Solubility of Fish Oil Components in Supercritical CO_2 and CO_2 + Ethanol Mixtures

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Solubility measurements for squalene, vitamin A palmitate, orange roughy oil, spiny dogfish liver oil, and commercial cod liver oil in supercritical carbon dioxide are reported over the temperature range 313−333 K and pressure range 200−300 bar. Solubility measurements are also reported for squalene, orange roughy oil, spiny dogfish liver oil, and cod liver oil in supercritical carbon dioxide + ethanol mixtures over the pressure range 200−300 bar at 333 K, and ethanol mass concentrations up to 12% by mass on a solute free basis. The use of ethanol as an entrainer substantially increased the solubility of all fish oil components. The increase in solubility, S, with ethanol concentration at fixed temperature and pressure was correlated by the empirical equation $S = S_0 \exp(kX)$, where S_0 is the solubility in pure CO_2 at given temperature and pressure, k is a fitted constant, and X is the mass fraction of ethanol on a solute-free basis.

Introduction

There is growing interest in the possibility of fractionating liquid lipid based oils by supercritical extraction to recover value added fractions (Catchpole et al., 1997; Bondioli et al., 1993; Brunner et al., 1991; Brunner, 1994; King et al., 1997; Ooi et al., 1996). The fractionation can be carried out continuously in packed columns. The design and operation of packed column extraction plants requires phase equilibrium data to determine the degree of fractionation possible and throughput at given scale of operation. In this work, the solubility of selected fish oils and representative fish oil components are measured in supercritical carbon dioxide. In addition, the enhancement in solubility of fish oils and selected fish oil components when using ethanol as an entrainer at concentrations of up to 12% by mass is determined. Previous work on the extraction of lipids from sunflower seeds (Cocero and Calvo, 1996), cocoa beans (Li and Hartland, 1996), fractionation of palm oil (Brunner and Peter, 1982; Ooi et al., 1996), and reported solubility of fatty acids (Iwai et al., 1996) and triglycerides (Geana and Steiner, 1995) in supercritical CO_2 + ethanol mixtures have shown that substantial increases in solubility are observed compared to pure CO_2. The fish oils and their components were selected to be representative of commercially available New Zealand fish species and to contain fractions of potential commercial value. Squalene is found in high concentrations in the livers of deep sea dog fish (Tsujimoto, 1932), vitamin A palmitate is the predominant form of vitamin A found in relatively large concentrations in surface dwelling sharks (Winholz, 1976), orange roughy oil contains predominantly wax esters and is obtained from the processing of waste deep sea orange roughy fish (Buisson et al., 1982), spiny dogfish (*Squalus acanthius*) liver oil contains both triglycerides and diacylglyceryl ethers (Malins et al., 1965), and cod liver oil is representative of typical polyunsaturated triglyceride fish oils found in many New Zealand species (Vlieg and Body, 1988).

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Experimental Section

Chemicals. Squalene was obtained by fractionating shark liver oil (Catchpole et al., 1997) to give 98% pure material. The purity was established by gas chromatography and refractive index measurements. Vitamin A palmitate was purchased from Sigma Chemicals at a stated purity of 95% by mass. Orange roughy oil obtained from the head and waste portions of the fish was supplied by NZ Fish Products and was deacidified using conventional alkali treatment to reduce the acid value to 0.15 before use. Spiny dogfish liver oil of low acid value obtained by careful cold pressing of the liver followed by filtering and chilling was supplied by MacCure Seafoods and was not further purified. The oil was analyzed by Iatroscan and found to contain approximately 50% by mass of triglycerides and diacylglyceryl ethers. Commercial grade cod liver oil was purchased from Healtheries NZ Ltd and was not further purified. Absolute ethanol at a stated purity of 99.82% by mass was supplied by Scharlau. Industrial grade carbon dioxide at >99.5% by mass purity was supplied by BOC Gases New Zealand Ltd.

