Purification of Polyunsaturated Fatty Acid Esters from Tuna Oil with Supercritical Fluid Chromatography

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ABSTRACT: The technical and economic feasibility of producing docosahexaenoic acid (DHA)- and eicosapentaenoic acid (EPA)-ethyl ester concentrates from transesterified tuna oil using supercritical fluid chromatography (SFC) was studied. A systematic experimental procedure was used to find the optimal values for process parameters and the maximal production rate. DHA ester concentrates up to 95 wt% purity were obtained in one chromatographic step with SFC, using CO_2 as the mobile phase at 65°C and 145 bar and octadecyl silane-type reversed-phase silica as the stationary phase. DHA ester, 0.85 g/(kg stationary phase · h) and 0.23 g EPA ester/(kg stationary phase · h) can be simultaneously produced at the respective purities of 90 and 50 wt%. The process for producing 1,000 kg DHA concentrate and 410 kg EPA concentrate per year requires 160 kg stationary phase and 2.6 tons/h carbon dioxide eluant recycle. The SFC operating cost is U.S. $550/kg DHA and EPA ethyl ester concentrate.

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KEY WORDS: Docosahexaenoic acid, eicosapentaenoic acid, polyunsaturated fatty acids, supercritical fluid chromatography, tuna oil.

Fish oils are a rich source of polyunsaturated fatty acids (PUFA). The interest is particularly in eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) because of their reported beneficial physiological activities. The EPA content of fish oil varies from 5 to 26 wt%, and DHA from 6 to 26 wt% of total fatty acids (1).

More concentrated n-3 fatty acids are needed to study their physiological effects and metabolism. PUFA concentrates have a market in pharmaceutical products, food additives, and in health supplements.

In fish oil, the EPA and DHA are preferentially located in the middle carbon of the glycerol backbone. However, triglycerides where two such long-chain fatty acids would be in the same backbone are expected to be minor constituents (2). It is therefore not possible to obtain fish oil products with very high EPA and DHA concentrations if the oil is fractionated as triglycerides. To make fractionation possible, the oil may first be transesterified with an alcohol to fatty acid monoesters. A number of methods have been developed to isolate EPA and DHA from transesterified fish oil, including molecular distillation, liquid chromatography, supercritical fluid extraction, and supercritical fluid chromatography (SFC) with carbon dioxide eluant (3,4). KD-Pharma GmbH, a German company based in Bexbach, is already operating a large-scale SFC plant in Tarragona, Spain. The company produces n-3 fatty acid esters from fish oil at over 95 wt% purity.

It is also possible to eliminate all saturates and the bulk of mono- and dienes from the re-esterified oil by mixing the oil with hot urea dissolved in ethanol. Upon cooling, the urea crystallizes, forming solid adducts with the saturates. The remaining solution can then be fractionated with supercritical (SC) CO_2 extraction to obtain polyunsaturates in high concentration (2). Table 1 presents the composition of urea-adducted menhaden oil. Although technically advantageous as a pretreatment, one should be aware that authors from the U.S. Food and Drug Administration recently called attention to the danger of the formation of the animal carcinogen ethyl carbamate by this method (5).

Fatty acid ethyl esters (FAEE) are soluble up to 10 wt% concentration in dense CO_2 at 230 bars and 80°C (6). Therefore, chromatography with SC CO_2 offers an attractive method to produce EPA and DHA esters at high purity using a nonflammable and nontoxic eluant. Using a SC eluant also brings the potential advantage of increased separation rate and therefore more compact equipment compared to using a liquid eluant. The reason for this is that mass-transfer rate is generally much higher in a SC fluid than in a liquid. SC CO_2 is essentially a nonpolar eluant that can be used with straight (normal) stationary phases like silica. However, due to fatty acid esters' low polarity, silicas retain them too weakly, leading to only partial separation between EPA and DHA esters (7). Reversed phases such as octadecylsilane-grafted silica (ODS) yield a good resolution of fatty acid esters with CO_2 eluant. Berger and co-workers separated n-3 fatty acid methyl esters using reversed-phase C18-silica with a CO_2 eluant (8). Starting from a concentrate containing 14.8 wt% EPA and 73.0 wt% DHA, they obtained three fractions whose respective EPA/DHA concentrations in weight percent were: 54.8/26.7, 2.7/78.0 and 18.7/78.8. Perrut and Breivik (9) obtained EPA and DHA in up to 55.5 and 77.7 wt% purities with CO_2 chromatography. Their starting material contained 28.5 wt% EPA and 26.9 wt% DHA.

