Solubility of Fish Oil Fatty Acid Ethyl Esters in Sub- and Supercritical Carbon Dioxide

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Mutual solubilities and K-values of fish oil fatty acid ethyl esters, prepared from sand launce oil, and sub- and supercritical carbon dioxide have been measured in an apparatus originally designed for phase equilibrium, density and gas-oil ratio measurements of reservoir fluids. The measurements were performed at pressures from 2 to 22 MPa at temperatures of 283.2, 313.2 and 343.2°K. Experimental temperatures, pressures, solubilities, K-values and densities are reported. The K-values of ethyl myristate, palmitate, oleate, eicosapentaenoate and docosahexaenoate are compared with published experimental binary and/or multicomponent data. Because both vapor and liquid solubilities are reported, such data are applicable in the design of supercritical extraction plants.

KEY WORDS: Carbon dioxide, DHA, EPA, fatty acid ethyl ester, fish oil, myristic acid, oleic acid, palmitic acid, phase equilibria, solubility, supercritical, ω3.

Fish meals and oils are receiving increased commercial and academic attention because of their nutritional content, such as vitamins, essential fatty acids and antioxidants. Disadvantages of conventional methods for extraction, fractionation and isolation of these components include the use of highly flammable or toxic solvents and energy-intensive vacuum distillation or high-temperature processing that results in degradation of thermally labile compounds. These factors have caused investigators to apply supercritical fluid techniques for the separation of such components (1,2).

Seasonal fat content of fish ranges between 0.5 and 18.8% (3), primarily as triglycerides, and essential fatty acids are about 20–42% of the fat content (4). Fish oils may be the most important source of long-chain ω3 unsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Recent reports suggest that ω3 fatty acids may have beneficial effects in treatment of rheumatoid arthritis (5), atherosclerosis (6) and cancer of the colon (7).

Supercritical fluid research and technology applications to fish oil can be divided into two categories. The first involves supercritical fluid extraction and/or fractionation, while the second includes fundamental measurements of solubilities and/or phase equilibria in supercritical fluid media. Zosel (8) has described the use of supercritical ethane to extract and fractionate triglycerides from fish liver oil with a recirculation apparatus. He found that the molecular mass and the iodine value increased over the duration of the extraction and fractionation process. Eisenbach (9) utilized the same experimental procedure to separate EPA ethyl esters from a mixture of fatty acid ethyl esters (FAEE) derived from codfish oil with supercritical carbon dioxide (SC-CO_2). Like Zosel, he found that the low-molecular-weight components were extracted preferentially, causing the high-molecular-weight components (like EPA ethyl ester) to be isolated in the final fractions. In a two-step process he achieved a yield of 79.8% of the initial EPA content with a purity of 91.9%, the impurities being predominately eicosenoic acid ethyl esters. Arai and Saito (10) have described the fractionation of a synthetic mixture of fatty acids and fatty acid methyl esters from sardine oil by adductive urea crystallization with SC-CO_2 as the solvent. By this method they were able to separate the fatty acid components by chainlength as well as by their degree of unsaturation.

Interest in fundamental measurements has increased in the last five years to be able to design and predict fractionation processes. Chrastil (11) has reported data on the solubilities of triglycerides and pure fatty acids in SC-CO_2. These measurements were performed in the temperature range of 313.2 to 353.2°K at pressures of 8.1 to 25.3 MPa. Inomata *et al.* (12) have measured phase equilibria of binary systems of CO_2 and fatty acid methyl esters at four temperatures in the range of 313.2 to 343.2°K at pressures of 1.01 to 20.42 MPa. Finally, Bharath *et al.* (13) have presented experimental phase equilibrium data for FAEEs in CO_2 at temperatures of 313.2 to 333.2°K over the pressure range of 1.14 to 21.07 MPa.

To concentrate fatty acids of different chainlengths and degree of unsaturation, for instance EPA and DHA, the acids have to be removed from the glycerol and be processed either as free fatty acids or as methyl or ethyl esters. Esters are much more soluble in dense CO_2 than the free fatty acids and are thus preferentially extracted. Because the EPA and DHA are consumed as reconstituted triglycerides, and because ethanol is much less toxic than methanol, ethyl esters are the obvious choice for downstream processing of fatty acids for later consumption as synthesized triglycerides.