Apparatus and Procedure. Measurements were carried out on the laboratory scale packed column apparatus shown schematically in Figure 1. Supercritical CO_2 was passed upward through the packed column (25.4 mm i.d.) consisting of three 0.5 m sections at the desired pressure (controlled to ±0.5 bar by a back pressure regulator) and temperature (controlled to ±0.5 °C by PID temperature controllers) at a mass flow rate of approximately 1 kg h^{-1}. Three sections were found to be sufficient for equilibrium to be achieved when measuring squalene solubilities and extracting squalene from shark liver oil in previous work (Catchpole and von Kamp, 1997; Catchpole et al., 1997). Oil was pumped into the top of the column by a plunger pump at a volumetric rate measurable by buret. Ethanol (also supplied by a feed buret) was compressed by a plunger pump, before being mixed with CO_2 prior to the column. Mixing was carried out by passing CO_2 and ethanol through a packed column PC of glass ballotini. Raffinate was collected from the bottom of the column at regular time

10.1021/je980184l CCC: $15.00 © 1998 American Chemical Society
Published on Web 10/22/1998

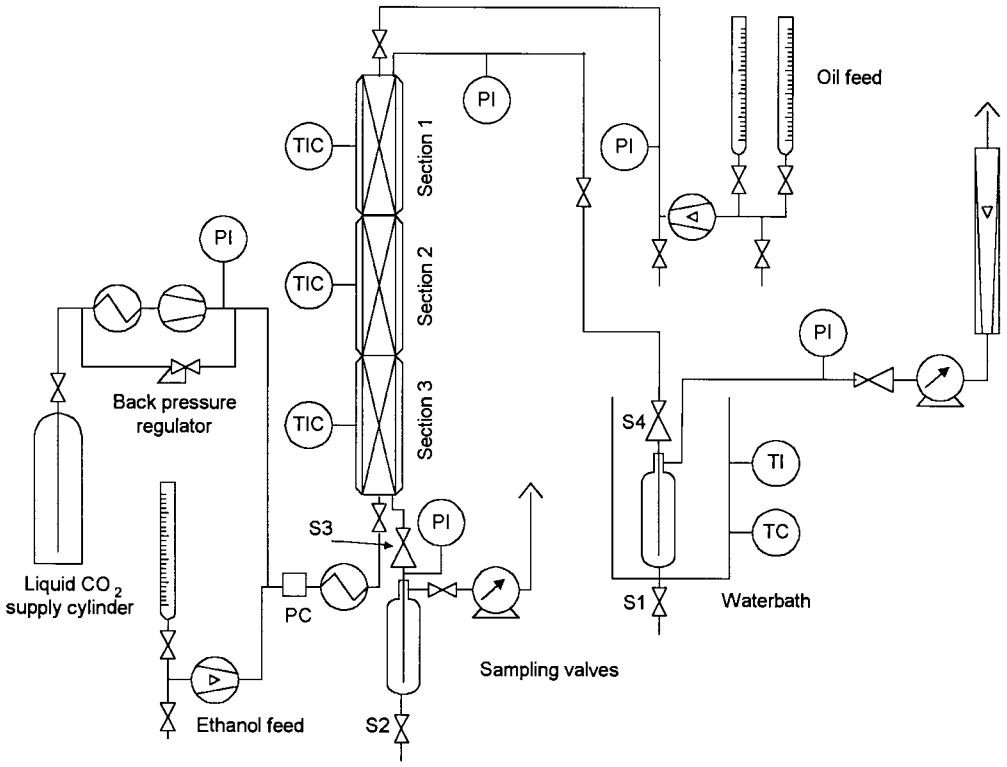

Figure 1. Schematic of solubility measurement apparatus.

