility, a single-step batch-fractionation process was designed to increase the 22:6 ethyl ester content from an initial value of 17.5% in the starting material to 55% in the final extract.

Keywords Supercritical fractionation · Ethyl esters fractionation · Hake liver oil · Fish oil · *Merluccius hubbsi* · DHA enrichment

I. Jachmanián (✉) · L. Margenat · A. I. Torres · M. A. Grompone
Laboratorio de Grasas y Aceites,
Departamento de Ciencia y Tecnología de Alimentos,
Facultad de Química, Universidad de la República,
Avda. Gral. Flores 2124, Casilla de Correo 1157,
11800 Montevideo, Uruguay
e-mail: ijachman@fq.edu.uy

Introduction

Several beneficial effects on prevention and therapy of certain diseases, mainly coronary disorders, have been attributed to an increased intake of n-3 polyunsaturated fatty acids, such as EPA (eicosapentaenoic acid, 20:5) and DHA (docosahexaenoic acid, 22:6), usually found in significantly high concentrations in oils of marine origin.

Different conventional processes, like vacuum distillation, urea complexation or hexane extraction, have been used to fractionate fish oils, focused on isolating a fraction enriched in these fatty acids. Such methods usually entail handling the raw material at high temperatures, thus promoting degradation of products of interest, in addition to hazards associated with the use of toxic and flammable organic solvents.

The use of supercritical solvents, CO_2 in particular, has been considered to be an attractive alternative to conventional methods, as it can be efficiently managed at mild temperatures without exposing the material to oxygen, thereby preserving the integrity of the polyunsaturated fatty acids of interest and avoiding solvent residues in the final product [1].

Much research has been reported on using supercritical CO_2 ($SCCO_2$) to isolate fish oil fractions enriched in n-3 polyunsaturated fatty acids [2–4]. Data have been reported on the partition coefficients and the solubility of fatty acid ethyl esters (FAEEs) obtained from different oils of marine origin [3], with or without the use of co-solvents [4].

Previous data reported on FAEEs solubility in supercritical CO_2 showed that solubility increased with solvent density. Staby and Mollerup [1], working with a mixture of FAAEs obtained from sand lauce oil, obtained solubilities between 0.51 and 257 mg/g CO_2, corresponding to solvent densities from 90 to 920 kg/m^3, respectively. Liang and

Yeh [5] determined the solubility of a modelled fish-like mixture of FAEEs, which varied from 1.7 to 121 mg/g CO_2, for solvent densities of 342 and 723 kg/m^3, respectively. Although some general similarities can be found among results reported in the literature, data concerning individual solubility and solvent selectivity differ for each fatty acid ethyl ester when comparing results obtained for oils from different sources [6], accordingly the behaviour of individual components has been suggested to depend on the overall mixture composition [3]. Since oil composition affects these parameters, new solubility and selectivity data must be obtained when a new raw material is studied, in order to ensure the proper design of a supercritical fractionation method.

Hake (*Merluccius hubbsi*), the major product processed by the Uruguayan fish industry, is a fishery resource distributed in the River Plate and southern Atlantic Ocean. Hake livers may vary in size from 5 to 10% of the fish weight, with oil content between 41 and 60%. Depending on the season, the DHA and EPA contents vary between 13 and 18% and between 5 and 9%, respectively [7]. Unlike most marine oils commercialized in the United States and Europe, hake liver oil has a high DHA/EPA ratio (between 1.8 and 2.5) [8]. No previous results have been reported on the selectivity of $SCCO_2$ in the fractionation of an ethyl ester mixture derivatized from this oil.

In the present work, the solubility of the mixture of FAEEs obtained from hake liver oil in $SCCO_2$ and the selectivity of the solvent were studied over a range of conditions.

The conditions resulting in a high selectivity were used to evaluate the efficiency of a batch-fractionation process designed to isolate a fraction enriched in n-3 polyunsaturated fatty acids family.

Experimental Procedures

Fish Oil

Vípez, a pharmaceutical formulation for human consumption, was provided by Laboratorio Landasur S.A. (Montevideo. Uruguay) and was used as the source of hake liver oil. This product was composed of pure hake liver oil enriched with vitamin E.

Hake Liver Oil Ethyl Esters

The fish oil was derivatized to the corresponding ethyl esters by acid-catalysis transesterification, by adding 10 ml of 100:4 (v/v) ethanol/H_2SO_4 to 2 g of oil and stirring the mixture and heating at 80 °C for 120 min in closed screw-cap tubes under nitrogen. After the reaction was completed, 5 ml of NaCl 5% were added and the ethyl esters were recovered by four extractions with 4 ml of hexane. The extracts were combined, washed several times with distilled water until neutrality and dried under a nitrogen stream.