In this work we have measured the phase equilibria of a multicomponent mixture consisting of CO_2 and the FAEEs derived from sand launces or sandeels (*Ammodytes lancea*). Recently, Nilsson *et al.* (14) published similar measurements of partition coefficients for FAEEs derived from menhaden oil, in SC-CO_2 with and without ethanol as cosolvent. Their measurements were made at 333.2°K and 12.5 MPa. Data of this kind are of considerable importance when designing countercurrent extraction processes, which requires knowledge of K-values. A literature survey of solubility and phase equilibrium measurements on fish oils in supercritical fluids has been given by Staby (15).

EXPERIMENTAL PROCEDURES

Equipment. A combined gravimetric and volumetric method was used to determine the mutual solubilities and K-values of fish oil ethyl esters and CO_2. The apparatus has previously been described by Staby and Mollerup (16). Briefly, the apparatus (D. B. Robinson Design & Manufacturing Ltd., Edmonton, Alberta, Canada) with standard features was originally designed for phase equilibrium, density and gas-oil ratio measurements, but was modified for measurements of supercritical fluid phase equilibrium properties. The apparatus consists of a phase equilibrium view-cell, an airbath, a displacement pump, pycnometers,

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a rocking mechanism, filling, cleaning and sampling lines, and a gasometer. The phase equilibrium cell is of the variable-volume static-cell type rated to 70 MPa, and the working temperature range is 278 to 453°K. The pressure is measured by a pressure transducer (Hottinger Baldwin Messtechnik GmbH, Darmstadt, Germany) with an accuracy better than ±20 kPa, and the temperature setpoint resolution is 0.1°K. The gasometer is used to measure the gas volume at ambient temperature and atmospheric pressure liberated from the pycnometer sample, and it has a volume capacity of 10 L.

The composition of the liquid part of the vapor and liquid samples was determined with an SFC-3000 chromatograph (Carlo Erba Instruments, Milan, Italy) in the gas-chromatographic mode. The chromatograph was equipped with a flame-ionization detector (FID), a cold on-column injection port connected to a retention gap (1.5 m × 0.32 mm) and an HP-FFAP (Hewlett-Packard Company, Avondale, PA) capillary column (25 m × 0.2 mm × 0.33 μm).

Methods. The experimental procedure has been described elsewhere in detail (15,16). In a typical experiment, a 10- to 20-g sample is withdrawn from the cell into an evacuated and weighed high-pressure pycnometer. The pycnometer is weighed before and after the sample is depressurized in the gasometer to atmospheric conditions, and the amount of liberated gas is measured. The remaining liquid fraction in the pycnometer is either poured out or rinsed out with *n*-heptane and analyzed by gas-liquid chromatography (GLC). A computer program is used to verify the mass balance of the sample and calculate the solubilities. The density of the sample is determined from the weight of the sample and the sample volume displaced after a correction for the dead volume in the sample line. An estimate of the relative uncertainty in the measured solubilities is between 3–5%. The relative uncertainty in the fish oil ester solubility at low densities may be larger because of the small amount of solute in the sample, often less than 0.1 g in a 10- to 20-g of sample. The uncertainty of the sample pressure being measured in the pressure readings is less than ±30 kPa and the temperatures are known within ±0.2°K. The uncertainty in the measured densities is probably less than ±30 kg/m^3. This large uncertainty is mainly due to the uncertainty in the dead volume correction in the sampling line because flashing may occur during sampling.

The FAEE composition of the fish oil fractions was determined by GLC. The cold on-column injection technique with a retention gap was used as described by Grob (17). The collected liquid fraction of the samples was diluted to approximately 0.05% with heptane, and 1.0 μL of the heptane solutions was injected onto the column through the cold on-column injection port. The analyses were performed with the FID temperature set at 270°C and the flow at 1 mL/min. The initial oven temperature of 95°C was held for 2 min, then increased at a rate of 3°C/min to 170°C followed by an increase of 1°C/min to 210°C, where it was held constant for 52 min. The resultant chromatographic peaks were identified by comparing their retention times to those of injected standards and by confirmation analyses (by Dr. Benny Jensen of The Technological Laboratory, Danish Ministry of Fisheries, Lyngby, Denmark). A computer program converts the chromatographic peak areas into relative amounts of each FAEE and calculates the K-values of each of the 29 components identified. As shown later, the chromatographic analysis achieves good separation but at an analysis time of 2 h compared to the approximately 30 min required by the AOCS standard Method Ce 1b-89 (18). The relative standard deviation of the major components (>1%) may be as large as 2% but generally it is less than 1%, while for minor components it may range between 4–10%. Other lipids, like cholesterol, were not detected by this method.

