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Supercritical Carbon Dioxide Fractionation of Nonesterified Alkoxyglycerols Obtained from Shark Liver Oil

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Ethanolysis of shark liver oil was carried out to generate a product enriched in nonesterified alkoxyglycerols and fatty acid ethyl esters. For the present study, the original oil contained very low amounts of squalene, and thus, unsaponifiable matter was mainly constituted by nonesterified alkoxyglycerols (NEAKG). A small percentage of monoesterified alkoxyglycerols (MEAKG) was also detected. Supercritical fluid extraction was employed to fractionate the mixture, achieving a complete elimination of esters and concentrating the alkoxyglycerol compounds in the raffinate product. Extractions were carried out in a countercurrent packed column, using extraction pressures in the range of 140−180 bar, temperatures from 45 to 65 °C, and a solvent-to-feed ratio of 15. NEAKG + MEAKG purity obtained in the raffinate at the best extraction conditions was around 78% w/w, and satisfactory yield (>60%) was also achieved. Therefore, the raffinate product can be re-esterified to design highly valuable ether lipid compounds.

KEYWORDS: Countercurrent extraction; supercritical fluids; alkoxyglycerol; ether lipids

INTRODUCTION

Ether lipids or alkoxyglycerols have been the subject of much attention because of their special physiological functions in humans (*1, 2*). Anticarcinogenic and immune stimulators properties have been attributed to dietary ingestion of these substances (*3, 4*). They are located in the human body mainly in the cells of the immune system and in higher doses in human breast milk. In some circumstances, the endogenous synthesis is reduced and the oral administration of alkoxyglycerols is recommended (*5, 6*).

Shark liver oil is a natural source of diesterified alkoxyglycerols (DEAKG), together with squalene and triacylglycerols. **Figure 1** compares the chemical structure of alkoxyglycerols and acylglycerols. Research has demonstrated that polyunsaturated fatty acids (PUFA) have specific physiological effects in humans (*7*). Shark liver oils contain relatively high amount of long chain PUFA residues (10–15% of fatty acid residues are PUFA) (*8, 9*), especially 5,8,11,14,17-eicosapentaenoic (EPA, C20:5) and 4,7,10,13,16,19-docosahexaenoic (DHA, C22:6) acids. The numerous health-promoting effects of EPA and DHA in human subjects have been recently reviewed (*10*).

Nonesterified alkoxyglycerols (NEAKG) can be obtained from commercial shark liver oil via saponification or ethanolysis reactions. Recently, our research group has been able to isolate NEAKG via saponification of shark liver oil (*11*). The transesterification reaction converts triacylglycerols to the corresponding fatty acid ethyl esters and DEAKG in NEAKG together with minor amounts of monoesterified alkoxyglycerols (MEAKG). Methanolysis of shark liver oil has been also reported (*12*). Then, these NEAKG can be re-esterified via enzymatic processes using health-promoting polyunsaturated fatty acids (PUFAs), and thus high value ether lipids can be designed (*5, 11*). Saponification or ethanolysis reactions are necessary because of the presence of triacylglycerols which can undergo similar esterification and transesterification reactions in the presence of lipases.

However, to obtain a product rich in alkoxyglycerols, squalene needs to be removed from the original shark liver oil previous to the saponification or ethanolysis reaction. For this matter, supercritical fluid technology has shown very interesting capability (*13*).

In this work, shark liver oil (squalene free) was transesterified with ethanol to produce a mixture that mainly contains fatty acid ethyl esters (FAEE), NEAKG, and MEAKG. Ethanolysis of fish oils have been extensively used to produce fatty acid ethyl esters rich in PUFAs (*14*). In the present study, countercurrent supercritical fluid extraction (CC-SFE) was employed to eliminate FAEE from the mixture, thus concentrating NEAKG and MEAKG in the raffinate product. In addition, fractionation of extract was also studied to selectively enrich one of the extracts in ω-3 fatty acid ethyl esters. The feed raw