Reichmann and Brunner (10) investigated SFC separation

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TABLE 1
Fatty Acid Compositions of Various Fish Oils (wt%)[a]

Fatty acid	Codfish oil ester (14)	Menhaden oil (15)	Urea-adducted menhaden oil (2)	Baltic herring oil (16)	Herring liver oil (17)	Sand launce ethyl ester (18)	Present study	
							Tunafish oil	Tunafish oil ethyl ester
14:0	5.8	10.8		6.8	6.9	6.2	3.1	
16:0	12.9	23.2		22.3	12.4	16.3	22.8	15.1
16:1	9.8	11.4		9.5	11.6	11.7	3.9	3.1
16:2		1.5		0.8		0.6		
16:3		2.2	5.2					
18:0	2.7	4.2		1.8	1.8	2.2	6.7	4.4
18:1	23.3	22.6		24.5	22.6	9.9	17.7	12.5
18:2	0.2	1.8		4.3	1.4	4.3	1.6	1.8
18:3		1.7		3.4	1.3	0	2.3	1.9
18:4	2.2	2.1		2.6	1.9	4.4	0.3	
20:0		0.4		0.1	0.1	0.1	0.3	
20:1	11.4	1.3		1.1	7.6	4.9	0.3	
20:2		0.6		0.6	0.5	0.3	0.3	1.7
20:4	0.5	2.3	1.4	0.8	0.5	1.2	1.5	
20:5 (EPA)	14.5	11.9	48.9	7.2	12.6	11.1	4.6	5.3
22:0		0.1					0.2	
22:1	8.6	0.2		0.1	5.2	7.5	0.8	
22:2								0.3
22:4	0.4	0.2						1.2
22:6 (DHA)	5.7	8.8	22.5	6.4	10.6	11.3	18.3	23.7
24:1						0.9	0.8	
C_{18} total	28.4	32.4		36.6	29.0	20.8	28.6	20.6
C_{20} total	26.4	16.5	50.3	9.8	21.3	17.6	7.0	7.0
C_{22} total	14.7	9.2	22.5	6.5	15.8	18.8	19.9	25.2
Percent EPA of all C_{20}	54.9	72.1	97.2	73.5	59.2	63.1	65.7	75.7
Percent DHA of all C_{22}	38.8	95.7	100.0	98.5	67.1	60.1	92.0	94.0

[a]DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

of fish oil ethyl esters with several types of reversed and normal stationary phases, using the separation of ester pairs ($C_{20:5}$/$C_{22:6}$) and ($C_{22:5}$/$C_{22:6}$) as a reference. Alumina stationary phase showed an especially good selectivity by the degree of saturation and chain length. They report that alkali treatment of the Al_2O_3 phase leads to substantial improvements in resolution.

The published reports and the operations of KD-Pharma GmbH clearly show that the separation of EPA and DHA ethyl esters from transesterified fish oil is technically possible using SC CO_2 eluant. However, the design of the economically most favorable purification process requires that the process variables be optimized for maximum productivity.

This paper describes a systematic procedure for developing an SFC separation method for producing EPA and DHA ethyl ester concentrates. The development was done in preparative, laboratory scale using the specific production rate of EPA and DHA ethyl esters as the target function, which was maximized. The specific production rate is the hourly production in grams of the desired compound per kilogram of stationary phase. The starting material was tuna oil, which is a low-value by-product of the fish meal industry. Statistical analysis of production rates calculated from nonpreparative SFC runs was first used to estimate the best operating conditions. A series of preparative SFC runs was then carried out at estimated optimal conditions to obtain the real production rate. The objective of this work was to study the technical and economic feasibility of producing EPA and DHA ethyl ester concentrates from by-product fish oil with SFC using a commercially available stationary phase and CO_2 eluant without co-solvents.