Materials. The CO_2 was supplied by Linde AG. (München, Germany) with a stated purity of 99.995%+. The ethyl esters from the fish oil of sand launce were supplied by Grindsted Products A/S (Århus, Denmark) with a stated purity of 98%. Both materials were used without further purification.

RESULTS AND DISCUSSION

Results obtained from the view-cell showed that the system at 313.2 and 343.2°K has a two-phase vapor/supercritical fluid (SCF)-liquid boundary below the system critical pressure. At 283.2°K the system has a two-phase vapor-liquid boundary below 4.2 MPa and a three-phase boundary between 4.2 and 4.7 MPa. Above 4.7 MPa, a two-phase liquid-liquid region at CO_2 compositions between 60 and 90% exists and a vapor-liquid region at higher CO_2 concentrations. The equilibrium cell was not charged to a constant overall composition, but the overall composition was kept at 60–80 wt% CO_2. The dependence of the overall composition on the results was in our case limited as the feed molar ratio of all components heavier than the supercritical gas were constant. The crude fish oil ester mixture had a yellow color in the optical cell. This yellow color was reflected in the ester mixture-rich liquid phase at all temperatures and pressures, and somewhat in the CO_2-rich vapor and liquid phase near the critical pressure.

The experimental pressures, P, mutual solubilities, s, and densities, ϱ, for the CO_2 + fish oil FAEE system are presented in Tables 1–3. The solubilities are reported as kg fish oil FAEE/Nm^3 CO_2 (CO_2 at 273.2°K and 0.1 MPa) for the vapor phase and as Nm^3 CO_2/kg fish oil FAEE for the liquid phase. Single runs were performed at each pressure. The critical pressures of the system are 5.90 MPa at 283.2°K, 15.64 MPa at 313.2°K and 22.00 MPa at 343.2°K. Figure 1 shows the fish oil ester solubility in CO_2 as function of the pure CO_2 density. The pure CO_2 densities are calculated from a computerized version of the tables by Younglove and Ely (19). The solubility isotherms presented in Figure 1 show the same trend as other natural products, such as soybean oil, in dense CO_2 (2). The gap in the 283.2°K curve represents the phase transition from vapor-liquid to liquid-liquid equilibrium. At the two other temperatures the enhanced fish oil ester solubility above the critical density of CO_2 (470 kg/m^3) is easily seen.

The experimental vapor and liquid phase densities presented in Tables 1–3 show that the densities of the liquid phases are almost constant with increased admixture of CO_2 at $x_{CO_2} < 80\%$ and that the densities are highest at the lowest temperature. At $x_{CO_2} > 80\%$ the density suddenly decreases as the supercritical region is entered, and at $90\% < x_{CO_2} < 95\%$ the density curves cross over.

The composition and retention times of the fish oil

TABLE 1

Mutual Solubilities and Densities for the Carbon Dioxide/Fish Oil Ethyl Ester System and Pure Carbon Dioxide Densities at 283.2°K

Run number	P (MPa)	S_{Vapor} (kg/Nm^3)	ϱ_{Vapor} (kg/m^3)	S_{Liquid} (Nm^3/kg)	ϱ_{Liquid} (kg/m^3)
1	2.04	—	—	0.067	1000
2	3.59	0.001	90	—	—
2	3.57	—	—	0.178	970
3	4.20	0.002	160	—	—
3	4.20	—	—	0.356	1200
4	4.67	0.245[a]	870[a]	—	—
4	4.37	—	—	0.802	1300
5	4.26	—	—	0.767	930
6	4.94	0.360[a]	920[a]	—	—
6	4.90	—	—	0.712	940
7	4.98	—	—	0.770	940
8	5.45	0.509[a]	920[a]	—	—
8	5.45	—	—	0.910	930
9	5.90	—	—	1.369	—

[a]Carbon dioxide-rich liquid phase.