intervals through valve S3. CO_2 and extract passed through a shut off valve, flow/pressure control valve S4 and into the separator, which was maintained at a pressure of 30 bar. Operation at 30 bar rather than atmospheric pressure was carried out to reduce the velocity in the separator to ensure that no entrainment of oil occurred. Extract was recovered at regular time intervals from this vessel. The mass recovered was measured on a balance to ±0.02 g accuracy. Ethanol was removed from raffinate and extract samples under vacuum. CO_2 leaving the separation vessel was reduced to atmospheric pressure and then passed through a flow totalizer and rotameter to determine the mass of gas that passed through over the same time interval. Solubility was calculated as the mass of extract recovered over the mass of CO_2 that passed through the flow totalizer over the given time period. All solubility determinations were carried out using a fixed mass flow rate of CO_2 and a sufficient excess of fish oil required to achieve saturation. At least four determinations were made per fixed temperature, pressure, and ethanol concentration combination, and the average value was taken. The errors involved in the measurements resulted in an uncertainty of ±0.05 g/kg. Liquid-phase concentration of CO_2 in solute was determined as described elsewhere (Catchpole and von Kamp, 1997).

Solubility measurements for squalene, vitamin A palmitate, orange roughy oil, spiny dogfish liver oil, and commercial cod liver oil in supercritical carbon dioxide were measured over the temperature range 313−333 K and pressure range 200−300 bar. A limited number of measurements were also made for the solubility of carbon dioxide in the oil phase for the squalene/CO_2 and cod liver oil/CO_2 systems. Solubility measurements were also carried out for squalene, orange roughy oil, spiny dogfish liver oil, and cod liver oil in supercritical carbon dioxide + ethanol mixtures over the pressure range 200−300 bar at 333 K, and ethanol mass concentrations up to 12% by mass on a solute-free basis.

Table 1. Solubility of Squalene in CO_2 and Liquid Mole Fraction of CO_2 in squalene

P/bar	S(323 K)/g·kg^{-1}	x	S(333 K)/g·kg	x
100	0.27	0.25		
150	10.63	0.27		
175	17.38	0.29		
200	23.84	0.31		
225	30.12	0.32	27.05	0.32
250			34.65	0.33
275			43.00	0.35
300			47.70	

Results and Discussion

Solubility Measurements using Pure Carbon Dioxide. The solubility of squalene in carbon dioxide was measured at selected pressure and temperature combinations to compare with existing reported measurements as a check on the reliability of the data and source of squalene used. Data were also obtained to extend these previous measurements. Agreement was within 1% by mass fraction of previous measurements (Catchpole and von Kamp, 1997). Unfortunately there is no other independent literature data for comparison. The measured data for the squalene/CO_2 binary system are listed in Table 1. The solubility data S_0 were correlated using a simple empirical density based equation:

$$\ln(S_0/\mathrm{g\cdot kg^{-1}}) = -28.24 - \frac{3936.6}{T/\mathrm{K}} + 6.54\ln(\rho_{CO_2}/\mathrm{kg\cdot m^{-3}}) \quad (1)$$

Equation 1 is slightly different from a similar equation published earlier (Catchpole and von Kamp, 1997) and gives a better fit over the extended range of conditions reported here. The equation is valid in the temperature range $313 \le T \le 333$ and CO_2 density range $350 \le \rho_{CO_2} \le 850$ kg m^{-3}. The liquid mole fractions are accurate to ±0.01

Phase equilibrium data for the system cod liver oil/CO_2 are reported in Table 2. Surprisingly, solubility measure-

Table 2. Solubility of Cod Liver Oil in CO_2 and Liquid Mole Fraction of CO_2 in Cod Liver Oil

P/bar	*S*(313 K)/g·kg^{-1}	*x*	*S*(333 K)/g·kg^{-1}	*x*
200	3.66	0.27	1.60	0.29
225	4.79	0.28	2.54	0.29
250	5.94	0.29	3.92	0.30
275			5.27	0.32
300			7.08	0.33