EXPERIMENTAL PROCEDURES

Materials. EPA and the ethyl ester of DHA were used as PUFA standards and purchased from Sigma-Aldrich Chemie (Deisenhofen). Their reported purities were 99%. EPA was esterified into the methyl ester by diazotization.

Carbon dioxide was food-grade quality (99.7% pure) from AGA Oy (Espoo, Finland). Absolute ethanol was from Primalco Oy (Helsinki, Finland). Anhydrous sodium sulfate was from Merck (Darmstadt, Germany).

Tuna (*Thunnus thynnus*) oil was obtained from Centro Technologico Gaiker (Zaimudio, Spain) from a Spanish fish-canning company. The oil contained 10.5 wt% water as determined with Karl Fischer titration. Its fatty acid composition was analyzed by first hydrolyzing the oil in NaOH-containing methanol, adding BF_3 in methanol to form methyl esters, extracting the methyl esters with *n*-heptane, drying the heptane phase with Na_2SO_4, and then analyzing the fatty acid ester mixture with gas chromatography–mass spectrometry (GC–MS) using the GC method described below. Commercial ethyl ester

made from sand launce (*Ammodytes lancea*) obtained from Grinsted Products (Aarhus, Denmark), was used in step 1 SFC runs where process variable levels were selected. Its reported fatty acid composition is presented in Table1.

Fish oil ethyl esters. The fatty acids in the tuna oil were converted to ethyl esters by transesterification with absolute ethyl alcohol. In the transesterification, 50 g tuna fish oil was first dried with anhydrous Na_2SO_4. Its water content after drying was 0.24 wt%. Dried oil was filtered and refluxed for 1.5 h with 350 g absolute ethyl alcohol. Freshly made sodium alcoholate was used as catalyst. The mixture was then extracted with *n*-hexane to obtain the fatty acid esters. The composition of the resulting fish oil ethyl ester mixture was analyzed by GC–MS and GC–flame-ionization detector (FID). The fatty acid compositions of the crude tuna oil and the transesterified oil are presented in Table 1. After transesterification, the DHA content of the oil was higher, the EPA content unchanged, and the oleic and palmitic acid contents lower than in the original oil. The authors suspect that the repeated extraction of the ethanol-containing aqueous layer with hexane did not remove all the lighter fatty acid esters. However, the slight change of the fatty acid composition during transesterification does not influence the SFC process development.

GC analysis. The fatty acids in the tuna oil, the transesterified oil, and the fractions collected from SFC runs were analyzed as ethanol solutions with a Hewlett-Packard (Palo Alto, CA) 9633 gas chromatograph, using an FID. The column was from J&W Scientific (Folsom, CA), DB-22, 30 m × 0.254 mm. Temperature program: 150°C; 5°C/min to 180°C, hold 25–30 min; 10°C/min to 250°C, hold 7 min. Injector: 250°C. Column flow 1 mL/min. Helium was used as the carrier gas. EPA and DHA ester GC peaks were located using the retention times of pure EPA and DHA esters.