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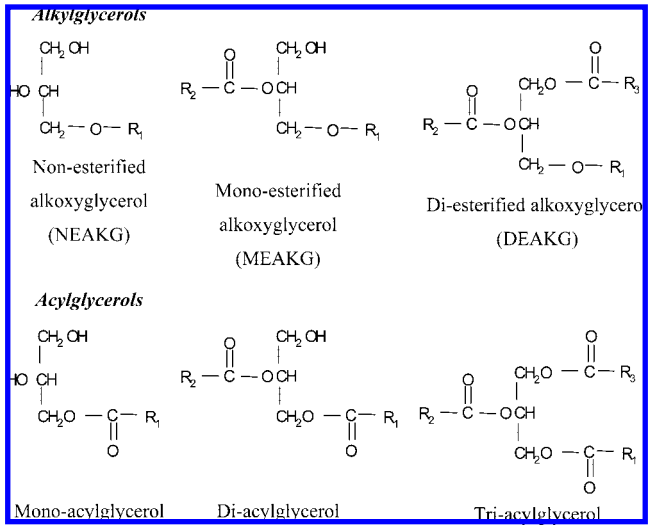

Figure 1. Chemical structure of alkoxyglycerol and acylglycerol compounds.

Table 1. Composition of Shark Liver Oil before and after the Ethanolysis Reaction and Simplified Composition Employed in the Thermodynamic Modeling

	before ethanolysis, % w/w	after ethanolysis, % w/w	composition employed in the modeling	
			component	% w/w
squalene	0.5	0.3		
cholesteryl esters	1.5	0.4		
FFA	0.0	<1.0	oleic acid	1.4
FAEE	0.0	71.8	ethyl oleate	71.8
DEAKG	44.6	0.0		
TAG	53.4	<1.0	triolein	1.5
cholesterol	0.0	0.3		
MEAKG	0.0	4.3	diolein	4.3
NEAKG	0.0	20.9	monolein	21.0

material contains around 72% of FAEE, 21% of NEAKG, and 4% of MEAKG (weight basis). Supercritical extractions were carried out in an isothermal countercurrent 3 m height packed column, without reflux, under semicontinuous operation. Pressures in the range of 140−180 bar and temperatures from 45 to 65 °C were explored. Furthermore, an internal reflux induced by thermal gradient was used to increase NEAKG and MEAKG recovery in the raffinate product.

MATERIALS AND METHODS

Materials and Analysis. *Sample and Reagents.* Shark liver oil (squalene free) was purchased from Lysi (Reykjavik, Iceland). The composition of the raw material before and after the ethanolysis reaction is given in **Table 1**. Carbon dioxide (99.98%), was purchased from AL Air–Liquide España S. A. (Madrid, Spain). All solvents used were HPLC grade from Laboratory-Scan (Dublin, Ireland).

Ethanolysis of Shark Liver Oil. The ethanolysis reaction was carried out in our laboratory as described in previous work (*11*). Shark liver oil was mixed with sodium ethoxide (5.25% w/v) in absolute ethanol at a ratio of 4:1 (v:v). The mixture was allowed to stand for 30 min at 60 °C while being stirred. Then, two washings were effected: first with a sodium chloride solution (0.5 M) at 50 °C, and second with distillate water. The volume utilized in these two washings was half of the volume of shark liver oil utilized. After the second washing, the mixture was centrifuged at 585*g*. Finally, the product of the ethanolysis reaction was dried with sodium sulfate and vacuum filtrated.

HPLC Analysis. The composition of the neutral lipids were determined on a kromasil silica 60 column (250 × 4.6 mm, Análisis Vinicos, Tomelloso, Spain) coupled to a CTO 10A VP 2 oven, a LC-10AD VP pump, a gradient module FCV-10AL VP, a DGU-14A degasser, and a evaporative light scattering detector ELSD-LT from Shimadzu (Izasa, Spain). The ELSD conditions were 2.2 bar, 35 °C, and a gain of 3. The flow rate was 2 mL/min. A splitter valve was used after the column, and only 50% of the mobile phase was directed through the detector. The column temperature was maintained at 35 °C. The mobile phase utilized has been previously reported by Torres et al. (*15*). This methodology permits one to analyze up to 18 different classes of neutral lipids including NEAKG, MEAKG, DEAKG, sterols, tocopherols, and mono-, di-, and triacylglycerols. Quantification was based on calibration curves effected with appropriate standards. Although the present methodology is also able to identify and quantify fatty acid ethyl esters (as a single lipid class), these compounds were much more precisely and accurately analyzed via gas chromatography.