Table 3. Solubility of Vitamin A Palmitate and Spiny Dogfish Liver Oil in CO_2

	S(333 K)/g·kg^{-1}	
P/bar	vitamin A palmitate	spiny dogfish liver oil
125	0.24	
150	0.93	
200	3.19	1.40
225		2.02
250	6.20	2.95
275	8.52	4.09
300	10.47	5.40

ments for cod liver oil do not appear to have been reported in the literature under the conditions used in this work. The solubility data were closely correlated by the empirical density based equation of del Valle and Aguilera at 313 K (1989), but around 20% lower than predicted at 333 K. This may be a result of the cod liver oil having a higher molecular mass and larger amounts of C20 and C22 fatty acids than the seed oil solubility data on which the correlation was based. The liquid mole fractions are accurate to ±0.01. Cod liver oil is a multicomponent mixture of mainly triglycerides. It is likely that some degree of fractionation based on molecular weight and polarity takes place. Unfortunately, chemical analysis of the extract, raffinate, and feed oil was not carried out. Cod liver oil solubility measurements were repeated three times (three separate experiments) at each pressure to establish reproducibility for solutes with low solubility. As with squalene, agreement was within 1% by mass fraction.

Solubility measurements for vitamin A palmitate and spiny dogfish liver oil were measured at 333 K only. The solubility of carbon dioxide in the liquid phase was not measured. The results are reported in Table 3. The measured solubility of vitamin A palmitate is of similar order of magnitude but lower than that reported for vitamin A in the free acid form (Johannsen and Brunner, 1997) at corresponding pressures at 333 K. The decrease in polarity of the ester over the free acid is counterbalanced by the large increase in molecular weight, leading to a modest decrease in solubility. However, the purity of the vitamin A free acid was only 70% by mass (Johannsen and Brunner, 1997), and it is not stated whether the impurities have similar or greater solubility in their work. The solubility of spiny dogfish oil is similar to, but less than that of cod liver oil at corresponding pressures. The fatty acid profile of the triglycerides in both oils are similar, which suggests that the difference in solubility is due to the diacylglyceryl ethers. The solubility data for vitamin A palmitate as a function of carbon dioxide density are shown in Figure 2. Also included in Figure 2 are the solubilities of squalene, orange roughy oil, spiny dogfish liver oil, and cod liver oil. The solubilities for all components and oils show a linear relationship when plotted in logarithmic coordinates as has been observed for other lipids (del Valle and Aguilera, 1989; Johannsen and Brunner, 1997).

Solubility Measurements Using CO_2 + Ethanol Mixtures. Solubility measurements for squalene, orange roughy oil, spiny dogfish liver oil, and cod liver oil at 333 K, pressures from 200 to 300 bar, and ethanol concentrations of up to 12% by mass on a solute-free basis are reported in Table 4. Owing to small variations in the quantity of ethanol supplied by the plunger pump and carbon dioxide flowing through the column, it was not possible to exactly set the required mass fraction of ethanol in carbon dioxide. Results are therefore reported for the average ethanol concentration over the four or more determinations at each pressure and concentration combination. Ethanol concentrations are accurate to ±0.05% by mass. The data point at 275 bar and 9.4 mass % ethanol for squalene is suspect, as there is some possibility of entrainment. The addition of ethanol to carbon dioxide increases the density of the fluid phase. Squalene has a relatively low density of 854 g kg^{-3} at 293 K (Winholz, 1976) compared to the value for fish oils of around 900 kg m^{-3}, which increases the likelihood of flooding at high pressures where the density difference between squalene and CO_2 becomes very low (Catchpole et al., 1997).