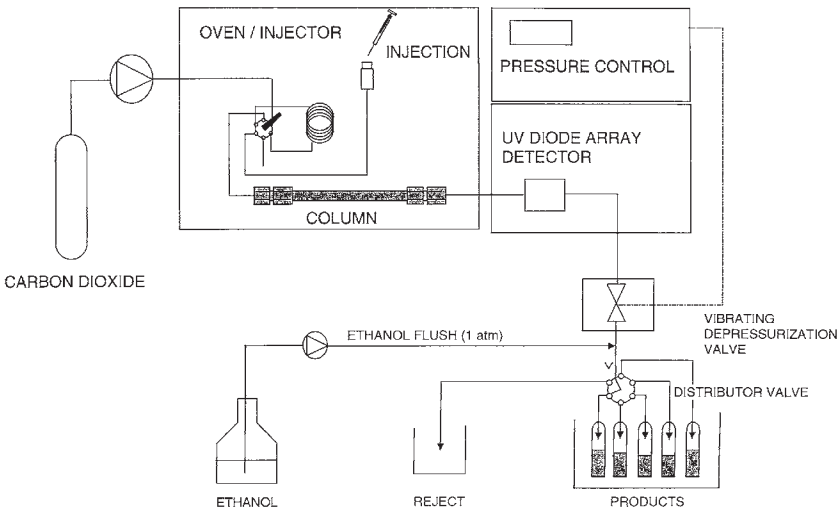
GC–MS analysis was done with the aforementioned method using a JEOL (Tokyo, Japan) SX-102 mass spectrometer. Fatty acid identification was done using a spectrum library and verified by using a known mixture of fatty acids. Margaric acid was used as internal standard.

Preparative supercritical chromatography. Preparative supercritical chromatography in milligram scale was done using modified Hewlett-Packard 1250-G SFC equipment. The columns were Kromasil 10-C18, 10 × 250 mm, and Kromasil 5-C18, 10 × 250 mm, obtained from Eka Chemicals AB (Bohus, Sweden). CO_2 as received was used as the mobile phase.

Transesterified fish oil was dissolved in ethanol at 10–50 wt% concentration. The ethanol solution was injected in the CO_2 eluant flow. To accommodate the large amount injected, the original automatic injector was replaced with a manual needle injection loop filler. Also, the original Hewlett-Packard restrictor valve was replaced with a JASCO (Tokyo, Japan) PB-880 vibrating restrictor valve system. From the restrictor valve the fluid was directed through a Rheodyne 7000 (Cotati, CA), six-port valve to vials that were immersed in an ice-water bath. From each injection, several successive fractions were collected and analyzed. The fractions were collected using the sequential-cut shaving technique, described previously (11). To flush the small, oily fractions quantitatively from the 1/16″ pipings, a small sidestream of ethyl alcohol was pumped continuously to a point in the piping immediately after the restrictor valve. The fractions were thus obtained as ethanol solutions. This is not necessary in industrial-scale SFC. The preparative SFC setup is depicted in Scheme 1.

RESULTS AND DISCUSSION

Selection of process variables. In industrial chromatography, much larger amounts of solutes must be injected per kilogram stationary phase than is customary in analytical work in order to achieve feasible production rates. However, increasing the injected amount decreases column efficiency, leading to decreasing resolution. At some column loads the required product purity cannot be achieved with any sacrifices in yield. For



SCHEME 1

a cost-effective chromatographic process, the process variables should be optimized so that the production rate per mass of stationary phase is at maximum while product purity is maintained at the required level.

The specific production rate in repetitive injection chromatography can be expressed in terms of the column load ratio:

$$\mathrm{PR} = \mathrm{LR} \cdot C_d \cdot \frac{Y/100\%}{t} \quad [1]$$

where PR is specific production rate [g of pure product/ (kg stationary phase · h)]; LR is load ratio (g injected solute/kg stationary phase); C_d is weight fraction of the desired component in starting material; Y is yield of the desired component of the injected amount (%); and t is injection interval (h).

Y in Equation 1 depends on column resolution, which is a function of column dimensions, stationary phase, and process variables in the following way:

$$R_s = 1/4(\alpha - 1)\sqrt{N}\left(\frac{k'}{1+k'}\right) \quad [2]$$

where R_s is resolution (dimensionless); α is separation factor (dimensionless); N is column plate number (dimensionless); and k' is retention factor (dimensionless).

Separation factor α is varied by changing the stationary and mobile phases and temperature. Plate number N is determined by column length, particle size, quality of packing, mobile phase, and linear velocity. In preparative chromatography, the plate number is strongly dependent on the column load ratio.