TABLE 2

Mutual Solubilities and Densities for the Carbon Dioxide/Fish Oil Ethyl Ester System and Pure Carbon Dioxide Densities at 313.2°K

Run number	P (MPa)	S_{Vapor} (kg/Nm^3)	ϱ_{Vapor} (kg/m^3)	S_{Liquid} (Nm^3/kg)	ϱ_{Liquid} (kg/m^3)
10	2.58	—	—	0.042	970
11	5.06	0.006	130	—	—
11	5.03	—	—	0.123	1000
12	7.42	0.009	210	—	—
12	7.42	—	—	0.233	910
13	8.99	0.010	480	—	—
13	8.99	—	—	0.377	940
14	10.35	0.076	710	—	—
14	10.28	—	—	0.468	910
15	12.57	0.198	800	—	—
15	12.55	—	—	0.558	910
16	15.00	0.432	860	—	—
16	15.03	—	—	0.900	920
17	15.64	—	—	1.369	—

TABLE 3

Mutual Solubilities and Densities for the Carbon Dioxide/Fish Oil Ethyl Ester System and Pure Carbon Dioxide Densities at 343.2°K

Run number	P (MPa)	S_{Vapor} (kg/Nm^3)	ϱ_{Vapor} (kg/m^3)	S_{Liquid} (Nm^3/kg)	ϱ_{Liquid} (kg/m^3)
18	5.13	—	—	0.064	910
19	10.12	0.005	250	—	—
19	10.04	—	—	0.170	880
20	12.49	0.006	390	—	—
20	12.49	—	—	0.248	900
21	15.03	0.026	540	—	—
21	14.97	—	—	0.336	930
22	15.19	—	—	0.335	900
23	17.50	0.072	650	—	—
23	17.49	—	—	0.423	910
24	19.95	0.177	740	—	—
24	19.94	—	—	0.551	900
25	21.50	0.345	800	—	—
25	21.52	—	—	0.750	880
26	22.00	—	—	1.369	820

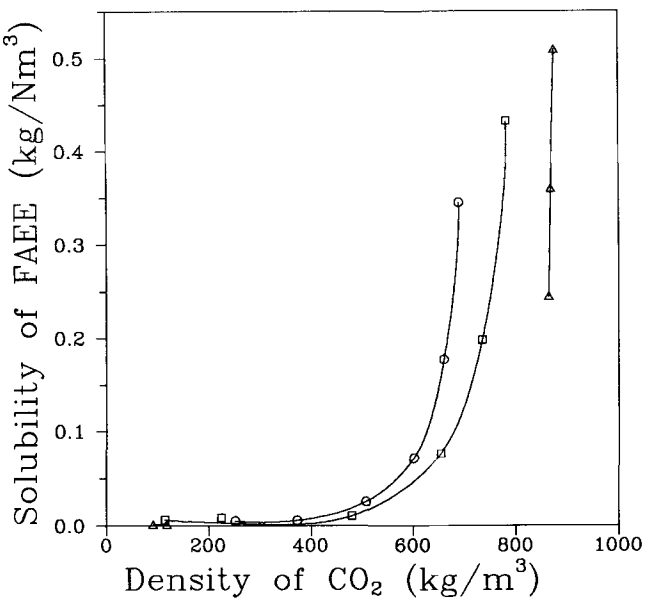

FIG. 1. Experimental solubility isotherms of the fish oil fatty acid ethyl ester (FAEE) mixture in carbon dioxide as a function of pure carbon dioxide density at (△) 283.2°K, (□) 313.2°K and (○) 343.2°K.