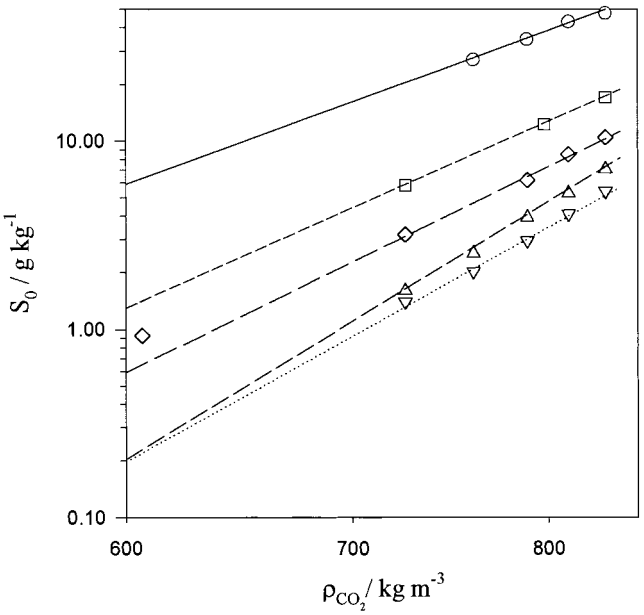

Figure 2. Solubilities S_0 of fish oils and associated components in supercritical carbon dioxide at 333 K as a function of density: ○, squalene; □, orange roughy oil; △, cod liver oil; ▽, spiny dogfish; ◇, vitamin A palmitate.

The use of ethanol substantially increases the solubility of squalene and the three fish oils. As an example, the solubility of squalene increases from 3.5% by mass at 250 bar and zero ethanol to 15.5% by mass at 10.3% by mass ethanol. Similarly, the solubility of orange roughy oil, which is predominantly long chain wax esters, is increased from 1.2 to 6.3% by mass at 260 bar as the ethanol concentration is increased from zero to 11.2% by mass. The rate of increase at fixed pressure with increasing ethanol concentration for cod liver oil, which is predominantly triglyceride based, is greater at low concentrations than both squalene and orange roughy oil. The solubility of all four liquids increased exponentially over the range 0−12% by mass ethanol with linear increases in the ethanol concentration (solute-free basis) at 200 bar, as shown in Figure 3. At higher pressures, the solubility increased exponentially for squalene, orange roughy oil, and spiny dogfish liver oil and linearly for cod liver oil with linear increases in ethanol concentration as shown in Figure 4. The cod liver oil solubility versus ethanol concentration follows the behavior reported for sunflower oil at high

Table 4. Solubility of Squalene, Orange Roughy Oil, Spiny Dogfish Liver Oil, and Cod Liver Oil in CO_2 + Ethanol at 333 K

		S/g·kg^{-1}			
P/bar	mass % ethanol	squalene	orange roughy	spiny dogfish	cod liver oil
200	0		5.79		
200	4.51			3.97	
200	4.86				4.81
200	4.99		14.43		
200	5.16	45.22			
200	7.53			7.21	
200	7.75				9.07
200	7.91		22.79		
200	8.47	72.07			
200	9.95			11.30	
200	10.89				14.91
200	11.01		35.36		
200	12.04	111.3			
250	4.16				11.58
250	4.26			7.99	
250	4.28	61.14			
250	6.51	85.28			
250	7.27				18.65
250	7.29			13.53	
250	9.74	146.3			
250	9.92				27.95
250	9.95			20.69	
250	10.28	155.3			
260	0		12.24		
260	5.00		26.23		
260	8.07		40.90		
260	11.17		63.41		
275	4.07	78.83			
275	4.31				15.70
275	4.37			9.97	
275	6.78	134.1			
275	6.92				21.15
275	7.11			16.58	
275	9.41	236.9			
275	9.61				32.60
275	9.72			24.68	
300	0		17.10		
300	3.98			12.25	
300	4.25				16.81
300	4.58		32.44		
300	6.56			18.58	
300	7.17				27.12
300	7.66		50.17		
300	9.53			28.05	
300	10.20				42.35
300	10.50		79.91		

pressures (Cocero and Calvo, 1996). The exponential increase in solubility S was correlated with the mass percent ethanol X as follows:

$$\ln(S/\text{g·kg}) = \ln(S_0/\text{g·kg}) + kX \qquad (2)$$

The validity of eq 2 has not been tested at temperatures other than 333 K and pressures lower than 200 bar, and so the variation in the correlating constant k with temperature and low pressure is not known. The constant k was fitted to each isobar for each solute and was found to be almost constant with pressure for both orange roughy at 0.15 and spiny dogfish liver oil at 0.21. k increased slightly with pressure for squalene but could be averaged to give a reasonable fit at 0.14. Ethanol is a useful entrainer for increasing the solubility of typical fish oil components, thus increasing the throughput in fractionation plant with fixed CO_2 flow rate capacity.