Gas Chromatography. For the analysis of fatty acid ethyl esters 1 μL of the diluted sample was injected into an Agilent (Avondale, PA) gas chromatograph (6890N Network GC System) coupled to an autosampler (Agilent 7683B). The capillary column was a 30 m HP-88 (Avondale, Pennsylvania) (0.25 mm i.d.). A 20:1 split was utilized. The temperatures of the injector and the detector were 220 and 250 °C, respectively. The temperature program was as follows: starting at 100 °C and then heating to 180 at 20 °C/min, followed by heating from 180 to 220 at 15 °C/min. The final temperature (220 °C) was held for 30 min. Identification of the various fatty acids was based on the retention times and relative area percentages of a PUFA No. 3 standard (#4–7085) obtained from Supelco. Shark liver oil was then transmethylated to properly identify the different species comprised of shark liver oil. A similar profile was obtained with their corresponding fatty acid ethyl ester, which permits identification and quantification of these species. Quantification was effected via an external standard of linoleic acid methyl ester.

SFE Equipment and Method. The CC-SFE pilot plant employed in this work is shown in **Figure 2**. The CC-SFE equipment is a homemade devise and comprises a countercurrent extraction column (316 stainless steel), two cascade separator cells (270 mL capacity), and a cryogenic trap at atmospheric pressure. The extraction column is 300 cm in height, 17 mm in internal diameter, and is packed with Fenske rings (3 × 0.5 mm). The extract can be fractionated by a cascade decompression in the two separator cells. All units are thermostatized. The packed column has three levels of sample introduction (top, middle, and bottom). In this work, the liquid sample was introduced through the middle point, with an effective packed height (from the introduction point of the liquid sample to the CO_2 feed point) of 180 cm. Both CO_2 and liquid feed samples were preheated at the exit of their respective pumps (Dosapro Milton Roy, France) before introduction into the extraction column. The pilot plant has computerized PLC (Programmable Logic Controllers) based instrumentation and a control system with several safety devices including valves and alarms.

During the extraction, a continuous flow of CO_2 was introduced into the column through the bottom. When the operating pressure and temperature were stabilized, the liquid sample was pumped (300 mL/h) during the entire extraction time (90 min). When the extraction was finished, CO_2 was pumped for another 30 min. The first separator cell (S1) was maintained at pressures of 100 or 120 bar and temperatures of 40, 45, or 55 °C, whereas the second separator cell (S2) was maintained at low pressure (<20 bar) to recover allof the remaining extracted material in this unit. The raffinate and liquid fractions collected in the separators were weighed and analyzed. The material balance closed in all experiments with an inaccuracy lower than 5%.

Replicates of some of the extractions were carried out to analyze the reproducibility of the methodology employed in the extraction assays. For example, extraction 4 was run in triplicate, and the standard deviation obtained in the FAEE, NEAKG, MEAKG, TAG, and FFA compositions was 1.54, 0.40, 0.63, 0.63, and 0.55 respectively. These values indicate a good reproducibility of the results and validate the experimental method utilized in the present study.

RESULTS AND DISCUSSION

Composition of the original and transesterified shark liver oil is given in **Table 1**. As can be observed in the table, shark