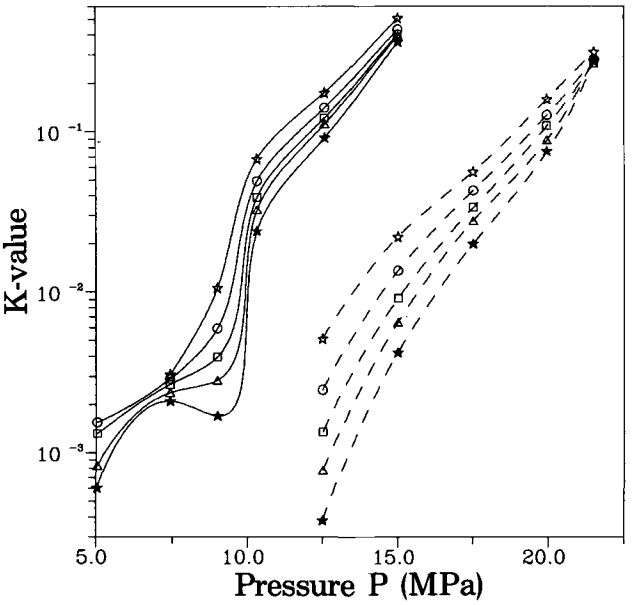

FIG. 2. K-values of the ethyl esters of (☆) myristic acid, (○) palmitic acid, (□) oleic acid, (△) eicosapentaenoic acid and (★) docosahexaenoic acid from the fish oil ester mixture as a function of system pressure at (——) 313.2°K and (---) 343.2°K.

TABLE 4

Chromatographic Retention Time, t_R, and Composition, x_i, of the Crude Fish Oil Fatty Acid Ethyl Ester Mixture

Component i	t_R (min)	x_i (mol%)	x_i (mass%)
$C_{10:0}$	15.40	0.5	0.4
$C_{12:0}$	22.50	0.3	0.2
$C_{14:0}$	29.79	8.9	7.5
$C_{14:1\omega5}$	32.05	0.6	0.5
$C_{15:0}$	34.00	0.6	0.5
$C_{15:1\omega5}$	36.48	0.2	0.2
$C_{16:0}$	39.13	19.9	18.5
$C_{16:1\omega7}$	40.52	13.4	12.4
$C_{16:2}$	43.86	1.5	1.4
$C_{16:3}$	45.71	0.7	0.6
$C_{16:4\omega3}$	49.31	0.9	0.8
$C_{18:0}$	50.74	2.1	2.2
$C_{18:1\omega9}$	52.06	10.1	10.2
$C_{18:1\omega7}$	52.52	2.3	2.3
$C_{18:2\omega6}$	55.10	2.9	2.9
$C_{18:3\omega6}$	57.06	0.4	0.4
$C_{18:3\omega3}$	59.60	1.3	1.3
$C_{18:4\omega3}$	61.71	3.8	3.8
$C_{20:0}$	64.12	0.2	0.2
$C_{20:1\omega9}$	65.57	3.8	4.2
$C_{20:2\omega6}$	68.97	0.3	0.3
$C_{20:4\omega6}$	72.61	0.2	0.2
$C_{20:4\omega3}$	76.74	0.6	0.7
$C_{20:5\omega3}$	78.93	9.3	10.0
$C_{22:1\omega11}$	81.81	5.4	6.5
$C_{22:1\omega9}$	82.36	0.9	1.0
$C_{21:5\omega3}$	90.82	0.4	0.4
$C_{22:5\omega3}$	105.12	0.4	0.5
$C_{22:6\omega3}$	110.02	8.3	9.6

FAEE mixture of sand launce are given in Table 4. Six duplicate chromatographic analyses of the fish oil ester mixture were performed and more than 95% of the total peak area was identified. The major components of the mixture are $C_{14:0}$, $C_{16:0}$, $C_{16:1\omega7}$, $C_{18:1\omega9}$, $C_{20:5\omega3}$ and $C_{22:6\omega3}$, corresponding to approx. 70% of the total mixture mass.

The K-values, given on a molar basis by

$$K_i = y_i/x_i \quad [1]$$

of some selected major components in the liquid fractions of the high-pressure samples at 313.2 and 343.2°K are presented in Figure 2. Two to three chromatographic analyses of the CO_2-free samples collected in the pycnometers after off-gassing in the gasometer were performed. The small pressure difference between the vapor and liquid sample that appeared in the majority of the measurements does not affect the calculated K-values.

The K-values presented in Figure 2 at 313.2°K above 9.0 MPa and at 343.2°K show the same trends, and it appears that the relative difference in the K-values becomes greater by reducing the pressure, but the absolute size of the K-values becomes smaller. Thus, optimal extraction conditions in regard to separation of the esters by chainlength would be 9.0 MPa at 313.2°K and 12.5 MPa at 343.2°K, corresponding to a density of 400–500 kg/m^3. The trend of the curves in Figure 2 below 9.0 MPa is caused by the sudden density increase of CO_2 near the critical pressure, where the relative difference between the K-values goes through an inflexion point at the critical pressure of CO_2.