Sample Preparation

Hake oil ethyl esters were supported on ground glass (100–150 mesh). 2 g of ethyl esters and 20 g of ground glass were placed inside a 125 ml round-bottom flask, 10 ml of dichloromethane were added and the mixture was mixed using magnetic stirring for 1 min. The solvent was removed by using a nitrogen stream and then under vacuum using a rotary evaporator until constant weight was achieved. The sample was finally transferred to the extraction cell.

Supercritical Extraction System

Supercritical extractions were performed in a dynamic-type system built in the laboratory (Fig. 1), equipped with a 300-ml capacity syringe pump (Thar Technologies, SP300-2), a 25-ml capacity extraction cell (Thar Technologies, 25 ml-ph) placed inside a temperature-controlled oven, a shutoff valve, a heated micro-metering valve (Sitec, 1-mm orifice), and a separation unit and a filtration unit (filled with glass wool) upstream of the flow meter and totalizer (Alborg, GMF 171).

Supercritical Extraction

The 25-ml extractor (E) was charged with 18 g of sample prepared as described above (which contained 1.6 g oil and 16.4 g ground glass). Once the oven temperature was adjusted, CO_2 was pumped until reaching the desired operation pressure. The system was kept at these conditions for 15 min before starting the extraction. Extractions started when the shutoff valve (V_2) was opened and the CO_2 flow was adjusted to between 0.6 and 0.8 g/min using the

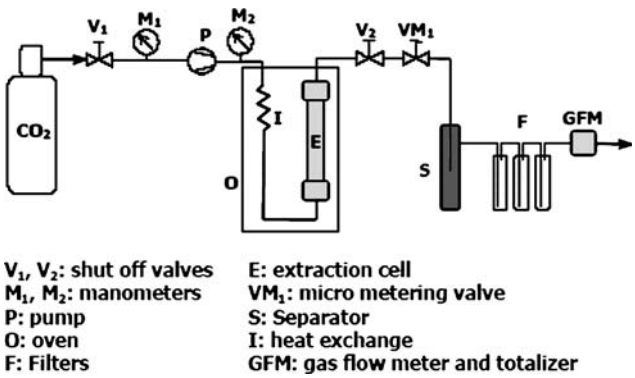

Fig. 1 Diagram of the extraction equipment

micrometering valve (VM_1). Different samples of extracted ethyl esters were collected after different extraction periods in vials located inside the separation unit (at room temperature), and their weights and compositions were determined. The CO_2 flow and the total amount of CO_2 corresponding to each extraction period were measured by the flow meter and totalizer (GFM). Extractions used for solubility determination were performed during short periods, in order to extract less than 30% of the esters. Longer extractions were performed for fractionation experiments, in order to extract more than 70% of esters Extractions were performed in triplicate at each condition, and mean results and standard deviations were determined.

Compositions of the Ethyl Ester Samples

The ethyl ester samples were directly analyzed by capillary gas chromatography, using a GC Shimadzu GC-14B, equipped with a FID detector and a capillary column Supelco SP 2330 (25 m × 0.5 mm × 0.25 mm). The temperature program started at 160 °C for 1 min, followed by a heating step (4 °C/min to 230 °C), and then held for 10 min. Nitrogen was used as carrier gas, at 40 kPa at the column head with a split ratio of 1:80. Peaks were identified and quantified by using standards and corresponding response factors.

Results and Discussion

Solubility

The masses of samples extracted after different periods were plotted against the masses of CO_2 pumped through the extraction cell (Fig. 2). The resulting straight line suggested that the solvent was saturated with substrate at the outlet of the extraction cell and that no mass transfer effects controlled the process, since curve plots are characteristic of a process controlled by mass transfer phenomena. This result was consistent with the low CO_2 flow used in the test and the low percentage of extracted material. Therefore, this procedure was used to determine the solubility at each extraction condition. The total solubility of hake liver oil ethyl esters was determined by the slope of the corresponding plot (S_T, in mg of ethyl ester/g of solvent). The lowest S_T value of 2.3 mg/g (Table 2) was obtained at 8.63 MPa pressure and 40 °C (density = 382.6 kg/m^3). Higher pressures and lower temperatures resulted in higher S_T values, with increased CO_2 density. The highest S_T value of 102.0 mg/g was obtained at 18.04 MPa pressure and 50 °C, corresponding to the maximum CO_2 density used in the test (757.9 kg/m^3). Between both extremes, a regular increase was observed