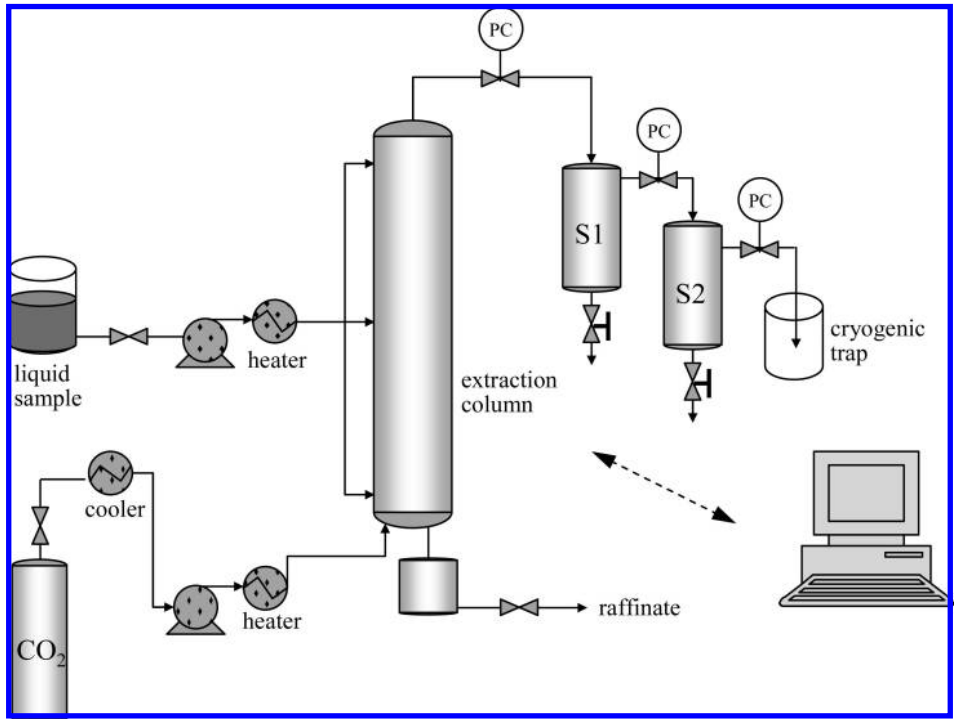

Figure 2. Experimental CC-SFE device.

liver oil acquired from the Lysi company contains around 0.5% w/w of squalene. Thus, elimination of squalene was not necessary.

However, the use of this oil to produce esterified alkoxyglycerols with specific PUFA chains via enzymatic reactions is still inadequate because of the presence of triacylglycerols, which can simultaneously undergo similar esterification and transesterification reactions. Hence, an ethanolysis reaction of this oil was effected to efficiently transform all triacylglycerols and esterified alkoxyglycerols (DEAKG) into FAEEs and NEAKG.

The residue obtained after the transesterification reaction (**Table 1**) contains 72% w/w of FAEE and ca. 25% w/w of NEAKG + MEAKG. The elimination of FAEE from this mixture can produce a product with a high concentration of NEAKG and MEAKG.

Solubility data of esterified or nonesterified alkoxyglycerols in SC-CO_2 is not available in the literature. Nevertheless, the NEAKG and MEAKG chemical structures are, respectively, very similar to the monoacylglycerol and diacylglycerol molecules (see **Figure 1**). Thus, solubility data of monoolein, diolein (*16*) and ethyl oleate (as representative of the FAEE fraction) in SC-CO_2 (*17*) was used to estimate adequate extraction conditions. In general, at temperatures of 40–60 °C and pressures around 140 bar, the solubility of ethyl oleate is 10 times grater than monoolein or diolein solubility. Thus, the elimination of FAEE seems to be achieved without difficulty.

Additionally, the minimum pressure required at different extraction temperatures can be estimated from the pressure versus composition phase diagram of the ethyl oleate + CO_2 binary mixture. **Figure 3** shows the vapor–liquid equilibria data as reported by Bharath et al. (*17*). For a particular isotherm, the region comprised inside corresponds to the two phase (liquid–vapor) region, whereas in the outside region the ethyl oleate + CO_2 mixture forms a homogeneous phase. For example, at 40 °C and at pressures higher than 140 bar, ethyl oleate can be completely dissolved in CO_2 to form a homogeneous supercritical phase. Besides the fact that the liquid raw

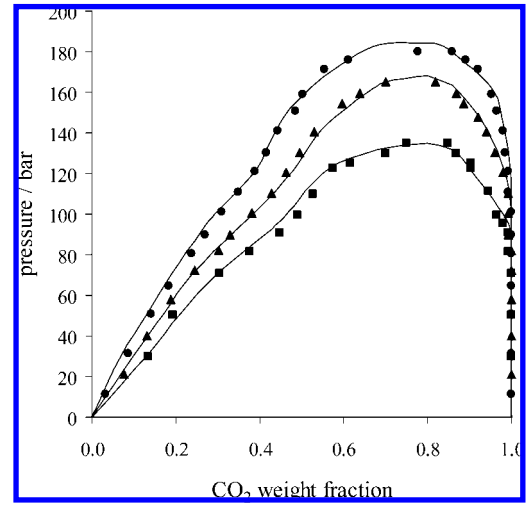

Figure 3. Vapor–liquid equilibria for the ethyl oleate + CO_2 binary mixture (17): (■) 40 °C; (▲) 50 °C; (■) 60 °C.

material is a complex mixture (i.e., is not pure ethyl oleate), it is expected that when the extraction temperature is 40 °C that pressures higher than 140 bar would favor the complete elimination of FAEE from the raw material. Similarly, taking into account the critical point corresponding to the 50 and 60 °C isotherms (see **Figure 3**), pressures higher than 160 and 180 bar would be required to achieve almost a complete elimination of FAEE from the raw material.