Figure 3 shows that the K-values of a series of esters with 16 carbon atoms but different degrees of unsaturation do not specifically depend on the degree of unsaturation. This trend is the same for all solutes of the same chainlength irrespective of their degree of unsaturation. Thus, we may conclude that the K-values vary primarily with the number of carbon atoms but not specifically with

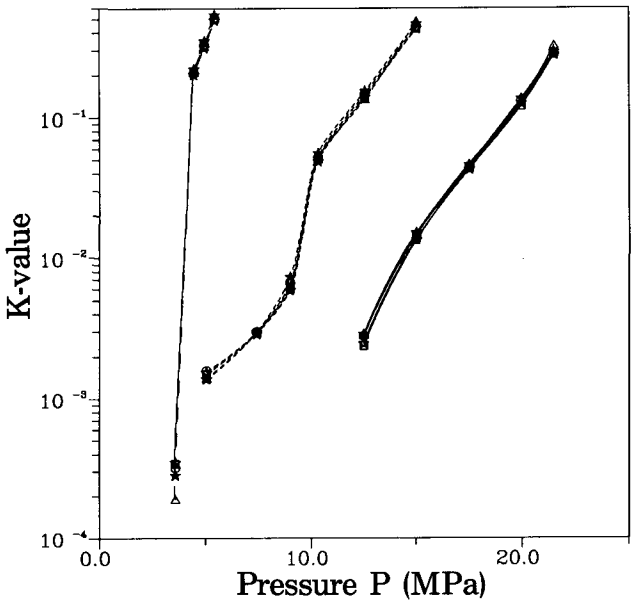

FIG. 3. K-values of the fatty acid ethyl esters of (★) $C_{16:0}$, (○) $C_{16:1\omega7}$, (□) $C_{16:2}$, (△) $C_{16:3}$ and (★) $C_{16:4\omega3}$ from the fish oil ester mixture as a function of system pressure at (— — —) 283.2°K, (- - -) 313.2°K, and (——) 343.2°K.

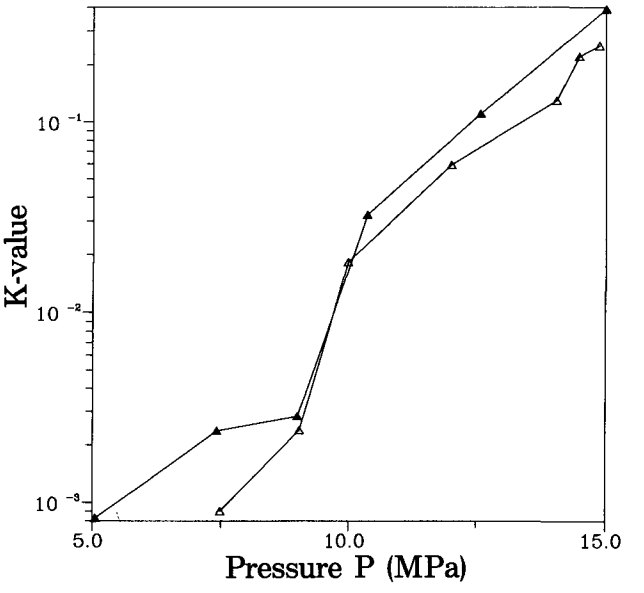

FIG. 5. Experimental K-values of eicosapentaenoic acid ethyl ester of this work (▲) compared with the binary data of Bharath *et al.* (Ref. 13) (△) as a function of pressure at 313.2°K.