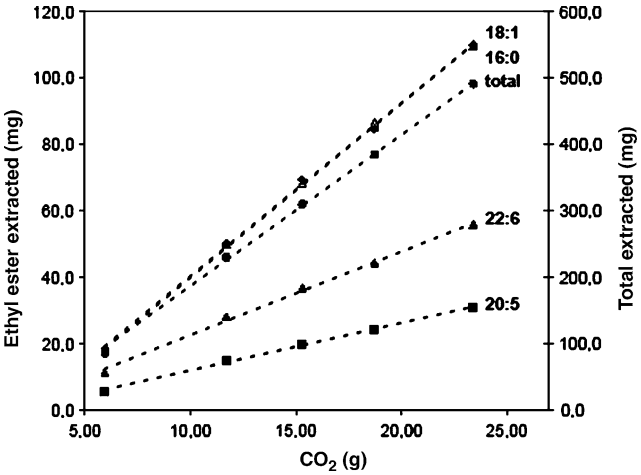

Fig. 2 Example of total extracted mass and mass of an individual component as function of solvent mass used in one extraction (performed at 70 °C and 16.16 MPa). Plot slope represents the solubility of each fatty acid ethyl ester: total (*filled circle*), 16:0 (*triangle*), 18:1 (*filled diamond*), 20:5 (*filled square*), 22:6 (*filled triangle*)

for the dependence of S_T on solvent density (Fig. 3). S_T values reported in the present work were similar to those previously reported for fish oils of different origin [1, 5, 9] (Table 1).

The composition of each extracted sample was analyzed by gas chromatography and similar plots were constructed (Fig. 2) for each ethyl ester. The solubility (S_i) of each individual ethyl ester (i) was also determined as the slope of the corresponding plot.

The individual solubilities of the different ethyl esters are also shown in Table 2. The solubilities of all compounds increased gradually with solvent density. Considering

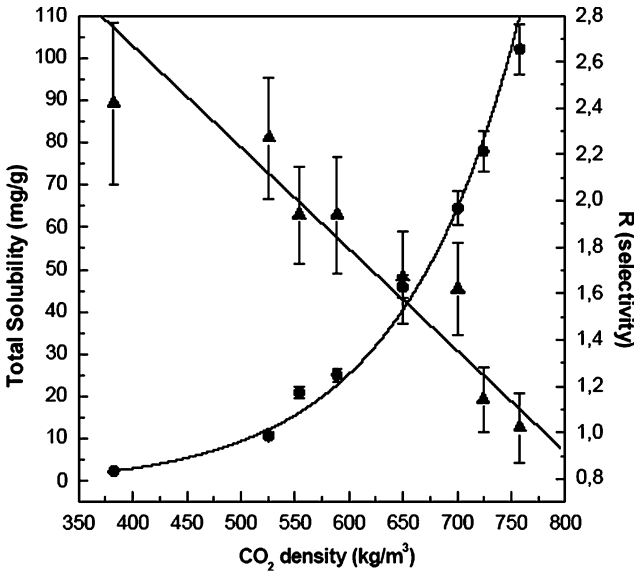

Fig. 3 Dependence of total solubility (S_T, *filled circle*) and selectivity (R, *filled triangle*) on $SCCO_2$ density

Table 1 Composition of the ethyl esters from hake liver oil (components with weight percentage higher than 1%)

Fatty acid ethyl ester	14:0	16:0	16:1	18:0	18:1	18:2	18:3	18:4	20:1	20:5	22:1	22:6
Starting material (wt%)	4.3	16.8	6.2	1.3	19.5	2.0	1.9	2.7	3.4	7.6	3.0	17.5

Table 2 Total solubility (S_T) and individual solubility (S_i) of the main fatty acid ethyl esters obtained from hake liver oil under different conditions

Experimental errors in solubility values are shown as '' ± (standard deviation)''