The different extraction conditions, that is, temperature and pressure, employed in the experimental assays are given in **Table 2**. A solvent-to-feed (S/F) ratio of 15 was used in all experiments. Also given in the table are the raffinate composition (CO_2 free basis) and yields of NEAKG and MEAKG obtained in the raffinate. The extract (top product) comprises the two liquid fractions recovered in S1 and S2 separators units. The compositions of the fractions collected in S1 and S2, together with NEAKG yield, are given in **Table 3**.

Table 2. SFE Carried out in the Countercurrent Packed Column: Composition (Weight Basis) and Yield (Mass in Raffinate/Mass in Feed) Obtained in the Raffinate (Bottom) Product

			% wt (CO_2 free basis) in raffinate					yield %	
exp.	T/°C	P/bar	FAEE	NEAKG	MEAKG	TAG	FFA	NEAKG	MEAKG
1	65	140	46.4	30.1	8.8	11.7	3.0	88.4	100
2	65	160	29.1	43.7	15.1	5.2	6.9	75.0	92.6
3	65	180	6.3	50.5	23.5	8.8	11.0	39.6	74.9
4	50	140	11.9	53.6	18.8	6.7	9.0	71.5	89.4
5	50	160	3.5	50.5	23.9	11.1	10.9	43.6	72.5
6	50	180	6.4	47.7	22.9	10.5	12.5	34.7	60.5
7	40	140	2.0	56.7	21.8	8.4	11.1	56.0	80.2
8[a]	40	140	33.9	37.8	12.1	4.0	12.2	82.9	100
9[a]	50	140	27.3	46.1	14.7	4.9	7.0	85.2	100

[a] Extraction carried out by employing a thermal gradient along the extraction column.

Table 3. Composition (Weight Basis) of the Fractions Collected in S1 and S2 Separator Units (Extract Product)

	% wt (CO_2 free basis) in S1				% wt (CO_2 free basis) in S2			
exp.	FAEE	NEAKG	MEAKG	NEAKG yield % in S1	FAEE	NEAKG	MEAKG	NEAKG yield % in S2
1	92.1	7.9	0.0	8.0	100.0	0.0	0.0	0.0
2	91.1	8.2	0.7	24.8	99.6	0.4	0.0	0.1
3	87.0	11.6	1.4	59.3	96.3	3.7	0.0	1.1
4	88.3	10.5	1.2	26.1	94.6	5.4	0.0	2.3
5	84.5	13.3	2.1	53.9	95.3	4.6	0.1	0.9
6	80.2	17.0	2.8	64.7	97.0	3.0	0.0	0.6
7	85.8	12.8	1.4	42.2	95.5	3.8	0.7	1.8
8[a]	92.0	8.0	0.0	17.1	93.5	6.5	0.0	0.0
9[a]	100.0	0.0	0.0	14.6	97.8	2.2	0.0	0.2

[a] Extraction carried out by employing a thermal gradient along the extraction column.

The experimental FAEE compositions reported in **Table 2** confirm the explanation given above, regarding the minimum pressure necessary to eliminate FAEE at the different extraction temperatures explored. According to this explanation, almost a total elimination of FAEE was achieved at 40 °C and 140 bar (exp. 7 in **Table 2**). Furthermore, increasing the extraction temperature to 50 or 65 °C requires an extraction pressure of, respectively, 160 or 180 bar, to achieve a raffinate with a FAEE concentration lower than ca. 6% w/w.

Increasing pressure at constant temperature (and constant solvent-to-feed ratio) improves the concentration of NEAKG and MEAKG in the raffinate and, as expected, also produces a decrease in the amount of NEAKG and MEAKG recovered. Particularly, MEAKG yields are always quite acceptable (>60%) in all experiments due to its high molecular weight and low volatility. Satisfactory NEAKG yields can be obtained at the lower pressure explored (140 bar), but around 65% of the feed NEAKG is extracted at 180 bar and 50 °C. In general, low extraction temperature greatly improves the concentration of both NEAKG and MEAKG in the raffinate, as can be deduced by observing the results obtained in experiments 1, 4, and 7 in **Table 2**, which were carried out at the constant extraction pressure of 140 bar. Nevertheless, temperatures lower than 40 °C are not recommended because of the observed increase of the raw material viscosity and consequent observed difficulties of maintaining a constant liquid flow into the packed column.