The retention factor k' depends on solvent strength, which in SFC can be varied by changing the pressure and density of the mobile phase. With a given column length, particle size, and stationary and mobile phases the resolution in SFC can thus be adjusted by changing the eluant linear velocity, pressure, temperature, and load ratio. To optimize these SFC process variables, a two-step statistical approach was used.

Step 1. Selection of process variable levels. Initial values for the four SFC process variables were selected based on the authors' previous experience in SFC studies. The variables and their ranges were: *u*, mobile phase linear velocity in packing (1.5–4.5 mm/s); *p*, column pressure (130–200 bar); *T*, column temperature (40–65°C); LR, column load ratio (1–10 g solute injected/kg stationary phase).

Three levels were given to each variable. A fractional factorial design was used to find process variable values for 20 SFC experiments. The experiments were done and the resulting SFC chromatograms were evaluated qualitatively and classified in three categories: good, fair, and not feasible.

It was found that 11 of the 20 combinations of process variable values resulted in chromatograms that would not be feasible in PUFA purification. The unacceptable values were identified, and reduced ranges for each of the four variables were set to avoid a large number of unacceptable value combinations in the next SFC experiments.

Step 2. Optimization of process variables. A new set of 20 SFC experiments was planned through fractional factorial design.The peaks that contained EPA and DHA esters were identified by injecting pure EPA and DHA esters separately and using their retention times for identification. Proper fraction collection intervals for obtaining pure EPA and DHA esters were visually estimated from each SFC chromatogram. The imaginary fraction collection times were selected so that there should be no overlapping of neighboring peaks during collection. Product yields were estimated from the segments of peak areas that were obtained during the imaginary fraction collection. The production rates for EPA and DHA esters were then calculated from Equation 1 using the data shown in Table 2. The calculated production rates are also shown in Table 2.

In order to optimize the conditions and to select the parameter values for the preparative work, a general linear model was fitted to the experimental data shown in Table 2. The best equation form was found by adding and removing first-order, second-order, and combination terms and fitting each equation to the data by the partial least squares (PLS) method. The best equation form was selected by comparing the fits using standard statistical tests. Principal component analysis (PCA) revealed that LR and temperature had the strongest effect on DHA and EPA production rates. Mobile- phase linear velocity and column pressure had lesser effects on the production rates.

The best-fitting general linear model for DHA and EPA PR was found to be the form of Equation 3:

$$\begin{aligned}\mathrm{PR} = {} & a + b \cdot p + c \cdot p^2 + d \cdot u^2 + e \cdot \exp(\mathrm{LR}) + f \cdot T^2 \\ & + g \cdot p \cdot \exp(\mathrm{LR}) + h \cdot p * T + i \cdot u \cdot T + j \cdot \exp(\mathrm{LR}) \cdot T\end{aligned} \quad [3]$$

where *a* through *j* are constant regression coefficients (PR of DHA/PR of EPA, respectively): *a*, –32.1/–3.4; *b*, 0.44/0.047; *c*, –0.002/–0.00023; *d*, –0.56/–0.025; *e*, –1.66E-10/0; *f*, –0.006/–0.0003; *g*, –4.24E-08/–2.84E-09; *h*, 0.005/0.00028; *i*, 0.039/0.002; *j*, 5.57E-07/2.06E-08.

The numerical values of the coefficients apply when the previously shown units are used for the variables. The standard error of the highest PR estimate from Equation 3 was ±0.67 g product/(kg stationary phase · h) for DHA and 0.05 g product/(kg stationary phase · h) for EPA. The standard errors of the coefficients were generally quite large, indicating a lot of noise in the data of Table 2. However, visual inspection of the calculated response surfaces from Equation 3 revealed clear trends that could be used for selecting the parameter ranges for preparative experiments in step 3.

Response surfaces were calculated from Equation 3 covering the range of process variables that were used in the step 2 experiments. Several response surfaces were drawn by alternatively changing two parameters and keeping the other two constant. The maximal production rates, calculated from Equation 3, were then found and the corresponding optimal process parameter values discovered. The best estimated SFC conditions for fish oil ester fractionation are u = 1.9 mm/s; p = 145 bar; column temperature = 65°C; LR = 4.95 g/kg.