CO_2 density (kg/m^3)	Pressure MPa	Temperature (°C)	S_T (mg/g)	S_i (mg/g)			
				16:0	18:1	20:5	22:6
382.6	8.63	40	2.3 ± 0.1	0.57 ± 0.02	0.42 ± 0.02	0.13 ± 0.01	0.17 ± 0.01
525.9	13.34	60	10.6 ± 0.5	2.48 ± 0.15	2.53 ± 0.10	0.62 ± 0.03	1.00 ± 0.03
554.0	16.16	70	20.9 ± 1.2	4.79 ± 0.25	4.83 ± 0.25	1.32 ± 0.05	2.31 ± 0.10
589.0	13.34	55	25.0 ± 1.5	5.37 ± 0.25	4.41 ± 0.20	1.41 ± 0.06	2.29 ± 0.10
649.6	13.34	50	45.9 ± 2.7	9.28 ± 0.50	10.8 ± 0.4	3.06 ± 0.11	5.78 ± 0.30
700.6	11.45	40	64.4 ± 4.0	13.1 ± 0.5	15.1 ± 0.6	4.32 ± 0.20	8.42 ± 0.44
724.0	18.04	55	77.9 ± 4.9	12.4 ± 0.6	16.9 ± 0.7	5.51 ± 0.21	13.4 ± 0.4
757.9	18.04	50	102.0 ± 6.0	17.5 ± 0.7	19.2 ± 0.8	8.01 ± 0.32	18.3 ± 0.7

the solubilities corresponding to major mixture components, both 16:0 and 18:1 (the shortest and more saturated components) showed the highest solubility at each condition tested. While the solubilities of 20:5 and 22:6 ethyl esters were very low at the lowest solvent density, 0.13 and 0.17 mg/g (CO_2 density = 382.6 kg/m^3), respectively, solubilities increased significantly with solvent density. For the highest CO_2 density (757.9 kg/m^3), the solubility of 22:6 ethyl ester achieved the maximum value, 18.3 mg/g, which was practically equal to that obtained at the same conditions for 16:0 and 18:1 ethyl esters: (17.5 and 19.2 mg/g, respectively). Conversely, 20:5 ethyl ester solubility remained low, 8.01 mg/g.

Selectivity

In order to compare the relative differences in solubility between the different FAEEs and to evaluate the selectivity of the solvent, the parameter R previously defined by Liang and Yeh [5] was determined. Thus, the ratios between the total concentration of target compounds (20:5 and 22:6 ethyl ester) and the total concentration of other major ethyl esters (16:0 and 18:1) were evaluated for both the extracted mixture and the starting ethyl ester mixture. Defined as relative separation efficiency, R was calculated according to the following equation:

$$R = \frac{\left(\frac{P_{16:0}+P_{18:1}}{P_{20:5}+P_{22:6}}\right)_e}{\left(\frac{P_{16:0}+P_{18:1}}{P_{20:5}+P_{22:6}}\right)_o}$$

where P_i was the weight percentage of the ethyl ester of fatty acid ''i'', determined by GC as described above, and the sub-indices ''e'' and ''o'' indicated that the corresponding expression must be evaluated considering the composition of the extracted sample or the composition of the original mixture, respectively. Concerning the efficiency of a separation process, a selectivity coefficient is a more relevant parameter than the solubility itself.

In Fig. 3, R values determined at different temperature and pressure conditions were plotted against the corresponding $SCCO_2$ density. R values higher than 1 were obtained under conditions resulting in low solvent densities (corresponding to high temperature and low pressure). The selectivity parameter (R) had a maximum value of 2.42 for a $SCCO_2$ density of 382.6 kg/m^3 (8.63 MPa and 40 °C). An R value higher than 1 means that the extracted material was more concentrated in esters of fatty acids 16:0 and 18:0 than the raw material, indicating that the solvent showed some discrimination against the esters of polyunsaturated fatty acids 20:5 and 22:6.

As shown in Fig. 3, as the density of $SCCO_2$ was increased by increasing the pressure and reducing the temperature, the selectivity parameter R approached 1. According to the definition of R, a value near 1 means that the composition of the extract and that of the raw material were similar, and that no significant selectivity was observed.

Therefore, for the lowest $SCCO_2$ densities, high solvent selectivity was found for ethyl esters of the shorter and more saturated fatty acids (16:0 and 18:1) as opposed to ethyl esters of the longer, polyunsaturated fatty acids (20:5 and 22:6). The above selectivity was less significant as solvent density increased.

Overlapped with R, Fig. 3 also shows total solubility (S_T) values. An inverse dependence on solvent density was