Limitations in the SFE experimental device available do not allow the use of external reflux to increase NEAKG and MEAKG yields. In turn, a thermal gradient was generated in the extraction column to induce an internal reflux. In this case (exp. 8 and 9 in **Table 2**) the 120 cm of packed column above the introduction point of the liquid feed material was maintained at a high temperature (65 °C) to decrease CO_2 density and thus reduce solubility. Then, an internal reflux was induced and a stripping section was generated into the column. The effect of this internal reflux was remarkable, taking into account the increase of NEAKG and MEAKG yields accomplished, but a higher FAEE content in the raffinate product also was obtained.

Table 4. ω-3 Enrichment (ω-3 FAEE in S1/ω-3 FAEE in S2) Obtained in the Cascade Fractionation System

exp.	S1 pressure (bar)	S1 temperature (°C)	CO_2 density (kg/m3)	ω-3 enrichment
4	100	40	630	8.20
4	120	55	506	4.63
4	100	45	500	4.51

The composition of both extracts, together with the NEAKG yield (amount of NEAKG in the extract/amount of NEAKG in feed material), obtained in S1 and S2 separator cells are given in **Table 3**. S1 was maintained at 100 bar and 45 °C (CO_2 density = 446 kg/cm^3) in all experiments reported in **Table 3**. Very low amounts of free fatty acids and triacylglycerols were detected in these fractions, and almost no MEAKG was recovered in S2 extracts. As can be observed in **Table 3**, the NEAKG and MEAKG concentration in S1 extracts increase with increasing extraction pressure, in accordance with the decrease of yield (particularly for NEAKG) observed in raffinates. Nevertheless, almost all the NEAKG extracted (see **Table 3**) was recovered in S1.

Both S1 and S2 extracts contain large amounts of FAEE, but the fractionation conditions (pressure and temperature) chosen in S1 unit can produce, in this separator, a FAEE fraction enriched in long chain ω-3 fatty acid ethyl esters (mainly docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and small amounts of docosapentaenoic acid (DPA)). **Table 4** shows the variation of this enrichment (ω-3 FAEE in S1/ω-3 FAEE in S2) as a function of CO_2 density, obtained for the 140 bar and 50 °C extraction (exp. 4 in **Table 2**) and different fractionation conditions. An increase of CO_2 density produced a high enrichment of ω-3 FAEE in S1, and thus this fraction

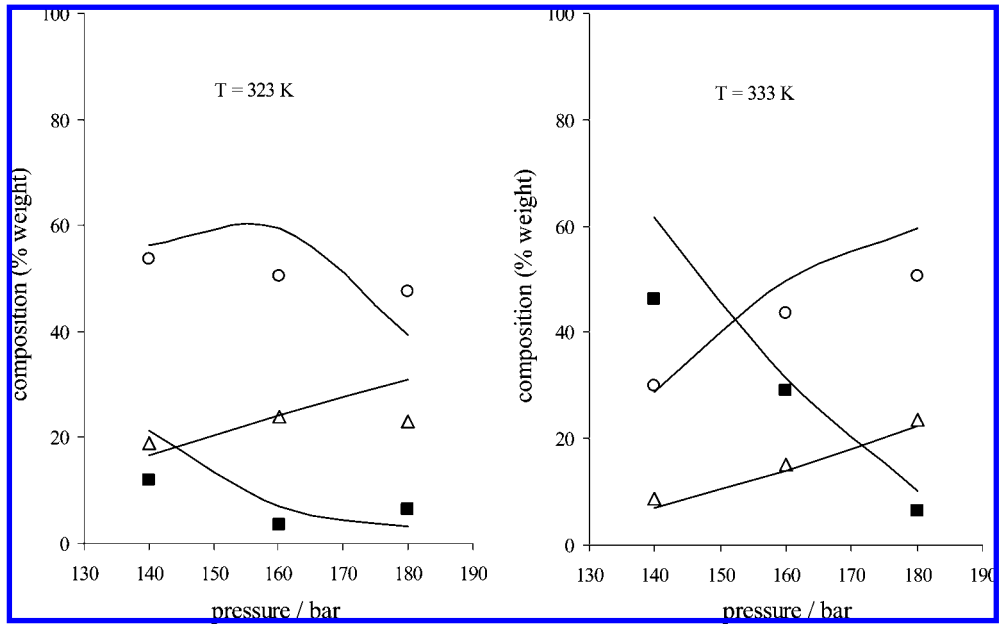

Figure 4. Comparison between the experimental compositions obtained in the raffinate for the FAEE (■), NEAKG (○), and MEAKG (△) and the GC-EoS calculations (solid lines).

can be employed as raw material to carry out the transesterification reactions of NEAKG to produce highly valuable DEAKG via enzymatic processes.