Under optimal conditions, the estimated (Eq. 3) maximal PR for pure DHA ethyl esters was 2.46 g DHA-ethyl ester/(kg stationary phase · h). For pure EPA, the estimated maximal pro-

TABLE 2
Process Parameters and Calculated Resulting DHA and EPA Production Rates from Step 2 SFC Experiments

Run no.	Pressure (bar)	Temperature (°C)	Fluid linear velocity (mm/s)	Column load ratio (g/kg)	Injection interval (min)	Visual yield of DHA (%)	Calculated prod. rate of DHA [g/(kg·h)]	Visual yield of EPA (%)	Calculated prod. rate of EPA [g/(kg·h)]
1	130	55	1.5	1.0	36	90	0.35	20	0.02
2	130	55	2.5	1.0	14	85	0.84	0	0.00
3	160	55	1.5	1.0	15	50	0.46	0	0.00
4	160	65	2.5	1.0	11	85	1.07	0	0.00
5	160	65	1.5	1.0	22	90	0.56	10	0.01
6	130	65	2.5	1.0	33	95	0.40	50	0.05
7	130	55	1.5	5.0	30	60	1.38	75	0.38
8	160	55	1.5	5.0	20	40	1.38	0	0.00
9	160	55	2.5	5.0	11	0	0.00	0	0.00
10	130	65	1.5	5.0	90	100	0.77	100	0.17
11	130	65	2.5	5.0	42	100	1.64	100	0.36
12	160	65	2.5	5.0	15	50	2.30	40	0.40
13	145	60	1.5	3.0	30	75	1.04	75	0.23
14	145	60	2.5	3.0	15	60	1.66	50	0.30
15	130	60	2.0	3.0	38	50	0.54	90	0.21
16	160	60	2.0	3.0	14	40	1.18	0	0.00
17	145	55	2.0	3.0	15	0	0.00	0	0.00
18	145	65	2.0	3.0	24	90	1.55	80	0.30
19	145	60	2.0	1.0	19	80	0.58	10	0.02
20	145	60	2.0	5.0	20	80	2.76	50	0.38

[a]SFC, supercritical fluid chromatography. See Table 1 for other abbreviations.

duction rate was 0.16 g EPA-ethyl ester/(kg stationary phase · h).

Step 3. Preparative verification of estimated PR. Transesterified tuna oil was injected in the SFC column at three load ratios: 1.25, 2.5, and 5.0 g crude ester mixture/kg stationary phase. The other process variables were kept at the estimated optimum conditions. The obtained SFC chromatograms at each column LR are depicted in Figure 1. At the highest load ratio of 5 g/kg the peaks became distorted and peak doubling was visible. This was apparently caused by overloading and not by column irregularities since the retention times were repeatable and at lower loading the peaks were symmetrical. Five fractions were collected from each EPA- and DHA-containing peak. The purities of EPA and DHA in each fraction are shown in Table 3.

It was observed that the PR was a strong function of the LR. At 5.0 g crude/kg stationary phase, the purest obtained fraction contained 87 wt% DHA. At 2.5 g/kg load ratio, more than 90 wt% pure DHA-ethyl ester fractions were collected. At the smallest, 1.25 g/kg load, the three purest fractions could be combined to obtain a product that constituted most of the peak and contained more than 95 wt% DHA. The corresponding production rates are shown in Figure 2.

The most critical impurities in obtaining pure DHA were other C_{22} esters. SFC separation between 22:5 and 22:6 was incomplete, but due to the marginal amount of 22:5 in the starting material, 22:5 did not interfere with DHA purification. At all load ratio levels, the first DHA fractions contained also 18:0 and 18:1 esters. This indicates that the low unsaturated C_{18} esters tend to tail. This was not observed with C_{20} esters nor with C_{18} esters of higher degree of unsaturation. The separation of 20:5 (EPA) from 22:6 (DHA), judged feasible by Reichmann and Brunner (10), was complete at each load ratio. The calcu-