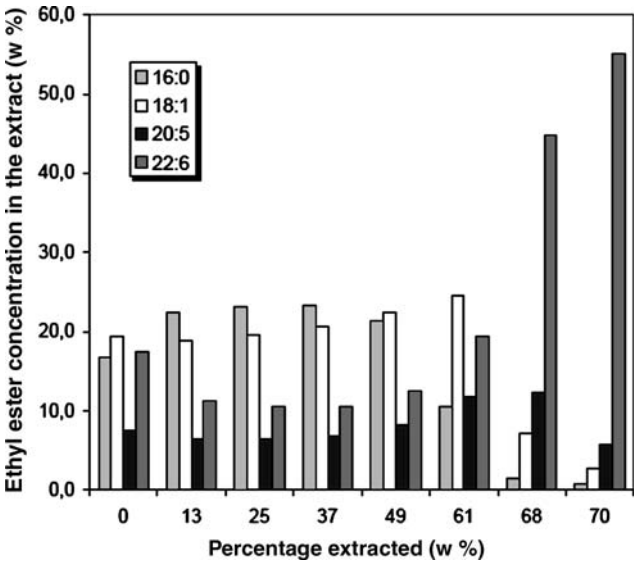

Fig. 4 Weight percentage of the major ethyl esters in the extracts obtained during the progress of long-period supercritical extraction performed at the most selective conditions (8.63 MPa and 40.0 °C): 16:0 *light gray bar*, 18:1 *white bar*, 20:5 *dark gray bar*, 22:6 *gray bar*

found for both parameters. Where selectivity was the highest the solubility was the lowest, and vice versa. These results are in agreement with results previously reported by Borch-Jensen and Mollerup [9] for the dependence of the partition coefficient of fatty acid ethyl esters on the CO_2 pressure. They found that isothermal increasing in solvent pressure caused the partition coefficients of the different FAEEs to converge to a value near ''1'', which actually means a reduction in solvent selectivity, which was consistent with our results.

Fractionation

Figure 4 shows results obtained for long extraction periods at the highest selectivity conditions (8.63 MPa and 40.0 °C). Note that the concentration of 22:6 ethyl ester in the extracted material remained relatively low through the extraction, increasing significantly towards the end of the period. When 70% of the mixture was extracted, the extract was composed of 55% 22:6 ethyl ester. In contrast, the concentration of both 18:1 and 16:0 ethyl esters were lower for long extraction periods than at the beginning of the extraction. Moderate enrichment was observed for 20:5 ethyl ester, from a 7.6% in the starting mixture to a maximum 12.2 % in the extracted material and decreasing after 68% of the sample was extracted.

According to our results, some discrimination against the target ethyl esters (20:5 and 22:6) was achieved at operating conditions that resulted in a high selectivity of the solvent. Discrimination against 22:6 was clearly higher, probably due to its higher carbon number and unsaturation. Therefore, a single batch process for the enrichment in 20:5 and 22:6 ethyl esters must involve the selective extraction of the shorter chain and more saturated FAEEs, such as 16:0 and 18:1, at the beginning of the extraction period. At long extraction periods the extract becomes richer in the longer and highly unsaturated ethyl esters.

Selecting preferred operating parameters, the mixture of the ethyl esters obtained from hake liver oil may be efficiently fractionated using supercritical CO_2 to obtain a fraction rich in long chain polyunsaturated fatty acid esters.

Acknowledgments This work was supported by a research grant from the Comisión Sectorial de Investigación Científica CSIC, Universidad de la República, Montevideo, Uruguay.

References

1. Staby A, Mollerup J (1993) Solubility of fish oil fatty acids ethyl esters in sub and supercritical carbon dioxide. J Am Oil Chem Soc 70:583–588
2. Catchpole OJ, Grey JB, Noermark KA (2000) Fractionation of fish oils using supercritical CO_2 and CO_2–ethanol mixtures. J Supercritical Fluids 19:25–37
3. Riha V, Brunner G (1999) Phase equilibrium of fish oil ethyl esters with supercritical carbon dioxide. J Supercritical Fluids 15:33–50
4. Fleck U, Tiegs C, Brunner G (1998) Fractionation of fatty acid ethyl esters by supercritical CO_2: high separation efficiency using an automated countercurrent column. J Supercritical Fluids 14:67–74
5. Liang JH, Yeh AI (1991) Process conditions for separating fatty acids esters by supercritical CO_2. J Am Oil Chem Soc 68:687–692
6. Nilsson WB, Seaborn GT, Hudson JK (1992) Partition coefficients for fatty acid esters in supercritical fluid CO_2 with and without ethanol. J Am Oil Chem Soc 69:305–308
7. Méndez E (1997) Seasonal changes in the lipid classes and fatty acid compositions of hake (*Merluccius hubbsi*) liver oil. J Am Oil Chem Soc 74:1173–1175
8. Grompone MA (1992) Aceites de pescado de interés nacional. Ing Quím 3:14–19
9. Borch-Jensen C, Mollerup J (1999) Phase equilibria of long-chain polyunsaturated fish oil fatty acid ethyl esters and carbon dioxide, ethane, or ethylene at reduced gas temperatures of 1.03 and 1.13. Fluid Phase Equilibria 161:169–189