Conclusions

The solubility of squalene, vitamin A palmitate, orange roughy oil, and commercial cod liver oil in supercritical CO_2 have been measured over the temperature range 313−333 K and pressure range 200−300 bar. The solubility behavior is similar to other lipids and can be correlated against solvent density. Solubility measurements have also been reported for the first time for squalene, orange roughy oil, spiny dogfish oil, and cod liver oil in supercritical carbon dioxide + ethanol mixtures over the pressure range 200−300 bar at 333 K, and ethanol mass concentrations up to 12% by mass on a solute-free basis. Ethanol acted as an entrainer and substantially increased the solubility of all fish oil components. The solubility in the mixed solvent was correlated with the mass fraction of ethanol on a solute-free basis, and the solubility in pure CO_2.

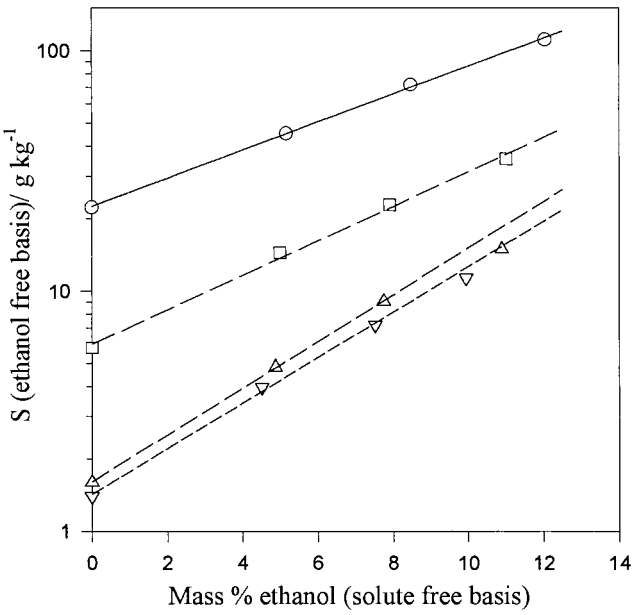

Figure 3. Solubilities S of squalene, orange roughy oil, cod liver oil, and spiny dogfish liver oil in CO_2 + ethanol mixtures at 333 K, 200 bar as a function of ethanol concentration; ○, squalene; □, orange roughy oil; △, cod liver oil; ▽, spiny dogfish.

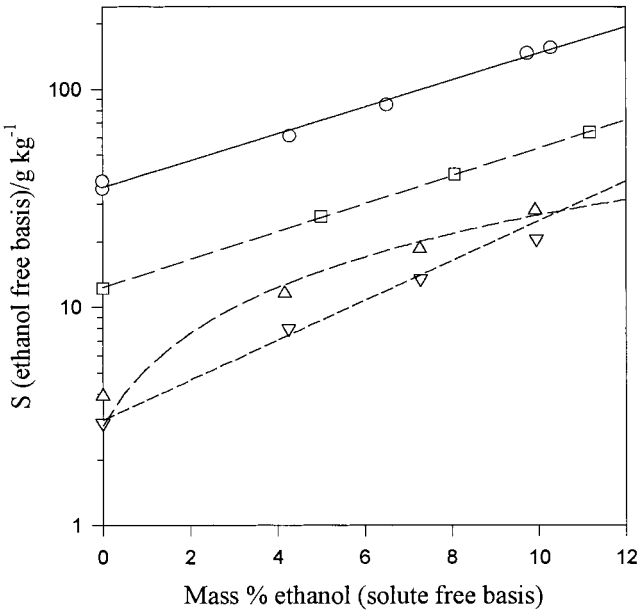

Figure 4. Solubilities S of squalene, cod liver oil, and spiny dogfish liver oil at 250 bar, and orange roughy oil at 260 bar in CO_2 + ethanol mixtures at 333 K as a function of ethanol concentration: ○, squalene; □, orange roughy oil; △, cod liver oil; ▽, spiny dogfish.

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Received for review August 6, 1998. Accepted September 11, 1998.

JE980184L