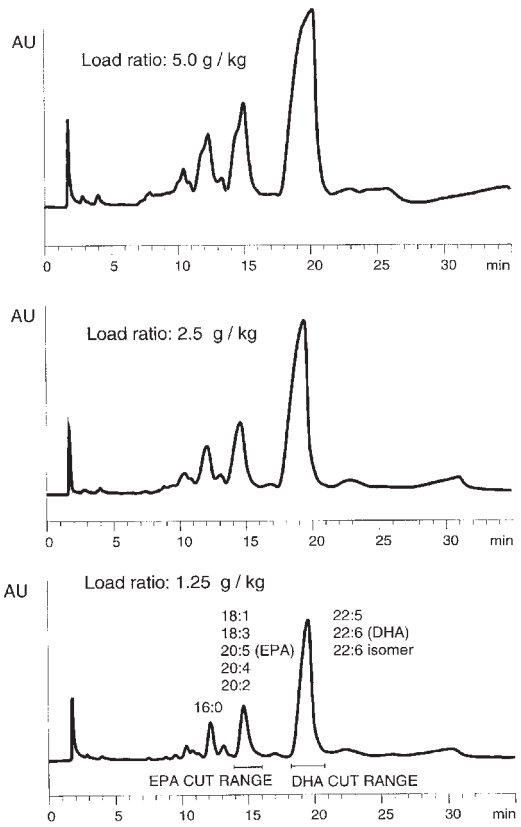


FIG. 1. Supercritical fluid chromatograms from preparative fish oil ethyl ester injections at different column load ratios. Fraction collection intervals for docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) concentrates are indicated. Kromasil 10-C18, 10 × 250 mm column. Pressure, 145 bar. Temperature 65°C. Mobile-phase linear velocity 1.9 mm/s. AU refers to absorbance units from the ultraviolet detector.

TABLE 3
DHA- and EPA-Ethyl Ester Fraction Purities (wt%) Obtained from Step 3 Preparative SFC Experiments[a]

	Load ratio (1.25 g/kg)		Load ratio (2.5 g/kg)		Load ratio (5.0 g/kg)	
Fraction	DHA purity	EPA purity	DHA purity	EPA purity	DHA purity	EPA purity
1	0.0	0.0	0.0	0.0	58.9	0.0
2	57.3	17.8	45.9	6.9	52.9	13.6
3	93.7	43.6	91.1	53.8	86.6	25.0
4	100.0	31.8	76.1	51.2	68.1	33.1
5	100.0	37.5	55.8	53.5	55.2	11.0

[a]For abbreviations see Tables 1 and 2.

lated SFC separation factor between EPA and DHA was good, $\alpha = 1.40$.

In the preparative runs, the measured production rate at 90 wt% purity was 0.85 g DHA-ethyl ester/(kg stationary phase · h). At 80 wt% purity the production rate was 1.9 g DHA ethyl ester/(kg stationary phase · h). These are lower than the estimated (Eq. 3) production rate, which was based on visual estimates of collection intervals from chromatograms. Visual estimation obviously does not reveal the elution of minor impurities within the main DHA peak. Therefore, it is imperative that fractions from the main peaks be collected and analyzed so that the real production rates can be calculated.

Purification of EPA-ethyl ester was far more demanding. The purest EPA fractions at 5.0 and 2.5 g/kg load ratios contained, respectively, 33.0 and 53.8 wt% EPA. Reducing the load to1.25 g/kg did not increase EPA purity. Similarly to the preparation of DHA, also in the separation of EPA the main impurities were stearic (18:0) and oleic (18:1) acid esters. At 50 wt% purity, the specific production rate of EPA was 0.23 g/(kg · h) while the calculated PR maximum from Equation 3 was 0.16 g / (kg · h).

The FAEE eluted primarily in the order of increasing carbon number with CO_2 mobile phase and ODS stationary phase. Within each carbon number, the most unsaturated esters eluted first.