The group contribution equation of state (GC-EoS) developed by Skold-Jorgensen (*18*) was used to simulate the phase equilibria behavior of the extraction experiments. The GC-EoS model is based on the group contribution approach (i.e., each component is represented by the chemical groups present in its chemical structure) and thus, it has predictive capability. The required parameters to carry out phase equilibria calculations are group parameters (pure group and binary group interaction parameters) together with some pure component parameters (critical temperature, critical pressure, and critical hard sphere diameter). For a detailed explanation of the model the reader is referred to the work of Skold-Jorgensen (*18*).

Recently, Fornari (*19*) presented a revision and summary of the GC-EoS parameter table, including all group parameters reported in the literature from the model development (1984) until present. Additionally, the GC-EoS was applied and optimized to represent vapor–liquid equilibria and solubility data of binary mixtures containing SC-CO_2 and several lipid-type substances, including ethyl oleate, monolein, diolein, triolein, and oleic acid, among others.

In this work, the lipid-type substances with critical parameters as reported by Fornari (*19*) were used to represent the multicomponent liquid raw material employed in the experiments (see **Table 1**). The FAEE, TAG, and FFA fractions were represented, respectively, by ethyl oleate, triolein, and oleic acid. NEAKG and MEAKG fractions were represented, respectively, by monolein and diolein, owing to the similarities in chemical structure (**Figure 1**) and thus, the expected similar solubility behavior. With this last assumption, the chemical groups that comprise the liquid sample + CO_2 mixture are: paraffin (CH_3, CH_2), olefin (CH=CH), ester (CH_2COO), alcohol (CH_2OH, CHOH), carboxylic acid (COOH), triglyceride ($(CH_2COO)_2CHCOO$), and carbon dioxide (CO_2). All pure group parameters and binary interaction parameters between these groups were taken from Fornari (*19*), except for the COOH interactions with the ester, triglyceride, and alcohol groups, which are not available in the literature and thus were set to be ideal. The lack of these interaction parameters can not significantly affect the calculations because the amount of FFA in the mixture is ca. 1% wt.

The representation of the liquid raw material, as described above, allows a rapid and first approach to simulate the SFE process experimentally studied in this work by applying the GC-EoS model in a predictive manner. The phase equilibria calculations were carried out by considering a countercurrent column with two theoretical stages and S/F = 15. **Figure 4** shows a comparison between the experimental compositions obtained in the raffinate for the FAEE, NEAKG, and MEAKG fractions and those predicted by the GC-EoS model. Despite the simplifications considered to represent the complex multicomponent raw material, satisfactory accordance between experimental and calculated values is observed, which provides a thermodynamic fundamental to the experimental compositions reported in **Tables 1** and **2**.

From the results presented in **Tables 2**−**4** the following conclusions can be derived. At 40 °C and 140 bar, nonesterified alkoxyglycerols and monoesterified alkoxyglycerols were concentrated in the raffinate (bottom) product from 25 to 78% w/w, but around 50% of the feed NEAKG was lost in the extract. Internal reflux was induced in the extraction column, by employing a thermal gradient, and NEAKG recovery in the raffinate product was greatly improved, but NEAKG and MEAKG concentration in the raffinate was reduced from ca. 78% to ca. 50%. Also, the possibility of recover the NEAKG extracted using a cascade fractionation of the extract was explored. Almost all of the extracted NEAKG was recovered in the first separator of the cascade decompression system (maintained at 100 bar and 40 °C). Furthermore, the FAEE fraction recovered in this separator (together with the extracted NEAKG) was considerably enriched in ω-3 fatty acid ethyl esters, and thus can be reutilized in a subsequent esterification step to produce structured ether lipids.