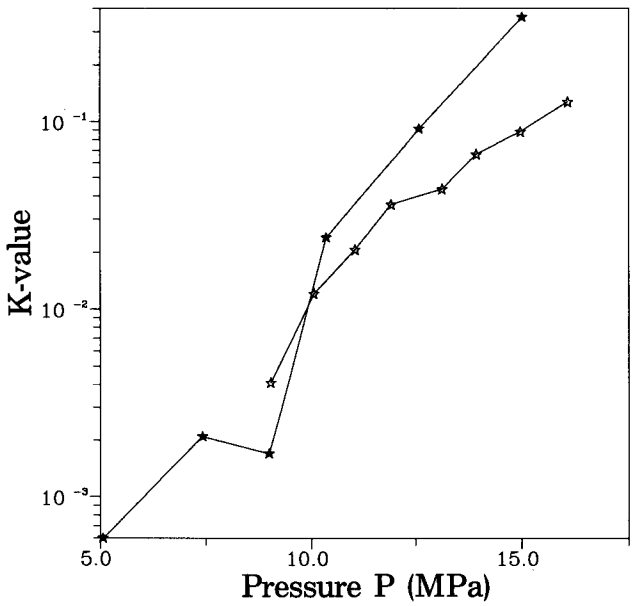

FIG. 4. Experimental K-values of docosahexaenoic acid ethyl ester of this work (★) compared with the binary data of Bharath *et al.* (Ref. 13) (☆) as a function of pressure at 313.2°K.

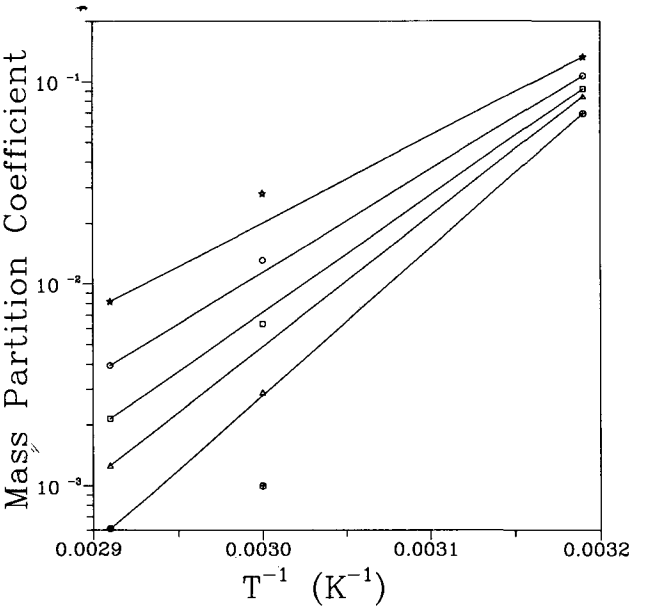

FIG. 6. Experimental mass partition coefficients of the ethyl esters of (☆) myristic acid, (○) palmitic acid, (□) oleic acid, (△) eicosapentaenoic acid and (★) docosahexaenoic acid of this work compared with the data of Nilsson *et al.* (Ref. 14) at 333°K as a function of inverse temperature at 12.5 MPa.

the degree of unsaturation. Thus, separation of FAEE mixtures in SC-CO_2 is carried out much more easily by carbon number than by degree of unsaturation.

Only a few data have been published on the phase equilibria of long-chain FAEE and SCF. Among these are the binary phase equilibrium data of Bharath *et al.* (13). Their K-values as a function of pressure at 313.2°K for the DHA and EPA ethyl esters in CO_2 are compared with our multicomponent data in Figures 4 and 5. K-values of the heavy DHA and EPA ethyl esters are, in general, greater when present in the fish oil ester mixture than in the binary mixture.

Recently, Nilsson *et al.* (14) have published multicomponent equilibrium data at 333.2°K and 12.5 MPa for menhaden oil FAEE in CO_2. Their data are presented as mass partition coefficients, defined as the ratio of the

weight concentration of component i in the CO_2-rich (vapor) phase to the weight concentration of component i in the lipid-rich (liquid) phase. The latter is inferred from mass balance considerations on a CO_2-free basis and not from direct measurements. The mass partition coefficients as a function of inverse temperature at approx. 12.5 MPa are presented in Figure 6. Here the observed lines represent an expected linear trend of the mass partition coefficients of the sand launce oil ester mixture between 313.2 and 343.2°K. As indicated in Figure 6, Nilsson *et al.* (14) obtain a better separation of their menhaden oil ester mixture at 333.2°K than we may expect in the present work. This is probably due to the rather large difference between the compositions of the two fish oil ester mixtures, the menhaden oil mixture having a larger amount of the polyunsaturated fatty acids.

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