The effect of LR on the experimentally verified PR at different purity levels is shown in Figure 2. The PR, calculated as pure PUFA, increase as column loading increases. However, when the LR exeeds a certain value at constant purity the production rate starts to decrease because the amount of collected fraction that meets the purity level decreases. When the required purity of the PUFA is set there is always an optimal column LR that gives the highest specific production rate.

Process design and purification costs. The process design and cost estimate are based on the results from the preparative SFC experiments carried out in Step 3. The production of DHA- and EPA-ethyl ester concentrates from transesterified tuna oil at 80–95 and 50 wt% respective purities requires only one supercritical chromatographic step. The flowsheet of an industrial SFC process, with descriptions of main equipment, has been described previously (12). An SFC process, which produces 1,000 kg DHA-ethyl ester and 400 kg EPA-ethyl

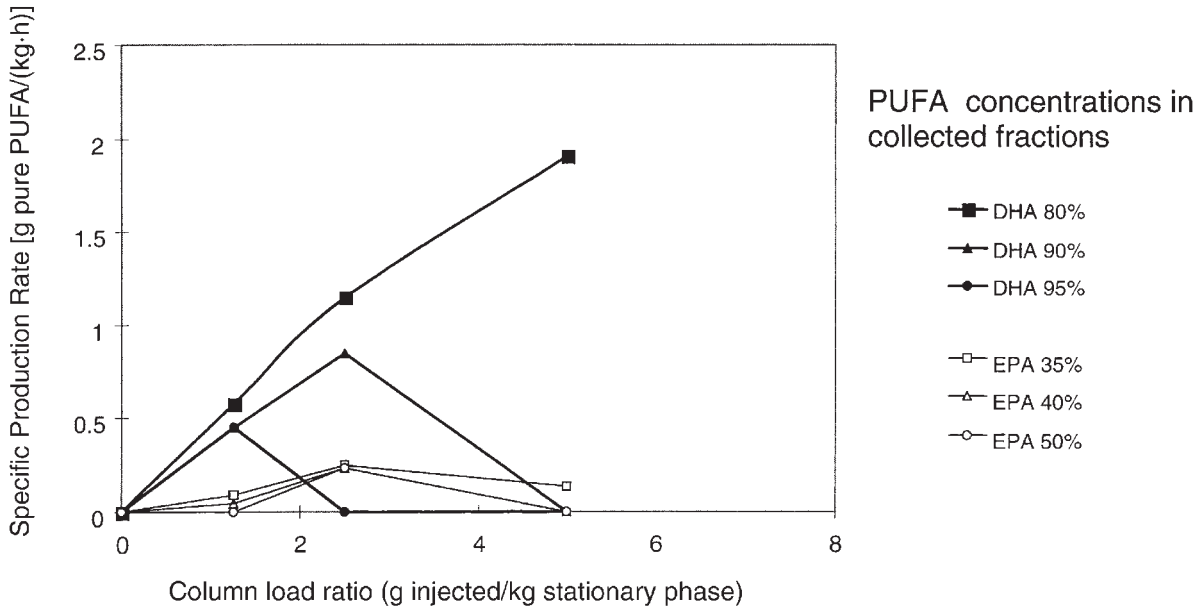


FIG. 2. The effect of column loading on the specific production rate of polyunsaturated fatty acids (PUFA) at different purities . Calculated from results obtained in Step 2 preparative SFC experiments. See Figure 1 for abbreviation.

ester concentrates per year requires that 2.6 tons of CO_2 per hour is circulated in the process. The ODS stationary phase requirement is 160 kg, which would preferably be packed in four parallel 600-mm i.d. columns. Main equipment for such an SFC process costs about U.S. $2 million. In assuming that the stationary phase would have to be replaced once a year, the total SFC operating costs are U.S. $550/kg DHA and EPA concentrate. The purification cost is sensitive to the lifetime of the stationary phase. The cost almost equals the US $200–500 range reported in 1994 by KD Pharma (4) and is considerably less than the US $ 4,000/DHA concentrate (95%) reported by Shisheido Corp. in 1996 (13) where a proprietary, silver-containing stationary phase was used.

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