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LITERATURE CITED

(1) Mangold, H. K. Chemistry and Biology, In *Ether Lipids*, Snyder F. L. Ed.; Academic Press: New York, 1972, 157–176.
(2) Brohult, A.; Brohult, J.; Brohult, S.; Joelsson, I. Effects of Alkoxyglycerols on the Frequency of Injuries Following Radiation Therapy for Carcinoma of the Uterine Cervix. *Acta Obstet. Gynecol. Scand.* **1977**, *56*, 441–448.
(3) Andreesen, R. Ether Lipids in the Therapy of Cancer. *Prog Biochem Pharmacol.* **1988**, *22*, 118–131.
(4) Palmblad, J.; Samuelsson, J.; Brohult, J. Interactions Between Alkylglycerols and Human Neutrophil Granulocytes. *Scand. J. Clin. Lab. Invest.* **1990**, *50*, 363–370.
(5) Mitre, R.; Etienne, M.; Martinais, S.; Salmon, H.; Allaume, P.; Legrands, P.; Legrand, A. B. Humoral defence improvement and haematopoiesis stimulation in sows and offspring by oral supply of shark-liver oil to mothers during gestation and lactation. *Br. J. Nutr.* **2005**, *94*, 753–762.
(6) Pugliese, P. T.; Jordan, K.; Cederberg, H.; Brohult, J. Some biological actions of alkylglycerols from shark liver oil. *J. Altern. Complementary Med.* **1998**, *4* (1), 87–99.
(7) Sardesai, V. M. Nutritional Role of Polyunsaturated Fatty Acids. *J. Nutr. Biochem.* **1992**, *3*, 154–166.
(8) Bradley, M.; Wetherbee, P.; Nichols, D. Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. *Comp. Biochem. Physiol., Part B* **2000**, *125*, 511–521.
(9) Jayasinghe, C.; Gotoh, N.; Tokairin, S.; Ehara, H.; Wada, S. Inter species changes of lipid compositions in liver of shallow-water sharks from the Indian Ocean. *Fisheries Sci.* **2003**, *69*, 644–653.
(10) Harris, W. S. Fish Oils Supplementation: Evidence for Health Benefits. *Cleveland Clin. J. Med.* **2004**, *71*, 208–221.
(11) Torres, C. F.; Vázquez, L.; Señoráns, F. J.; Reglero, G. An Efficient Methodology for the Preparation of Alkoxyglycerols Rich in Conjugated Linoleic Acid and Eicosapentaenoic Acid. *J. Am. Oil Chem. Soc.* **2007**, *84*, 443–448.
(12) Haraldsson, G. G.; Thorarensen, A. The generation of glyceryl ether lipids highly enriched with eicosapentaenoic acid and docosahexaenoic acid by lipase. *Tetrahedron Lett.* **1994**, *35*, 7681–7684.
(13) Mukhopadhyay, M. *Natural Extracts Using Supercritical Carbon Dioxide*; CRC Press: Boca Raton, Florida, 2000.
(14) Perretti, G.; Motori, A.; Bravi, E.; Favati, F.; Montanari, L.; Fantozzi, P. Supercritical carbon dioxide fractionation of fish oil fatty acid ethyl esters. *J. Supercrit. Fluids* **2007**, *40*, 349–353.
(15) Torres, C. F.; Vazquez, L.; Señorans, F. J.; Reglero, G. Study of the analysis of alkoxyglycerols and other non-polar lipids by liquid chromatography coupled with evaporative light scattering detector. *J. Chromatogr. A* **2005**, *1078*, 28–34.
(16) Nilsson, W. B.; Gauglitz, E. J.; Hudson, J. K. Solubilities of methyl oleate, oleic acid, oleyl glycerols, and oleyl glycerols mixtures in supercritical carbon dioxide. *J. Am. Oil Chem. Soc.* **1991**, *68*, 87–91.
(17) Bharath, R.; Inomata, H.; Arai, K. Vapor-Liquid Equilibria for Binary Mixtures of Carbon Dioxide and Fatty Acid Ethyl Esters. *Fluid Phase Equilib.* **1989**, *50*, 315–327.
(18) Skjold-Jørgensen, S. Gas Solubility Calculations. II. Application of a New Group-Contribution Equation of State. *Fluid Phase Equilib.* **1984**, *16*, 317–351.
(19) Fornari, T. Revision and summary of the group contribution equation of state parameter table: application to edible oil constituents. *Fluid Phase Equilib.* **2007**, *262*, 187–209.

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